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Transfer of maternal colostral leukocytes promotes development of the neonatal immune system Part II. Effects on neonatal lymphocytes☆

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Abstract

It has been established that maternal leukocytes, conditioned by the mammary environment, cross the neonatal gut and circulate in the newborn calf. However, the impact of these cells on the development of neonatal immunity remains to be determined. This study examined the effects of maternal colostral leukocytes on development and maturation of neonatal adaptive immunity by examining the expression of surface markers on neonatal lymphocytes. At birth, neonatal calves were fed whole colostrum, or colostrum that had the maternal cells removed (cell-free colostrum), from their respective dams. Peripheral blood samples were collected at regular intervals over the first 4 weeks of life and lymphocytes were evaluated for surface expression of cellular markers. The results of these studies demonstrated that calves receiving whole colostrum had fewer CD11a positive lymphocytes in circulation during the first 2 weeks of life and this marker was expressed at a lower density than calves receiving cell-free colostrum. In addition, calves receiving whole colostrum also had a higher percentage of lymphocytes expressing the activation markers CD25 and CD26 by 7 days after birth. During the first week of life, lymphocytes from calves receiving whole colostrum had a higher density of MHC class I expression on their surfaces than cells from calves receiving cell-free colostrum. In general, these results indicate that transfer of maternal cells with colostrum allows for more rapid development of lymphocytes and maternal cells appeared to enhance their activation.

Keywords: Bovine; Calves; Colostrum; Immunity; Leukocyte

1. Introduction

To ease the transition of the neonate into the inhospitable, competitive environment of the outside world, the mother provides colostrum; a mixture of relatively high concentrations of easily digestible nutrients, hormones, and important immunological components. These immunological components include immunoglobulin, cytokines, and substantial numbers of leukocytes (Mallard et al., 1998; Duhamel et al., 1987;

Abbreviations: C, whole colostrum; CFC, cell-free colostrum; PBMC, peripheral blood mononuclear cells.

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Concha, 1986). While the protective role of colostral immunoglobulin has been well studied, our understanding of the mechanisms underlying protection by maternal colostral leukocytes has proven much more challenging.

When activated, leukocytes originating in bronchial-associated and mucosal-associated lymphoid tissues migrate to distant lymphoid and non-lymphoid tissues. As animals near parturition, these cells also migrate to the mammary gland (Chernishov and Slukvin, 1990; Nickerson, 1985; Norcross, 1982), where their phenotype and function are altered (Reber et al., 2006; Harp and Nonnecke, 1986; Ho and Lawton, 1978; Parmley et al., 1977). Of significant note is the up-regulation of homing and trafficking markers on these cells as they are incorporated into colostrum. Changes in these cell surface markers likely aid in the migration of these cells from the neonate's intestine across the gut epithelial barrier into the circulation, and in the expression of a memory phenotype (Reber et al., 2006; Taylor et al., 1994). The ability of colostral leukocytes to traffic into the peripheral blood of the neonate has been demonstrated in several species including cattle, swine, baboons, and sheep (Reber et al., 2006; Williams, 1993; Jain et al., 1989; Tuboly et al., 1988; Sheldrake and Husband, 1985; Schnorr and Pearson, 1984). However, upon entering the neonate, the role of transferred maternal colostral leukocytes in protection of the neonate remains undetermined.

The uptake of maternal lymphocytes into the neonate's circulation may result in the transfer of antigen-specific immunity to the neonate; the results of our most recent studies suggest that these cells may provide immediate, transitory antigen-specific activity (Donovan et al., 2007). However, these cells also appear to play an important role in the development of the neonatal immune system. Our earlier studies demonstrated that transferred maternal colostral leukocytes influence neonatal immune responses as indicated by their ability to modulate the neonatal responses to foreign leukocytes in mixed leukocyte responses (Reber et al., 2005). In those studies, calves receiving maternal colostral leukocytes developed tolerance to maternal cells, but preserved responses to cells from unrelated cows. In contrast, calves receiving cell-free colostrum did not exhibit these differential responses.

To investigate the effects of maternal colostral leukocytes on development of cells involved in neonatal adaptive immunity, we examined the effects of transferred cells on the phenotype and function of neonatal lymphocytes. At birth, calves were fed whole colostrum (C) or cell-free colostrum (CFC), and expression of important cellular markers was examined during the first 4 weeks of life.

2. Materials and methods

2.1. Humane treatment of animals

Animals in this project 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 number A2005-10027-c2.

2.2. Animals and treatments

Ten multiparous, Holstein dairy calves from the University of Georgia Dairy Facility were used for this project. To obtain calves immediately at birth, a monitoring system (Foalert Inc., Acworth, GA) was used to alert investigators at initiation of parturition. Monitors were sutured to the vulvar lips of dams 7 days prior to anticipated parturition. Each calf received colostrum from its respective dam; five calves received C and five received CFC. Each calf consumed at least four pints of their respective colostrum treatment within 4 h of birth, and a second four pints approximately 12 h later. All calves were fed a milk replacer diet after colostrum treatment.

Forty-five milliliters of heparinized blood and 5 ml serum samples were obtained via jugular venipuncture from calves at birth (prior to feeding colostrum), 24 h after birth, 48 h after birth, and once weekly for 4 weeks. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood by single step buoyant density floatation over Histopaque 1083 (Sigma, St. Louis, MO) as previously described (Reber et al., 2005). Expression of cell surface markers was evaluated by monoclonal antibody staining and flow cytometry. Serum antibody concentrations were determined at each sample time.

2.3. Colostrum preparations

Whole colostrum was collected from each dam of calves in this study. Calves receiving C were bottle-fed colostrum from their respective dam. Cell-free colostrum was prepared by centrifuging the colostrum for 40 min at 1500 × *g* at 26 °C in 1 l bottles. The soft, lipid layer that formed at the top of the supernatant 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, placed in double zip lock storage bags, and rapidly frozen between stainless steel plates pre-frozen to −80 °C. Once the colostrum was frozen, the bags containing the colostrum were placed in a water bath at 50 °C until the colostrum became slushy. They were then placed in a water bath at 37 °C until completely thawed and bottle-fed to calves in the CFC treatment group.

2.4. Serum antibody ELISA

Concentrations of IgG in serum samples from the calves were measured using a sandwich ELISA. Rabbit anti-bovine IgG antibody (B5645; Sigma, St. Louis, MO) diluted 1:400 in sodium carbonate buffer at pH 9.0 was used to coat Immulon 4HBX plates (Thermo Corp., Milford, MA) at 4 °C overnight. The plates were washed three times with phosphate-buffered saline containing 0.5% Tween 20. Samples were diluted 1:10 in diluent (phosphate-buffered saline containing 0.5% Tween 20) for screening and IgG positive samples were further diluted to determine titer endpoint. The samples were placed in quadruplicate wells for assessment, and incubated for 1 h at room temperature. The plates were again washed three times, after which bound IgG was detected using a rabbit-anti-bovine IgG conjugated to horseradish peroxidase (A5295; Sigma, St. Louis, MO). The detection antibody was diluted 1:1000 in diluent and the wells were incubated with detection antibody for 30 min. The plates were washed three times with wash buffer, after which bound detection antibody was detected using 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) substrate (A-9941; Sigma, St. Louis, MO). The plates were incubated for 30 min to allow color development and measured using a plate reader with a 405 nm filter. The quantity was determined relative to a six-point serial dilution of bovine gamma globulin standard (I5506; Sigma, St. Louis, MO).

2.5. Phenotyping and analysis

The phenotype of isolated PBMC was evaluated using monoclonal antibodies specific for cell surface receptors associated with cellular differentiation and function (Table 1). Antibody concentrations were optimized using a step-wise dilution series. The highest dilution yielding maximal (peak) signal intensity was used for each antibody. Isolated PBMC were evaluated for cell viability using trypan blue exclusion prior to staining; greater than 90% of PBMC were viable in all samples. Cells were placed in round bottom 96-well plates (5×10^5 cells/well) and stained for 20 min at 4 °C. A 1:100 dilution of primary antibodies was made in FACS buffer (0.2% bovine serum albumin and 0.1% sodium azide in phosphate-buffered saline), and 20 μl was placed in each well. Cells were washed three times with cold, sterile FACS buffer, and labeled with FITC-conjugated goat anti-mouse IgG (Sigma, St. Louis, MO) as a secondary antibody for 20 min at 4 °C. Cells were stained using 20 μl of diluted (1:100) secondary antibody per well. Cells were washed three times with cold, sterile FACS buffer, suspended in 100 μl of cold FACS buffer, and added to 400 μl of cold FACS fixative (4% formalin in FACS buffer). Samples were stored at 4 °C until analyzed by flow cytometry using a Coulter Epics XL-MCL flow cytometer. Samples were analyzed with respect to cells stained with secondary antibody only. Expression above that of the secondary antibody control was considered to be positive. Flo Jo analysis software (Tree Star Inc., San Carlos, CA) was used for flow cytometry analysis and all samples were analyzed as a batch with a single set of parameters across the whole set of files to insure a fair comparison. At least 10,000 cells were analyzed per sample, and samples were gated on forward vs. side scatter and analyzed according to regions: lymphocytes, activated lymphocytes, and monocytes. The three regions for analysis were established by staining with monoclonal antibodies specific for monocytes, granulocytes, B cells, and T cells (Table 1). Cells within the lymphocyte gate stained for B cells and T cells (CD3). T lymphocytes were examined as a total population; we were unable to stain for the lymphocyte subsets T helper (CD4), T cytotoxic (CD8), and gamma–delta T cells (WC1) individually due to limitations of the number of cells

Table 1
Monoclonal antibodies for characterization of neonatal leukocytes

Specificity	Source	Clone
Gating antibodies		
Monocytes	VMRD	BAQ151A
Granulocytes	VMRD	MM20A
B cells	VMRD	BAQ155A
T cells (CD3)	VMRD	MM1A
Differentiation and activation antibodies		
CD11a/18 (LFA-1)	VMRD	BAT75A
CD25	VMRD	CACT108A
CD26	VMRD	CACT114A
CD172a	Serotec	MCA2041S
MHC I	VMRD	H58A
MHC II	VMRD	TH14B

Changes in cellular differentiation and activation markers were measured during the first 4 weeks of life to characterize the effects of maternal colostral leukocytes on neonatal lymphocytes.

and time. Cells within the activated lymphocyte gate stained with antibodies specific for B cells and CD3, but were larger and more granular (forward vs. side scatter) than cells in our lymphocyte gate. Activated lymphocytes increase in size and granularity (Sweat et al., 2005; Grossman et al., 2004; Castillo-Olivares et al., 2003) we therefore designated these cells to be "activated". Although we did not measure a large proportion of cells staining positive for CD25, the majority of our CD25 positive cells were located within this gate, further supporting their designation of activated lymphocytes. Purification using Histopaque 1083 eliminates the majority of granulocytes; however, a small number of granulocytes were identified in the purified PBMC population. These cells fell into the activated lymphocyte region.

2.6. Statistical analysis

Outliers were removed from analysis using a Q-test (Skoog and West, 1969). Statistical differences were determined at each time point using an unpaired t-test. Analysis yielding $p \leq 0.05$ was considered significant.

3. Results

3.1. Total leukocytes

Lymphocyte, activated lymphocyte, and monocyte populations were examined for 4 weeks after neonatal calves were fed C or CFC. The number of PBMC recovered per milliliter of blood collected increased steadily throughout the first 4 weeks of life in neonatal calves (Fig. 1A). The average cell density of calves at birth was 2.2×10^6 PBMC/ml, and increased to 3.3×10^6 PBMC/ml by 4 weeks of age. For both treatment groups, the percentage of lymphocytes was highest at birth, averaging 83% of the total PBMC population (Fig. 1B). This percentage declined rapidly during the first 48 h, then stabilized over the next 4 weeks. The lymphocyte density also decreased during the first 48 h, then increased throughout the remainder of the 4-week period as the total PBMC density increased (Fig. 1C). The activated lymphocyte population was relatively low at birth (0.5%) and increased only slightly throughout the first 4 weeks of life (Fig. 1D); the number of activated lymphocytes followed a similar trend (Fig. 1E). The monocyte lineage population averaged 10.6% of the total PBMC population at birth and increased steadily over the 4 weeks (Fig. 1F), calves receiving CFC had an increased percentage of circulating monocytes during the first 2 weeks of life ($p < 0.05$). The density of monocytes in the peripheral blood increased steadily over the first 4 weeks of life (Fig. 1G); calves receiving CFC had a significantly higher number of circulating monocytes at 48 h post-feeding ($p < 0.05$).

3.2. Transfer of maternal antibody

All calves had relatively low concentrations of IgG in serum at birth, but these concentrations increased rapidly by 24 h after colostrum consumption (approximately 4000 mg/dl, data not shown). Serum IgG concentrations declined slowly in both treatment groups over the remainder of the 4-week study period. There were no significant differences in serum concentrations of IgG between the two treatment groups at any sample time.

3.3. Expression of adhesion molecules, (CD11a)

Cells expressing CD11a on their surface segregated into three distinct patterns based on receptor density. The lowest density (329–482 linear fluorescence units after expansion of the log scale) of CD11a was primarily on lymphocytes, activated lymphocytes expressed CD11a at moderate density (490–600), and monocytes had the highest level of expression (601+). Similar differential expression patterns of CD11a based on marker intensity have been documented in humans (Bikoue et al., 1996, 1997; Desroches et al., 1990). Each expression pattern of CD11a was examined individually: CD11a low, CD11a moderate, and CD11a high.

At birth, 4.4% of lymphocytes expressed a low level of CD11a on their surface (Fig. 2A). The percentage of cells increased quickly during the first week of life reaching 46.7% by day 7. Although calves in both treatment groups had similar percentages of CD11a positive lymphocytes throughout most of the study, calves receiving colostral leukocytes showed a significant decrease in CD11a positive lymphocytes in the peripheral blood at day 14 ($p < 0.05$). The density of expression on lymphocytes varied little over the 4 weeks (data not shown).

The majority of activated lymphocytes expressed a moderate density of CD11a (Fig. 3A). Calves receiving C had fewer CD11a positive activated lymphocytes than calves receiving CFC during the first week of life ($p < 0.05$). At 48 h post-colostrum feeding, these cells also expressed a significantly higher density of CD11a, whereas calves receiving CFC expressed a significantly higher surface density ($p < 0.05$, Fig. 3C).

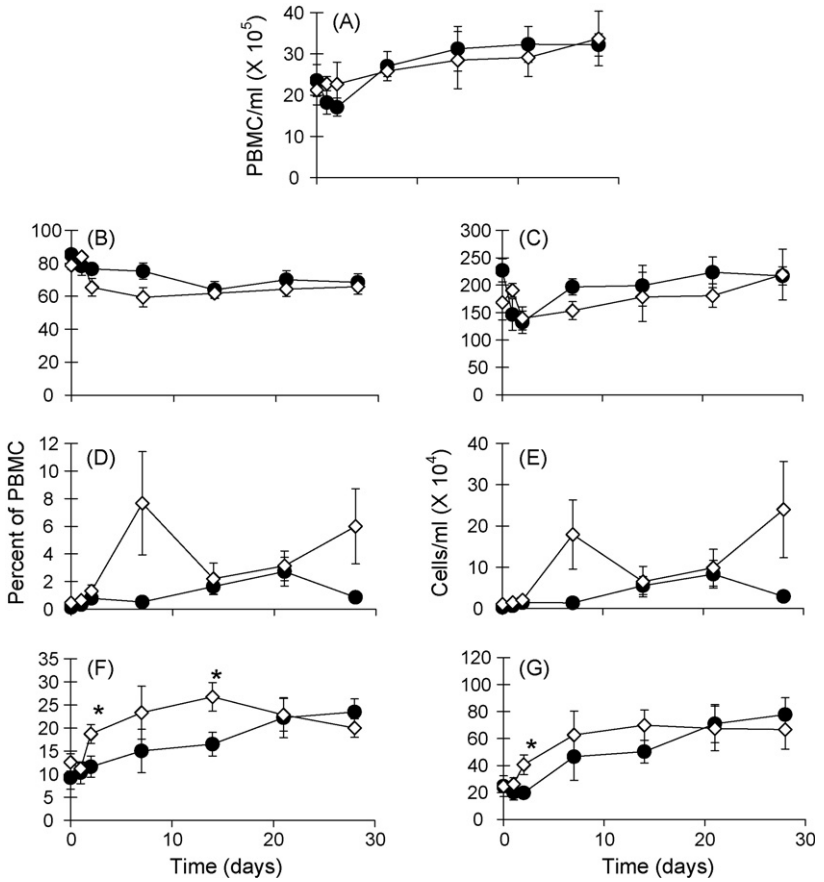

Fig. 1. Calves were fed whole (●) or cell-free (◇) colostrum at birth and changes in peripheral blood cell types were analyzed. Peripheral blood mononuclear leukocytes were collected periodically during the first 4 weeks of life and (A) number of PBMC/ml was determined for each treatment group. PBMC were also analyzed by flow cytometry using forward vs. side scatter. The percent of (B) lymphocytes, (D) activated lymphocytes, and (F) monocytes were evaluated. The number of (C) lymphocytes, (E) activated lymphocytes, and (G) monocytes in the peripheral blood were determined during the same period of time. Significant differences were observed in the number and percentage of monocytes during the first 2 weeks of life ($p < 0.05$). Error bars represent the standard error of the mean, $n = 10$.

3.4. Expression of effector molecules (CD25, CD26, and CD172a)

The percentage of CD25 positive cells was relatively low for all groups of calves. The percentage of CD25 positive activated lymphocytes remained below 14% of the total activated lymphocyte population throughout the study. On day 7, calves receiving C expressed a significantly higher percentage ($p < 0.05$, 12.9% vs. 1.8%) of CD25 positive activated lymphocytes than calves receiving CFC. Similarly, the percentage of CD26 positive cells was relatively low for all groups of calves, remaining below 13% of the total activated lymphocyte population throughout the study. Calves receiving C expressed a significantly higher percentage of CD26 positive activated lymphocytes than calves receiving CFC at 24 h ($p < 0.05$, 11.9% vs. 2.6%) and on day 7 ($p < 0.05$, 12.2% vs. 1.3%).

Calves receiving C maintained a lower percentage of activated lymphocytes expressing CD172a for the first 2 week of the study than calves receiving CFC (Fig. 4A). The percentage of activated lymphocytes expressing CD172a peaked on day 21 in calves receiving C, and was significantly higher than calves receiving CFC ($p < 0.05$). There was no significant difference in CD172a expression between the treatment groups by the end of the study.

3.5. Expression of antigen presentation molecules (MHC I and MHC II)

Lymphocytes expressing MHC class I at birth was less than 20% of PBMC for all groups of calves (Fig. 5A), but increased quickly during the first 7 days of life. On day 7, calves receiving CFC had a lower percentage of MHC class I positive lymphocytes than

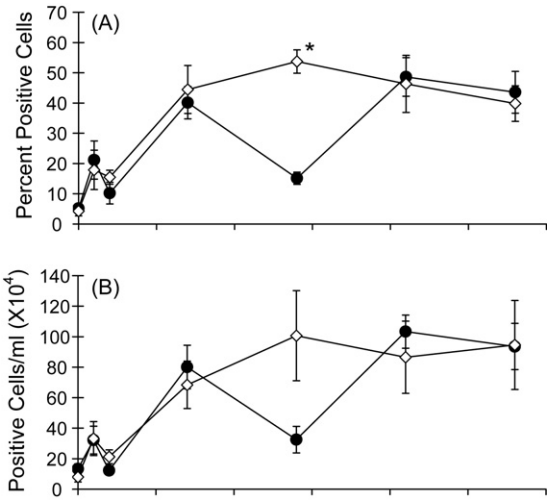

Fig. 2. Neonatal leukocytes segregated into a low, moderate, or high density of CD11a. Lymphocytes expressing a low density of CD11a were examined in neonatal calves during the first 4 weeks of life. Calves were fed whole (●) or cell-free (◇) colostrum at birth and the mean change in the (A) percent of positive cells and (B) number per ml are presented as determined by flow cytometry. The percentage of lymphocytes expressing a low density of CD11a was significantly higher in calves fed cell-free colostrum than calves fed whole colostrum at 14 days post-colostrum feeding ($p < 0.05$). Error bars represent the standard error of the mean, $n = 10$.

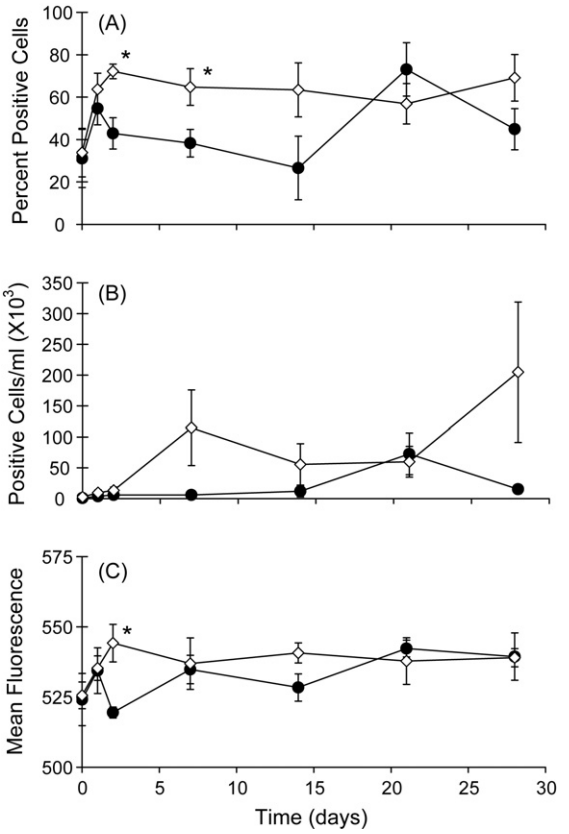

Fig. 3. Neonatal leukocytes segregated into a low, moderate, or high density of CD11a. Lymphocytes expressing a moderate density of CD11a were examined in neonatal calves during the first 4 weeks of life using flow cytometry. Calves were fed whole (●) or cell-free (◇) colostrum at birth and the mean changes in the (A) percentage of activated lymphocytes expressing a moderate density of CD11a were examined. The percentage was significantly higher in calves fed cell-free colostrum than calves fed whole colostrum at 2 and 7 days post-colostrum feeding ($p < 0.05$). The (B) mean circulating number per milliliter of activated lymphocytes expressing a moderate density of CD11a is also represented. The (C) average for the mean per cell fluorescence of activated lymphocytes in circulation was examined. Calves fed cell-free colostrum expressed a higher mean surface fluorescence on activated lymphocytes than calves fed whole colostrum at 2 days ($p < 0.05$). Error bars represent the standard error of the mean, $n = 10$.

calves receiving C ($p < 0.05$). The cell density of MHC class I positive lymphocytes increased steadily in both treatment groups throughout the course of the study (Fig. 5B). Calves receiving C also expressed a higher surface density of MHC class I on their lymphocytes during the first week of life (Fig. 5B; $p < 0.05$).

The percentage of MHC class II positive lymphocytes averaged 0.9% of PBMC at birth for all groups of calves, increasing continuously throughout the course of the study, reaching 13.1% of PBMC by week 4 (data not shown). The cell density showed a similar trend averaging 1.7×10^4 MHC class II positive lymphocytes/ml at birth, reaching 3.0×10^5 MHC class II positive lymphocytes/ml at 4 weeks of age (data not shown). The density of expression of MHC class II on lymphocytes was relatively constant throughout the course of the study. There were no differences in MHC class II expression between the two treatment groups throughout the course of the study.

4. Discussion

This project was performed to examine the effects of maternal colostral leukocytes on development and maturation of neonatal lymphocytes. All calves in this project had relatively little circulating antibody at birth, but received an equivalent level of IgG transfer via maternal colostrum, as indicated by increases in serum concentrations of IgG (about 4000 mg/dl). Differences in cellular development and maturation can, therefore, be attributed to the transfer of colostral cells.

Calves deprived of colostral leukocytes (CFC treatment) had higher numbers of lymphocytes expressing CD11a and lymphocytes that expressed a higher density of CD11a on their surface (Fig. 2) than lymphocytes from calves receiving colostral leukocytes. This was most clearly seen on activated lymphocytes. CD11a is a

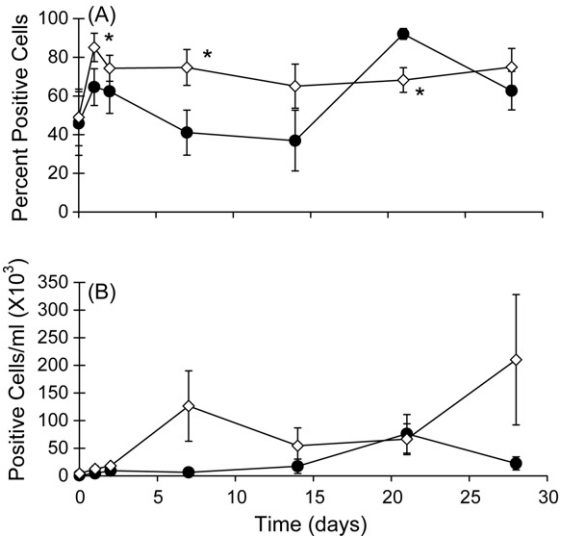

Fig. 4. Calves were fed whole (●) or cell-free (◇) colostrum at birth, and the (A) average percentage and (B) circulating number of activated lymphocytes expressing CD172a were determined during the first 4 weeks of life by flow cytometry. Calves receiving cell-free colostrum exhibited a higher percentage of activated lymphocytes expressing CD172a at 1 and 7 days post-colostrum feeding ($p < 0.05$). By 3 weeks post-feeding of colostrum, the percentage of CD172a positive activated lymphocytes was significantly lower in calves fed cell-free colostrum than calves fed whole colostrum ($p < 0.05$). Error bars represent the standard error of the mean, $n = 10$.

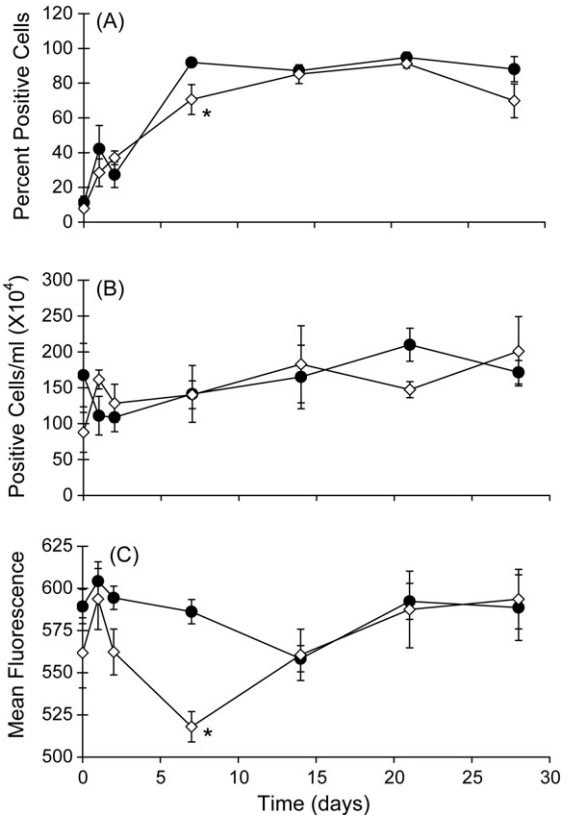

Fig. 5. Expression of MHC class I on neonatal lymphocytes was examined during the first 4 weeks of life. Calves were fed whole (●) or cell-free (◇) colostrum at birth and peripheral blood leukocytes were collected and (A) percentage and (B) number of MHC class I positive lymphocytes were examined by flow cytometry. The percentage of MHC class I positive lymphocytes was significantly higher in calves fed whole colostrum than calves fed cell-free colostrum at 1 week post-colostrum feeding ($p < 0.05$). At the same time point, calves fed whole colostrum also expressed a (C) higher density of surface MHC class I on lymphocytes than calves fed cell-free colostrum ($p < 0.05$). Error bars represent the standard error of the mean, $n = 10$.

cellular adhesion molecule that is extremely important for mobilization of leukocytes. Up-regulation of CD11a on cellular surfaces is an indication of an ensuing immune response. Although none of the calves on this project exhibited signs of clinical disease, up-regulation of CD11a may be an indication of systemic inflammation or sub-clinical disease, suggesting an increased susceptibility of calves deprived of colostral leukocytes to environmental stress and infectious agents. Further, it has previously been shown in other species that exposure to a psychological or physiological stressor leads to a robust leukocyte redistribution, a phenomenon that is at least partly mediated by activation of the sympathetic nervous system (Mills et al., 2003; Benschop et al., 1996). The results of several recent studies in rats and humans have linked this redistribution of leukocytes to up-regulation of CD11a. For example, experimentally induced hypertension in rats up regulated CD11a expression on T lymphocytes (Kim and Vaziri, 2005), and CD11a was up regulated on lymphocytes of healthy male volunteers during aerobic exercise (Gannon et al., 2001; Goebel and Mills, 2000). Similarly, CD11a levels were higher on lymphocytes in mildly hypertensive individuals than in normotensive individuals (Mills et al., 2003), and exposure to a stressor (*i.e.* delivering a public speech) further increased the level of expression of CD11a in both groups. In that study, expression of CD11a remained higher in the hypertensive individuals (Mills et al., 2003). Further, CD11a was up regulated on T lymphocytes of a study that examined a small number of patients with infectious mononucleosis (Pallis et al., 1993). Our previous studies demonstrated that transfer of maternal colostral leukocytes enhanced development of neonatal immune responses as indicated by the calves' ability to modulate responses to foreign leukocytes in mixed leukocyte responses (Reber et al., 2005). Calves deprived of maternal colostral leukocytes in those experiments lagged behind in immune development by 1–2 weeks. It is possible that the neonatal immune system considers

this immature state (*e.g.*, low receptor expression, sub-optimal cytokine production, cellular unresponsiveness) to be an indication of physiological stress.

CD25 positive lymphocytes remained below 14% throughout the study, a finding that is consistent with other reports examining immune system development in neonatal calves. For example, Kampen et al. (2006) reported that colostrum-fed calves have a mean CD25 expression of 3–8% of CD4 positive cells during the first 6 months of life. In that study, smaller percentages of CD25 positive cells were also demonstrated on other lymphocyte subpopulations.

Of particular interest in the present study was the expression of CD172a (MyD1, SIRPα) on neonatal lymphocytes. CD172a is a surface receptor belonging to a group of signal-regulatory proteins and, although its function is not entirely understood, it purportedly involves stimulation of T cell responses, possibly through co-stimulation or control of T cell/APC binding (Brooke et al., 1998; Howard et al., 1997; Kharitonenkov et al., 1997). In the present study, 15% of neonatal monocytes expressed CD172a at birth, and this increased quickly to 52% by 2 days after birth (data not shown). These changes occurred in calves in all groups, suggesting that the presence of maternal leukocytes is not required for increased expression of CD172a on monocytes as the age of the calves increased. In contrast, the percentage of CD172a positive-activated lymphocytes was higher during the first 2 weeks of life in calves that received CFC (Fig. 4A). The pattern of CD172a expression appears to mirror CD11a expression in this same population of lymphocytes; likely the result of their related cellular function. Until recently, expression of CD172a was thought to be restricted to monocytes, macrophages, and granulocytes. However, the results of more recent studies indicate that antigenic stimulation results in proliferation of a population of CD172a positive cells (Waters et al., 2006; Maue et al., 2005). While the consequences of this differential expression are as yet not understood, it should become clearer as the function of CD172a becomes better characterized.

The percentage of cells expressing MHC class I rapidly increased during the first week of life in all groups of calves (Fig. 5A). Lymphocytes from calves that received C had an increased density of surface expression of MHC class I during the first week of life (Fig. 5C); and the same trend was observed on neonatal monocytes (Reber et al., 2008, companion paper), and in an earlier study conducted by others (Menge et al., 1999). Although MHC class II expression is the primary surface marker affecting mixed leukocyte responses, MHC class I expression plays a significant, but subordinate role. Given the equivalent levels of expression of MHC class II between the two treatment groups, the differential expression of MHC class I surface density may account for our previously reported differences in responsiveness in mixed leukocyte cultures (Reber et al., 2005).

Although the importance of feeding colostrum to provide protective antibody has long been recognized, it is becoming increasingly evident that colostrum also helps to constitute the cellular arm of the neonatal immune system by providing maternal colostral leukocytes. An effective cellular immune response requires antigen-specific effector cells as well as antigen presenting capacity. In this study, we determined that uptake of maternal colostral leukocytes affects development of neonatal lymphocytes, as evidenced by an increased antigen presenting capacity (*i.e.*, via up-regulation of MHC class I). Additionally, lymphocytes from calves receiving maternal colostral leukocytes had a lower expression of markers associated with lymphocyte activation and general physiological stress. Overall, these findings demonstrate the importance of maternal colostral leukocytes on development of neonatal cellular immunity.

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