Suppression of Mitogenic Response of Peripheral Blood Mononuclear Cells by Bovine Mammary Secretions

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ABSTRACT
Effects of bovine mammary secretions collected at different stages of the lactation cycle on blood mononuclear cell response to mitogens were evaluated. Mammary secretion skims and wheys collected 7 and 28 d following cessation of milking, at parturition, and during early lactation were used. Colostrum and mammary secretions obtained 7 d after milk cessation were associated with greatest inhibition of mononuclear cell blastogenesis. Milk collected during early lactation caused the least inhibition. Mammary secretion wheys caused greater inhibition of blastogenesis than skims. Dilution of mammary secretions reduced blastogenic inhibition. Phytohemagglutinin-stimulated mononuclear cells were less inhibited by mammary secretion than Concanavalin A-stimulated cells. Suppression of mononuclear cell activity, particularly during early involution and at parturition, may influence susceptibility of the bovine mammary gland to new IMI during the nonlactating period.

Introduction
Bovine mammary glands are highly susceptible to new intramammary infection (IMI) during early involution (15) and near parturition (10). In contrast to transitional periods, the fully involuted mammary gland appears to be resistant to new IMI (10). Increased susceptibility to new IMI during functional transitions has been attributed to several factors, such as increased intramammary pressure caused by fluid accumulation (7), absence of regular “flushing out” of pathogens during the milking process (26), and changes in the biochemical composition of mammary secretions (3). Cells present in mammary secretions may also influence susceptibility of the mammary gland to new IMI during the nonlactating period.

Total cell concentrations increase during the 1st wk of involution, remain elevated during the dry period, and decrease following parturition (11). Polymorphonuclear neutrophils (PMN) and macrophages predominate during early involution and decrease in concentration as involution progresses. However, the concentration of lymphocytes is highest in secretions from the fully involuted mammary gland (11).

An important factor in resistance to infection is the ability of lymphocytes to initiate and maintain an immune response. Human and bovine peripheral blood lymphocytes (PBL) are more responsive to in vitro mitogenic stimulation than mammary gland lymphocytes (MGL) (9, 20). Reasons for this remain unclear. However, hyporesponsiveness of MGL may be associated with components of mammary secretion. In support of this hypothesis, bovine and human mammary secretions suppress response of PBL to mitogens (6, 8, 9).

Suppressed activity of bovine mononuclear cells could affect dynamics of IMI during involution. Depressed ability to mount a specific immunoglobulin or cytotoxic response to bacterial invasion may contribute to observed susceptibility of the mammary gland to new IMI during involution, especially during physiological transitions. The purpose of this study was to determine if mammary secretions obtained at various stages of the lactation cycle influenced bovine blood mononuclear cell blastogenesis.
MATERIALS AND METHODS

Animals

Five multiparous lactating Holstein cows from the University of Tennessee dairy research herd served as blood donors. Animals had been lactating between 2 and 14 wk prior to initiation of experiments and were producing approximately 31 kg of milk (range 24 to 40 kg/d). Animal age ranged from 38 to 58 mo.

Secretions

Colostrum, milk collected 14 d after parturition, and mammary secretions obtained 7 (D + 7) and 28 d (D + 28) after milk cessation were evaluated. Only secretions from mammary glands free of major mastitis pathogens based on microbiological analysis were used. Skim samples were prepared by centrifugation (37,000 \( \times \) g for 30 min) to remove fat and cellular debris. Wheys were prepared by lowering the pH of skim samples to 4.5 with glacial acetic acid followed by centrifugation at 37,000 \( \times \) g for 45 min. Skim and whey samples were adjusted to pH 7.0 with 1 N NaOH, filter sterilized (.45 \( \mu \)m filter; Gelman Sciences Inc., Ann Arbor, MI), and stored frozen until required. Total protein concentration of skims and wheys was determined by Lowry analysis (14).

Mitogens

Concanavalin A (Con A) and phytohemagglutinin (PHA; Sigma Chemical Co., St. Louis, MO) were utilized to induce mononuclear cell blastogenesis. Optimal concentrations of Con A and PHA were determined prior to these experiments. Concanavalin A (5 \( \mu \)g/ml) and PHA (25 \( \mu \)g/ml) were reconstituted in sterile ultrapure water and diluted in RPMI 1640 medium (Sigma Chemical Co., St. Louis, MO).

Isolation of Mononuclear Cells

Bovine peripheral blood mononuclear cells were isolated by the method of Nonnecke and Kehrli (16). Bovine blood (80 ml) was collected by jugular venipuncture into 17 ml of modified Alsever's solution and diluted with two parts phosphate-buffered saline (pH 7.2, .15 M). Diluted blood (12 ml) was overlaid carefully on 3 ml Ficoll-sodium diatrizoate with specific gravity 1.083 g/ml (Histopaque 1083; Sigma Chemical Co., St. Louis, MO). Following centrifugation (400 \( \times \) g at 18°C) for 50 min, the mononuclear cell band was recovered and washed twice (300 \( \times \) g for 15 min/wash at 18°C) in Ca\(^{2+}\) and Mg\(^{2+}\)-free Hank's balanced salt solution (Gibco, Grand Island, NY). Residual red blood cells were lysed by hypotonic shock treatment using sterile phosphate-buffered water (pH 7.2). Following a single wash in Hank's balanced salt solution, cells were resuspended in RPMI 1640 medium containing 10 mM HEPES buffer, 2 mM L-glutamine, 100 U penicillin G and 100 \( \mu \)g streptomycin sulfate/ml. Mononuclear cell recovery and viability were determined by hemacytometer count using trypan blue exclusion. No attempt was made to purify mononuclear cells by removal of monocytes due to prior reports that bovine and ovine monocytes enhance response of lymphocytes to mitogens (2, 17).

Cell Quantification

Purity of isolated mononuclear cells was evaluated by differential microscopic count of cytocentrifuge smears stained with Wright-Giemsa. Aliquots of cells from Ficoll gradients were processed also for light and electron microscopy using the method of Sordillo and Nickerson (23). Briefly, cells were fixed in 2.5% glutaraldehyde in .1 M cacodylate buffer (pH 7.0 at 37°C) for 2 h and postfixed in .1 M cacodylate-buffered osmium tetroxide (pH 7.0 at 5°C) for 1.5 h. Following dehydration in a graded series of ethanol washes, cell pellets were embedded in epoxy resin. Thick sections (.5 to 1 \( \mu \)m) were prepared and stained with toluidine blue for light microscopy. Ultrathin sections (60 nm) were prepared and stained for 20 min with 5% uranyl acetate in 50% methanol for electron microscopy. Representative lymphocytes and monocytes isolated by density gradient centrifugation are presented in Figure 1.

Cell Culture Procedures

Cells were cultured in 96-well flat bottom microtiter plates (Corning Cell Wells; Corning Glass Works, Corning, NY). Fifty microliters of RPMI 1640 containing 2 \( \times \) 10\(^5\) viable mononuclear cells were added to each well. Ad-
ditional components added to each well were: 100 µl of mitogen, 50 µl of fetal calf serum (heat inactivated and diluted in RPMI 1640 to a final concentration of 10% FCS per well) and 50 µl of bovine mammary secretion. Serial fourfold dilutions of mammary secretions in RPMI 1640 were added also to cultures to determine the effect of decreasing protein concentration of secretions on mononuclear cell blastogenesis. Control wells received 150 µl of RPMI 1640 medium instead of mitogen and mammary secretion. Stimulated control wells received 50 µl of RPMI 1640 instead of mammary secretion.

![Figure 1. Electron micrographs of bovine peripheral blood mononuclear cells isolated by density gradient centrifugation. a. Representative bovine lymphocyte; (X11,400) b. Representative bovine monocyte; X14,980.](image-url)
Microtiter plates were incubated for 72 h at 37°C in an atmosphere of 95% air and 5% CO$_2$. After 72 h, 1 μCi of methyl-$[^3]$H]thymidine (ICN Radiochemicals, Irvine, CA) diluted in 50 μl of RPMI 1640 medium was added to each well. Following 18 h of incubation, mononuclear cells were harvested onto glass fiber filters using a multiple automated sample harvester (Mini-MASH II; MA Bioproducts, Walkersville, MD). Filters were placed in plastic scintillation vials and 2 ml of scintillation cocktail (Scintiverse II; Fisher Scientific Co., Fairlawn, NJ) were added to each vial. Retained radioactivity was counted for 10 min/vial in a liquid scintillation counter (Isocap 300 Liquid Scintillation System; Searle Analytic Inc., Des Plaines, IL). Data were expressed as mean counts per min × 10$^3$ of three replicate cultures. Data were expressed also as an index of inhibition where Index = mean counts per min experimental/mean counts per min stimulated control.

Mononuclear cell viability was determined by trypan blue exclusion at 24, 48, and 90 h of culture. Viability counts were performed on duplicate wells for control, Con A-stimulated control, PHA-stimulated control, and D + 28 skim and whey experimental cultures.

Statistical Analysis

Count data were transformed to loge for statistical analysis. However, actual count data are presented. Experimental design was split block and data were analyzed by least squares analysis of variance (24) using the following model:

$$Y_{ijk} = \mu + C_i + D_j + S_k + DS_{jk} + \epsilon_{ijk}$$

where $\mu$ = overall mean cpm, $C_i$ = cow, $D_j$ = dilution of secretion, and $S_k$ = secretion. In order to characterize further differences in effects of individual secretions on bovine blood mononuclear cell blastogenesis, a series of contrasts were made between effects of different secretions using the same statistical model.

RESULTS

Cell Cultures

Bovine blood mononuclear cell isolation procedures resulted in a population of >99% pure mononuclear cells comprising approximately 75% lymphocytes and 25% monocytes based upon differential microscopic cell counts of cytocentrifuge smears. Light microscope counts on thick sections from cell pellets were corroborated by EM preparations of mononuclear cells isolated from two cows, which indicated that approximately 70% of cells were lymphocytes and 30% were monocytes with <1% granulocytic contamination.

Mean viability of mononuclear cells recovered from density gradients prior to initiation of cultures was 81.3%. Viabilities of various cell preparations at intervals throughout the 90 h culture period are presented in Table 1. Only in the case of PHA-stimulated cells did cell viability decrease to below 60% at 90 h of culture. Viability of PHA-stimulated cells was consistently lower than Con A-stimulated cells. However, mean counts per minute of PHA-stimulated cells was 8.5 times higher than control values. Addition of mammary secretion to cultures did not appear to affect adversely mononuclear cell viability compared with addition of mitogen alone.

Total protein concentrations of undiluted mammary secretions, secretion protein concentration per well, and results of incubation of bovine blood mononuclear cells with undiluted bovine mammary secretions are presented in Tables 2 and 3. Colostrum contained the greatest amount of total protein, while normal milk contained the least. Mammary secretion wheys contained 10 to 20 mg/ml less total protein than mammary secretion skims. However, mammary secretion wheys (Tables 2 and 3) were associated with greater inhibition (P<.05) of mononuclear cell blastogenesis than skim preparations.

Individual cow variation in magnitude of inhibition of mononuclear cell response to mitogens by mammary secretions was significant. Milk skim and whey caused the least inhibition of mononuclear cell response to mitogens (Tables 2 and 3). In contrast, greatest inhibition of both Con A- and PHA-stimulated cells was caused by secretions obtained at D + 7 and at parturition. Inhibition of PHA-stimulated cells by secretions collected from fully involuted mammary glands was lower (P<.05) than for secretions obtained during early involution (Table 3). Similarly, colostral skim and whey caused greater (P<.05) inhibition of
TABLE 1. Mean viability of selected cell preparations through 90 h of culture.$^1,2$

<table>
<thead>
<tr>
<th>Hours of culture</th>
<th>Control</th>
<th>Con A$^3$</th>
<th>PHA$^4$</th>
<th>Con A + D + 28 skim</th>
<th>Con A + D + 28 whey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$</td>
<td>SE</td>
<td>$\bar{x}$</td>
<td>SE</td>
<td>$\bar{x}$</td>
</tr>
<tr>
<td>24</td>
<td>76.7</td>
<td>2.1</td>
<td>74.3</td>
<td>1.4</td>
<td>68.4</td>
</tr>
<tr>
<td>48</td>
<td>77.4</td>
<td>2.3</td>
<td>66.8</td>
<td>2.5</td>
<td>61.4</td>
</tr>
<tr>
<td>90</td>
<td>80.3</td>
<td>2.0</td>
<td>64.2</td>
<td>3.9</td>
<td>38.4</td>
</tr>
</tbody>
</table>

$^1$ Data expressed as mean percent viability.

$^2$ Viability obtained by hemacytometer count of duplicate wells of a culture plate using trypan blue exclusion.

$^3$ Concanavalin A.

$^4$ Phytohemagglutinin.

$^5$ Twenty-eight days following cessation of milking.

Mononuclear cell response to PHA than did milk skim and whey. Of the skim preparations used, colostral skim was associated with the greatest inhibition of Con A-stimulated mononuclear cell blastogenesis (Table 2). In contrast, early involution whey caused the greatest inhibition of Con A-stimulated cells. However, differences in inhibition of Con A-stimulated cells by early involution secretion and colostrum were not significant for both skim and whey preparations. Greatest stimulation of bovine mononuclear cells was associated with Con A. Based on indices of inhibition, PHA-induced mononuclear cell blastogenesis appeared to be less inhibited by skims than was Con A-induced blastogenesis (Tables 2 and 3). A similar trend was observed with wheys, although differences in inhibition of Con A- and PHA-induced mononuclear cell blastogenesis were less marked.

TABLE 2. Effect of undiluted bovine mammary gland secretions on Concanavalin A-induced bovine peripheral blood mononuclear cell blastogenesis (n = 5 cows).

<table>
<thead>
<tr>
<th>Preparation of secretion</th>
<th>Secretion</th>
<th>Total protein concentration of secretion (mg/ml)</th>
<th>Protein concentration/well (μg/μl)</th>
<th>$[^3]$H Thymidine incorporation X$^1$ SEM</th>
<th>Index$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skim</td>
<td>Early involution</td>
<td>84.1</td>
<td>16.8</td>
<td>1.4</td>
<td>.6</td>
</tr>
<tr>
<td></td>
<td>Midinvolution</td>
<td>74.2</td>
<td>14.8</td>
<td>4.6</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>Colostrum</td>
<td>87.6</td>
<td>17.5</td>
<td>1.0</td>
<td>.6</td>
</tr>
<tr>
<td></td>
<td>Milk</td>
<td>22.7</td>
<td>4.5</td>
<td>20.4</td>
<td>6.6</td>
</tr>
<tr>
<td>Whey</td>
<td>Early involution</td>
<td>64.4</td>
<td>12.9</td>
<td>.2</td>
<td>.03</td>
</tr>
<tr>
<td></td>
<td>Midinvolution</td>
<td>60.8</td>
<td>12.2</td>
<td>1.8</td>
<td>.6</td>
</tr>
<tr>
<td></td>
<td>Colostrum</td>
<td>69.4</td>
<td>13.9</td>
<td>.6</td>
<td>.2</td>
</tr>
<tr>
<td></td>
<td>Milk</td>
<td>5.5</td>
<td>1.1</td>
<td>5.8</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>Stimulated control</td>
<td>...</td>
<td>...</td>
<td>158.8</td>
<td>27.4</td>
</tr>
</tbody>
</table>

$^1$ Data are expressed as mean counts per minute X 10$^3$.

$^2$ Data are expressed as an index of inhibition where index = mean counts per minute experimental ÷ mean counts per minute stimulated control.
TABLE 3. Effect of undiluted bovine mammary gland secretions on phytohemagglutinin-induced bovine peripheral blood mononuclear cell blastogenesis (n = 5 cows).

<table>
<thead>
<tr>
<th>Preparation of secretion</th>
<th>Secretion</th>
<th>Total protein concentration of secretion (mg/ml)</th>
<th>Protein concentration/well (μg/μl)</th>
<th>[3H] Thymidine incorporation index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skim</td>
<td>Early involution</td>
<td>84.1</td>
<td>16.8</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>Midinvolution</td>
<td>74.2</td>
<td>14.8</td>
<td>12.6</td>
</tr>
<tr>
<td></td>
<td>Colostrum</td>
<td>87.6</td>
<td>17.5</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>Milk</td>
<td>22.7</td>
<td>4.5</td>
<td>19.0</td>
</tr>
<tr>
<td>Whey</td>
<td>Early involution</td>
<td>64.4</td>
<td>12.9</td>
<td>.2</td>
</tr>
<tr>
<td></td>
<td>Midinvolution</td>
<td>60.8</td>
<td>12.2</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Colostrum</td>
<td>69.4</td>
<td>13.9</td>
<td>.7</td>
</tr>
<tr>
<td></td>
<td>Milk</td>
<td>5.5</td>
<td>1.1</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>Stimulated control</td>
<td>...</td>
<td>...</td>
<td>66.2</td>
</tr>
</tbody>
</table>

1 Data are expressed as mean counts per minute × 10^3.
2 Data are expressed as an index of inhibition where index = mean counts per minute experimental / mean counts per minute stimulated control.

Effects of serial fourfold dilutions of mammary secretions on mononuclear cell blastogenesis are presented in Tables 4 and 5. Dilution of secretions reduced (P<.05) inhibition of mitogen-induced mononuclear cell blastogenesis. Dilution of mammary secretion appeared more effective in removing inhibition of Con A-induced mononuclear cell blastogenesis by colostral and milk skims as opposed to early and midinvolution secretion skims (Table 4). In contrast, dilution of secretions appeared most effective in removing inhibition of PHA-induced mononuclear cell blastogenesis by early and midinvolution secretion skims (Table 5).

No similar trends could be established for secretion wheys.

TABLE 4. Effect of dilution of mammary secretions on Concanavalin A-induced bovine peripheral blood mononuclear cell blastogenesis (n = 5 cows).

<table>
<thead>
<tr>
<th>Secretion</th>
<th>Dilution of skim</th>
<th>Dilution of whey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1:4</td>
</tr>
<tr>
<td>Early involution</td>
<td>.009</td>
<td>.086</td>
</tr>
<tr>
<td>Midinvolution</td>
<td>.029</td>
<td>.068</td>
</tr>
<tr>
<td>Colostrum</td>
<td>.006</td>
<td>.195</td>
</tr>
<tr>
<td>Milk</td>
<td>.129</td>
<td>.567</td>
</tr>
</tbody>
</table>

1 Data are expressed as an index of inhibition where index = mean counts per minute experimental / mean counts per minute stimulated control.

DISCUSSION

Human colostrum (8, 9) and bovine colostrum and dry secretion (stage of involution not reported) (6) have been shown to suppress peripheral blood lymphocyte responsiveness to both T- and B-cell mitogens. Of the secretions used in our study, colostrum and early involution mammary secretion caused greatest inhibition of Con A- and PHA-induced bovine peripheral blood mononuclear cell blastogenesis. Milk obtained during early lactation, which contained the least total protein of all secretions used, caused the least inhibition. Inhibition of mononuclear cell blastogenesis by mammary secretions in this study may have
TABLE 5. Effect of dilution of mammary secretions on phytohemagglutinin-induced bovine peripheral blood mononuclear cell blastogenesis (n = 5 cows).1

<table>
<thead>
<tr>
<th>Secretion</th>
<th>Dilution of skim</th>
<th>Dilution of whey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1:4</td>
</tr>
<tr>
<td>Early involution</td>
<td>.067</td>
<td>.546</td>
</tr>
<tr>
<td>Midinvolution</td>
<td>.190</td>
<td>.598</td>
</tr>
<tr>
<td>Colostrum</td>
<td>.095</td>
<td>.324</td>
</tr>
<tr>
<td>Milk</td>
<td>.288</td>
<td>.556</td>
</tr>
</tbody>
</table>

1 Data are expressed as an index of inhibition where index = mean counts per minute experimental ÷ mean counts per minute stimulated control.

been, in part, a result of nonspecific blockage of mitogen receptor sites by mammary secretion proteins. However, wheys, which contained 10 to 20 mg/ml less total protein than skims, were associated with greater inhibition of mononuclear cell blastogenesis than skims. The final concentration of undiluted milk whey in culture wells was approximately 1 μg/μl, but milk whey significantly depressed Con A- and PHA-induced mononuclear cell blastogenesis. Therefore, suppression of mononuclear cell blastogenesis by mammary secretions would appear to be of a more specific nature.

Reasons for increased suppression of mononuclear cell blastogenesis by mammary secretion wheys compared with skims are unclear. It may be that acid precipitation of casein affects proteins other than casein, causing them to become more inhibitory to mononuclear cell blastogenesis. However, increased suppression by wheys may be associated with Ca²⁺. A large percentage of Ca in mammary secretion is associated with casein micelles (18). Changes in pH disrupt casein micelles and may result in the release of free ionic Ca²⁺ (13), which at high concentrations (10⁻³ M) is cytotoxic (18). One possible mechanism of action of mitogens such as Con A is increased cellular uptake of Ca²⁺ (27). In our study, mammary secretion pH was decreased to 4.5 to precipitate caseins, and then increased to 7.0. Therefore, increased suppression of mononuclear cell response to mitogens by mammary secretion wheys compared with that of skims may be a result of higher concentrations of free ionic Ca²⁺. However, specific measurement of Ca²⁺ concentrations in mammary secretion skims and wheys would be required to prove this hypothesis.

Proteinaceous components of bovine mammary secretions, which have been implicated in suppression of in vitro response of lymphocytes to mitogens, include lactoferrin (Lf) (21) and secretory component (8). The concentration of Lf increases in bovine mammary secretions as involution progresses (29). If Lf alone were responsible for variation in mononuclear cell activity, then inhibition of cellular response to mitogens should have been greater for mammary secretions collected at 28 d of involution. However, such was not the case. Significantly greater inhibition of mononuclear cell blastogenesis by mammary secretions compared with that of skims may be indicative of the presence of an as yet unidentified immunosuppressant present in greater concentration or in a more active form in mammary secretions from early rather than fully involuted bovine mammary glands.

Crago et al. (8) reported that of the components of human colostrum tested, only free secretory component (FSC) significantly inhibited human PBL response to pokeweed mitogen via suppression of B-cell cytoplasmic Ig. Bovine colostral secretory IgA concentrations are elevated compared with those of dry secretion and milk (4), and therefore, FSC concentration might also be assumed to be elevated. However, no data are available concerning inhibition of T-cell mitogen-induced bovine lymphocyte blastogenesis by FSC, and thus no conclusions can be made about the role of FSC as an immunosuppressant in bovine colostrum. The concentration of Lf in colostrum is low (29). Therefore, it is also unlikely that the immunosuppressive effects of colostrum were due solely to Lf.
A possible explanation for increased immunosuppression by colostrum compared with milk may be related to factors of serum origin. Suppression of bovine peripheral blood mononuclear cell response to mitogens during the peripartum period has been observed (22). Hormones such as prolactin, estrogens, and adrenal glucocorticoids, are involved in lactogenesis (28) and may possess immunosuppressive properties (5, 12). Increased concentrations of hormones in colostrum, which may be both lactogenic and immunosuppressive, may contribute to increased suppression of mononuclear cells by colostrum.

Marked suppression of bovine blood mononuclear cell response to PHA compared with Con A was observed in this study. Concanavalin A and PHA preferentially stimulate T-cells (19). Differences in stimulation of mononuclear cells may reflect differences in effectiveness of Con A and PHA as T-cell mitogens. However, Stobo and Paul (25) proposed the existence of two distinct subsets of murine T-cells. One subset of T-cells responded equally well to Con A and PHA while the other subset responded best to Con A. Using differential erythrocyte rosetting techniques, Belden and Streikauskas (1) demonstrated the presence of subsets of bovine blood T-cells that responded differently to mitogens and in the mixed lymphocyte reaction. Wilson et al. (30), using similar rosetting techniques, identified similar subsets of bovine mammary gland T-cells. Evidence is unclear as to whether different subsets of bovine T-cells are entirely different populations of T-cells. Each subset may be a different maturational stage of bovine T-cell ontogeny. However, differences in response of bovine blood mononuclear cells to Con A and PHA in our study may possibly reflect the presence of subpopulations of T-cells found in bovine blood. Lessened ability of mammary secretions to inhibit PHA stimulation of mononuclear cells may indicate relative insensitivity of a PHA-responsive subpopulation of bovine T-cells to immunosuppressive factors in mammary secretions. Inhibition of Con A-induced mononuclear cell blastogenesis by colostral and milk skims was reduced by dilution of secretions. Inhibition of PHA-induced blastogenesis by early and midinvolution skims was reduced also by dilution. Variation in ability of secretions obtained from involuted mammary glands to inhibit mononuclear cell blastogenesis may be due to differences in concentration of immunosuppressive substances. A further understanding of the nature of immunosuppressive substances in mammary secretions, the mechanisms by which these substances inhibit mitogenic stimulation of mononuclear cells, and the nature of subpopulations of bovine mononuclear cells is required to substantiate these hypotheses.

The bovine mammary gland is susceptible to infection during physiological transitions from lactation to involution and from involution to colostrogenesis. Limited data are available concerning the role of mononuclear cells, in particular lymphocytes, in the defense of the mammary gland against IMI during physiological transitions. Our study suggests that mammary secretions collected during times of highest susceptibility to new IMI are most inhibitory to bovine blood mononuclear cell blastogenesis. Increased suppression of mammary mononuclear cell blastogenesis during early involution and the peripartum period could contribute to increased susceptibility of the mammary gland to infection. However, further studies of mammary mononuclear cell function throughout involution are required before definitive conclusions can be formed about the role of mononuclear cells in variation in rates of IMI during involution.

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