Alterations in bovine neutrophil function during the periparturient period

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SUMMARY

Neutrophils from 8 Holstein heifers were evaluated for function during the periparturient period. Random migration, ingestion of bacteria, superoxide anion production, native (nonenhanced) chemiluminescence, iodination, and antibody-dependent, cell-mediated cytotoxicity by neutrophils were determined. Foremilk samples were evaluated for bacteria. Significant ($P < 0.05$) increases in random migration of neutrophils, iodination, and chemiluminescence were evident 2 weeks before parturition and then decreased dramatically by the first week after parturition. These impairments of neutrophil function after parturition may be manifested as a severe cumulative deficit in the native defenses afforded by the neutrophil.

Infectious mastitis begins with the establishment of an intramammary infection (IMI). Bovine mastitis caused by opportunistic environmental pathogens (mostly coliform bacteria and streptococci other than Streptococcus agalactiae) has a high incidence in early lactation. The rates of new IMI caused by environmental pathogens are highest during the first and last 2 weeks of a 60-day nonlactating period of dairy cows.[1,2] The rate of IMI during these periods of peak susceptibility is 2 to 12 times higher than that at any other time.[1] Most coliform and environmental streptococcal infections that are established in the nonlactating period and are present at parturition result in clinical mastitis soon afterward.[1,3] The proportion of all cases of clinical coliform mastitis that develop during the first 2, 4, and 8 weeks of lactation has been reported to be 25, 45, and 60%, respectively.[4,5]

Factors contributing to the increased susceptibility to IMI and the progression to clinical disease have not been fully elucidated. Neutrophils can egress rapidly from the blood into the mammary gland in response to an irritant.[6] Phagocytosis and killing of bacteria by neutrophils in conjunction with humoral factors are critical defense mechanisms of the mammary gland.[7,8] Conditions that may compromise immune mechanisms in the bovine udder include physiologic stress of lactation,[9] a decrease in the number of circulating neutrophils capable of phagocytosis after parturition,[10,11] dietary aflatoxin,[12] and a delayed udder inflammatory response elicited by bacteria infecting cows in early lactation.[13,14] Dystocia, ketosis, and milk fever also have been associated with coliform mastitis after calving.[15,16]

Investigation of immunosuppression and coliform mastitis in sows revealed decreased neutrophil chemokinesis, ingestion of bacteria, and iodination to be associated with the susceptibility to postpartum mastitis caused by Escherichia coli.[17] The purpose of the study reported here was to determine the alterations in bovine neutrophil function during the periparturient period.

Materials and Methods

Animals and experimental design—Eight healthy Holstein heifers (principals) were evaluated during their periparturient period (ranging from 51 days before to 25 days after parturition). Estrus and breeding were synchronized[a] to minimize the duration of the calving period to be studied. Eight mixed-breed steers were used as controls to adjust for the day-to-day variability typically seen with immune cell function assays. For each immune cell function, the results of the steers were averaged for each day of sampling; then, the results from individual principals for that day were converted to a percentage of the daily average for the controls (percentage of controls). Beginning about 5 weeks before the expected calving time, samples were obtained once a week. The frequency of sampling was increased to a Monday-Wednesday-Friday schedule about one week before expected parturition and continued at that frequency for at least one week after parturition. At that time, the frequency of obtaining samples was reduced to once a week for the next 3 weeks (Table 1).

Bacteriologic examination of milk—Foremilk samples from individual quarters were obtained at the beginning of the experiment and during lactation. Foremilk (0.1 ml) was spread over blood agar plates, which were examined for bacterial growth after 24 and 48 hours' incubation at 39 C.

Hematologic studies—Leukocyte counts in blood were determined by electronic counting of blood obtained by jugular venipuncture into tubes containing EDTA. Cytocentrifuge films were prepared and stained with a combination Wright/Giemsa stain, and > 200 cells were counted to determine the percentage of cell types. Lymphocytes and monocytes were counted together and termed mononuclear cells.[18]

Time period	N	n	Neutrophils (%)	Eosinophils (%)	Granulocytes (%)
< = Wk−5	4	14	88	9	97
Wk−4	6	12	84	11	95
Wk−3	5	11	89	9	98
Wk−2	7	11	77	21	98
Wk−1	8	21	89	8	97
Day 0*	3	3	92	5	97
Wk 1	8	24	90	6	96
Wk 2	8	18	88	8	96
Wk 3	7	8	89	6	95
Wk 4	3	3	87	11	98

* Day 0 represents the day of calving and is not included in the values of either the week before or the week after calving.
N = No. of heifers, n = No. of blood samples.

Leukocyte preparation—Neutrophils were separated by hypotonic lysis from packed RBC, as described.[19] The remaining cells, usually > 95% granulocytes (neutrophils + eosinophils), were resuspended to 5.0 × 10⁷ granulocytes/ml in Hanks balanced salt solution without Ca^{2+} or Mg^{2+} for functional analysis.

Neutrophil function assays—Procedures for evaluating ingestion of ¹²⁵I-labeled *Staphylococcus aureus*,[19] iodination,[19] cytochrome C reduction,[20] and antibody-dependent, cell-mediated cytotoxicity[21] were performed as described, except that all assays were run at 39 C and *S aureus* strain Newbould 305 (ATCC No. 29740) was used for neutrophil bacterial ingestion. Tests for neutrophil function were performed in duplicate, except for chemiluminescence, which was performed in single vials (one for resting and one for stimulated chemiluminescence) for each animal. All solutions were prepared with ultrapure, pyrogen-free, double-distilled 18 Mohm water.[b]

Random migration under agarose by neutrophils was evaluated as reported with modification for data analysis.[22] A large image of the migration area was traced using a microscope with a drawing attachment, and the area of the image produced was measured with a digitizing pad.[c] Using morphometric analysis,[d] the area of the projected image was mathematically converted to the actual migration area under agarose after the area of the center well was subtracted.

Native (nonluminol dependent) chemiluminescence by stimulated neutrophils was measured using a liquid scintillation counter[e] in the out-of-coincidence mode, with the sample chamber heated to 39 C. The standard reaction mixture for determining stimulated chemiluminescence contained 1.0 × 10⁷ neutrophils and 2.0 mg of opsonized zymosan (prepared as described)[19] in 10 ml of Earle balanced salt solution without phenol red. Nonspecific activation of neutrophils by the media was monitored by omitting opsonized zymosan to determine resting neutrophil chemiluminescence. The reaction was started by adding neutrophils to the vial. Light emission from each vial was measured for 15 seconds at 10-minute intervals over a 90-minute period. Results were converted to the area under the curve.

Statistical analysis—All data analyses were done with the Statistical Analysis System.[23] Results from principals were converted to a percentage of control for each sampling day and then were coded relative to their actual calving dates. Within each

b Industrial Water Purification Service, Culligan USA, Division of Culligan International Co, Northbrook, Ill.

c HIPAD Digitizer, Houston Instruments, Graphics Division of Bausch & Lomb Inc, Austin, Tex.

d Bioquant II, Image Analysis System Software, Version 2.1, R & M Biometrics Inc, Nashville, Tenn.

e Model LS-8000, Beckman Instruments Inc, Irvine, Calif.

week relative to the day of calving (day 0) values from individual principals were averaged and analyzed by fitting the general linear model: y = mean + week + principal + error. In this model, the data were blocked by week (a 7-day period before or after, but not including the day of calving), by principal (representing animal differences), and error represented the residual variation of principals after fitting the model. Statistical significance of differences between week −2 and each of the 3 successive weeks was judged by F tests of the week effect. This analysis was done for raw data and percentage of control values for the principals.

Percentage of control data was plotted as averages of 2-day periods relative to parturition to evaluate the duration of time when immune function may have changed. Data were analyzed by linear regression to determine the duration of significant changes in normalized principal immune cell function over selected periods. All probabilities were considered significant at the 0.05 level. Three principals were tested on the day they calved. The day-0 data were not included in the plotted weekly averages, but were included in the linear regression analyses.

Results

General observations at parturition—All principals gave birth to a single healthy calf, which was removed from the dam's environment within 8 hours after birth.

Udder health status—After calving, 2 principals (No. 1 and 2) had an *E coli* IMI. Principal 1 had clinical coliform mastitis 2 days after calving (abnormal milk, fever, mild anorexia, and dehydration). Three quarters were infected, and these infections persisted for 2, 14, and 16 days, respectively. Intramammary infection was detected in one quarter of principal 2 at the first milking after calving. Clinical signs of coliform mastitis were not evident. This IMI persisted for 11 days. Both principals recovered without treatment. Intramammary infections caused by coagulase-negative *Staphylococcus* spp were detected in 1 quarter of each of 3 other principals (No. 3, 4, and 5). One infection was evident at parturition, and the others were detected 2 and 3 days after calving; the infections persisted for 16, 18, and 25 days, respectively. None of the staphylococcal infections resulted in abnormal milk secretions or signs of illness.

Periparturient changes in leukogram—Before calving, total WBC count increased (*P* < 0.002) and peaked 2 weeks

Cells evaluated	Week interval	b*	SE of b	P value
Total WBC count†	−6 to −2	0.016	0.005	0.002
	−2 to 3	−0.004	0.002	0.04
Total mononuclear cell count†	−6 to −2	0.006	0.003	0.02
	−2 to 0	−0.01	0.007	0.08
Total neutrophil count†	−6 to 0	0.07	0.01	0.0001
Total eosinophil count†	−2 to 0	−0.22	0.1	0.04
Mononuclear cells in blood (%)	−6 to 0	−0.004	0.001	0.0008
	0 to 4	0.003	0.002	0.19
Neutrophils in blood (%)	−6 to 0	0.005	0.001	0.0002
	0 to 4	−0.004	0.003	0.09
Eosinophils in blood (%)	−3 to 0	−0.001	0.0006	0.02
	0 to 4	0.001	0.0005	0.04

* b = slope of value vs time. † Linear regressions based on log_{10} cells/mm³ of blood.

Neutrophil function		\multicolumn{8}{Weekly mean}								P value of week −2 vs week:		
		−2			−1			1			2			−1	1	2
Random migration	RD*	12.0	±	1.2	10.9	±	1.7	8.4	±	0.6	9.5	±	1.4	NS	NS	NS
	POC	139	±	15	105	±	11	92	±	5	113	±	8	NS	0.04	NS
Antibody-dependent, cell-mediated toxicity	RD†	92.2	±	3.6	90.0	±	2.8	83.0	±	6.7	96.0	±	1.0	NS	NS	NS
	POC	105	±	3	105	±	3	92	±	7	103	±	2	NS	NS	NS
Cytochrome C reduction	RD‡	0.377±		0.023	0.382±		0.019	0.395±		0.013	0.427±		0.011	NS	NS	NS
	POC	101	±	3	101	±	3	96	±	1	100	±	3	NS	NS	NS
Native chemiluminescence	RD§	1,640		±140	1,680		±150	1,430		±250	1,320		±110	NS	NS	0.002
	POC	144	±	21	134	±	18	103	±	16	95	±	8	NS	NS	0.05
Ingestion	RD‖	59.4	±	1.7	67.0	±	1.3	57.5	±	2.7	52.9	±	3.4	0.02	NS	NS
	POC	114	±	3	120	±	4	111	±	4	107	±	5	NS	NS	NS
Iodination	RD¶	44.3	±	5.0	42.0	±	4.2	26.3	±	2.9	29.7	±	2.4	NS	0.007	0.007
	POC	109	±	7	114	±	7	84	±	8	102	±	7	NS	0.002	NS

* Migration area in mm^2. † Percentage release from ^{51}Cr-labeled chicken erythrocytes. ‡ Optical density, 550-nm wave length. § Mean stimulated area under the curve. ‖ Percentage of 60 *Staphylococcus aureus* ingested/neutrophil. ¶ nM NaI/10^7 neutrophils/h.

Data are expressed as mean ± SEM. NS = not significant at $P < 0.05$.
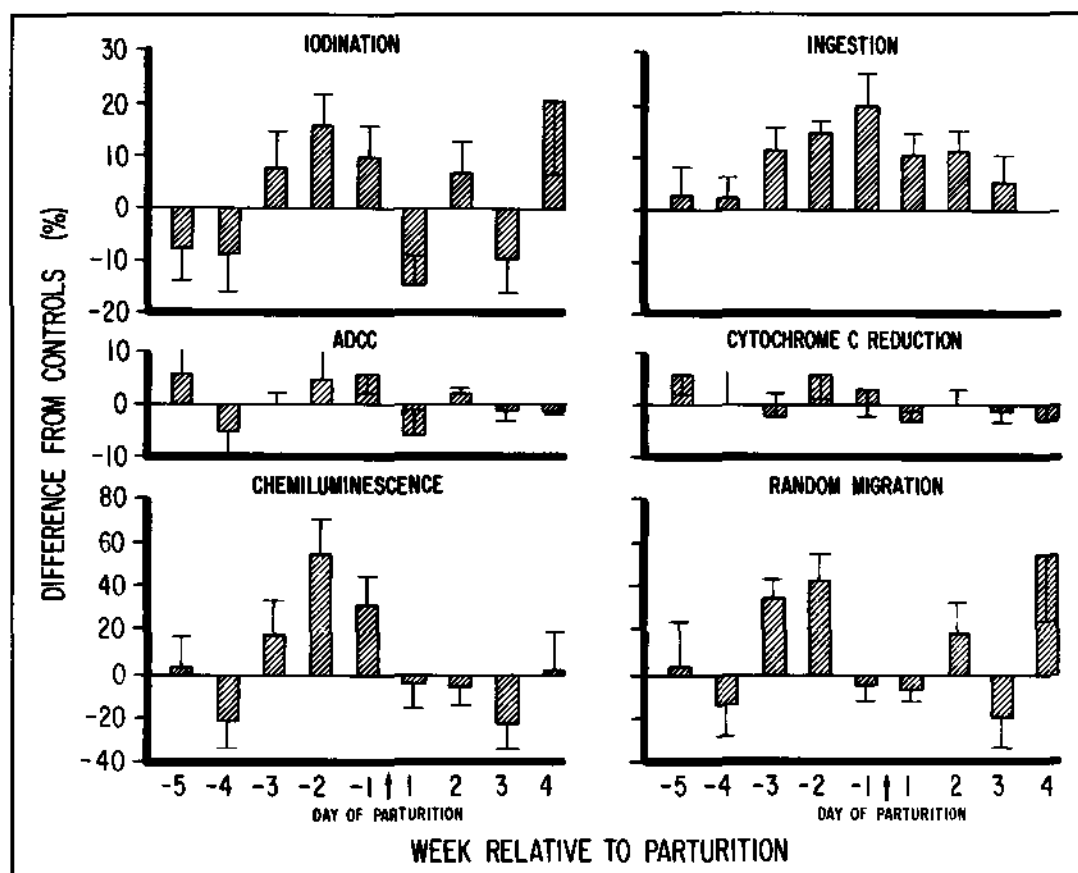

Fig 1—Neutrophil function assay results relative to the day of parturition from 8 Holstein heifers. Bars represent the mean difference from the controls for all measurements determined each week on all principals. Weekly standard errors are shown with each bar. ADCC = antibody-dependent, cell-mediated cytotoxicity.

before parturition (Table 2). This increase was caused by the combined absolute increases in the number of circulating neutrophils before calving ($P < 0.0001$) and circulating mononuclear cells from −6 to −2 weeks before parturition ($P < 0.02$). At 1 week after calving, the absolute number of circulating neutrophils decreased to about two-thirds the concentration detected 1 week before parturition.

Periparturient changes in neutrophil function—Hypotonic lysis of blood from principals (Table 1) yielded an average of 87% neutrophils (range, 77 to 92%). Most of the contaminating cells were eosinophils. Granulocyte purity was 96% for the experiment (range, 95 to 98%). When averages of raw data from week −2 were compared with those from the 3 following weeks, a significant increase in bacterial ingestion was observed by week −1

TABLE 4—Regression analyses of neutrophil function values for selected time intervals, based on means of 2-day periods* relative to day of calving (data not shown)

Function assay	Linear regression time interval (days)†	b‡	SE of b	PR > F
Neutrophil random migration	−47 to −14	0.02	0.008	0.004
	−14 to 0	−0.05	0.02	0.006
	0 to 25	0.02	0.01	0.08
Neutrophil iodination	−40 to −14	1.3	0.6	0.04
	−14 to 8	−2.0	0.6	0.002

* Only the assays of neutrophil function with significant linear changes over time are shown. † Days relative to parturition (parturition = day 0). ‡ Slope of normalized data vs time in days.

TABLE 5—Grand mean comparisons of neutrophil function of principals vs controls over the experiment and a components of variance analysis of the principals' neutrophil function values assuming date and animal effects to be random, independent effects contributing to the overall variation observed in the listed assays

Neutrophil function	Means		Random effect	Fraction of variance	
	Steer (SD)	Heifers (SD)		Raw data	Normalized data
Antibody-dependent, cell-mediated cytotoxicity*	89.3(0.4)	88.8(1.7)	Date†	0.26	0.06
			Animal	0.04	0.06
Chemiluminescence‡	1,389(40)	1,440(50)	Date	0.01	0.10
			Animal	0.18	0.16
Ingestion§	54(0.5)	60(0.9)	Date†	0.47	0.09
			Animal	0.09	0.16
Iodination‖	37.3(0.7)	37.4(1.1)	Date†	0.36	0.12
			Animal	0.09	0.13
Chemokinesis¶	10(0.4)	10(0.5)	Date†	0.11	0.16
			Animal	0.06	0.06
Cytochrome C#	0.397(0.004)	0.395(0.008)	Date†	0.15	0.06
			Animal	0.04	0.01

* Percentage release from ^{51}Cr-labeled chicken erythrocytes. † Average date fractional component of variance for raw data from principals for these assays = 0.27, 4.2 times greater than the animal effect. Normalizing the principals' data with the controls' values each day for these assays reduced the date component to the same fraction of variance (0.1) due to animal differences in these assays. ‡ Stimulated area under the curve × 1,000. § Percentage of 60 *Staphylococcus aureus* ingested per neutrophil. ‖ nM NaI/10⁷ neutrophils/h. ¶ Migration area in mm². # Optical density, approx 550-nm wavelength.
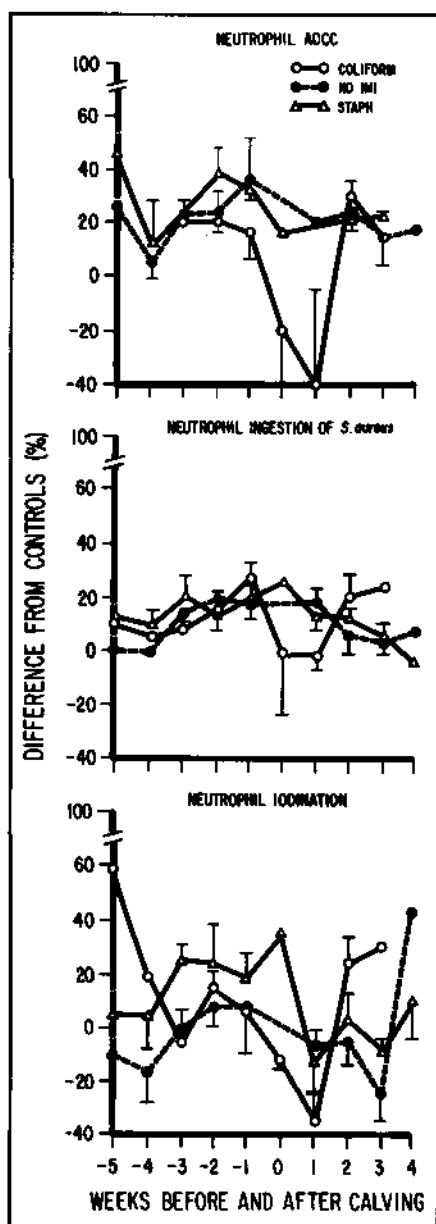

Fig 2—Neutrophil antibody-dependent, cell-mediated cytotoxicity (ADCC), bacterial ingestion, and iodination assay results shown by udder health status of 8 Holstein heifers during the periparturient period. Two principals had coliform (*Escherichia coli*) infections (O——O), 3 had *Staphylococcus epidermidis* infections (△——△), and 3 had no intramammary infections (IMI) detected (●——●). Data points represent the difference from controls by week for the 3 infection status groups during the periparturient period. Weekly standard errors are shown with each point.
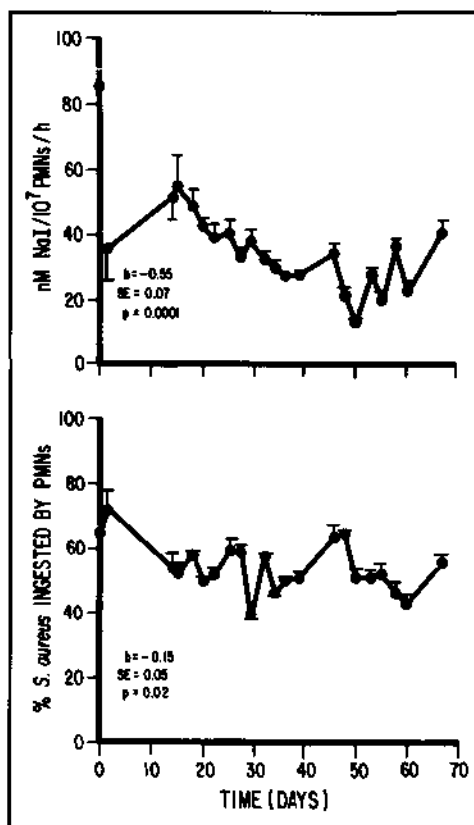

Fig 3—Plots of the raw data from 8 controls for neutrophil iodination and ingestion of *Staphylococcus aureus* assays vs the 67 days during which the study was conducted. These were the only 2 assays with significant negative slopes. Slope (b), SE, and P values for b of each assay is shown. Bars on data points represent the daily SE for the 8 controls.

(which decreased to values observed in week −2 during week 1; Table 3). A decrease in neutrophil iodination ($P < 0.007$) was observed during week 1 . Resting chemiluminescence values did not indicate nonspecific activation of neutrophils in the assay. Because resting values were essentially constant, stimulated chemiluminescence values were not adjusted. By week 2, stimulated chemiluminescence values were significantly ($P < 0.002$) decreased, whereas iodination values remained low ($P < 0.007$). During week 1, a decrease in random migration was observed, but was not significant ($P < 0.07$; Fig 1).

Comparison of normalized data from week −2 with those from the subsequent weeks revealed general agreement with raw data comparisons from week 1 (a trend of decreased random migration and significantly decreased iodination). Percentage of control values indicated a trend of decreased cytochrome C reduction that was not significant ($P < 0.07$). Comparisons of normalized data from week −2 with those from week 2 also agreed with raw data for decreased stimulated chemiluminescence; however, normalized iodination values returned to prepartum values rather than decreasing. Ingestion of bacteria by neutrophils was not significantly changed when analyzed as a percentage of controls.

Linear regressions of normalized neutrophil function values were performed on the basis of changes observed in data plots of averages of 2-day periods relative to calving (Table 4). Random migration by neutrophils increased up to 2 weeks before parturition ($P < 0.004$) and then decreased rapidly until the day of calving ($P < 0.006$). By the fourth week after calving, neutrophil random migration function had increased and, thus, appeared to recover slightly, but not significantly ($P < 0.08$). Ingestion of S aureus by neutrophils of principals increased from 7% greater than controls at 3 to 4 weeks before parturition to 24% greater than controls for the 4 days before calving. At parturition, ingestion decreased to 11% greater than controls and remained on that level for 2 weeks after parturition. Stimulated chemiluminescence by neutrophils of principals increased from 37% less than controls beginning at day −28 to 60% greater than controls by day −9, and then decreased to 9% less than controls on the day of calving and remained about the same as controls for 12 days after parturition. One-way analysis of variance comparison of stimulated chemiluminescence for the period between days −18 and −2 with that for the period between days 0 and 10 indicated a significant decrease in the oxidative metabolic capacity of neutrophils of principals during the first 10 days after parturition ($P < 0.002$). The ability of principals' neutrophils to catalyze the iodination reaction increased from 17% less than controls beginning around day −28 to 24% greater than controls around day −14 ($P < 0.04$). From day −14 to day 8, there was a linear decrease ($P < 0.002$) in the iodination activity of neutrophils. For the first 8 days after calving, neutrophil responses were 15% less than controls in the iodination assay and then recovered. Cytochrome C reduction and antibody-dependent, cell-mediated cytotoxicity by neutrophils did not change significantly during the periparturient period.

Concomitant with the observed increase in certain neutrophil functions about 2 weeks before parturition, there was an increase in eosinophil contamination of the neutrophil preparations (Table 1). The Pearson correlation coefficient between eosinophil contamination of the neutrophil preparations and iodination ($r = 0.21$) was found to be significant ($P < 0.02$). The correlation coefficient suggests that not more than 4.3% of the variance in the iodination results can be attributed to eosinophil contamination. Because the eosinophil contamination was stable except for week −2, data from that particular week contributed greatly to the correlation.

Immune cell function based on udder health status—As a group, principals with IMI had the most dramatic suppression of the following neutrophil functions: antibody-dependent, cell-mediated cytotoxicity, ingestion, and iodination (Fig 2). The 2 principals with coliform IMI had more severely suppressed neutrophil antibody-dependent, cell-mediated cytotoxicity, ingestion, and iodination than did the staphylococcal-infected group of principals; statistical tests of these differences were not calculated because of the few *df*.

Correlations between immune cell functions—The principals' neutrophil-stimulated chemiluminescence results were significantly correlated ($r = 0.43$; $P < 0.0001$) with the iodination results. Superoxide anion production measured by cytochrome C reduction also was correlated ($r = 0.23$; $P < 0.02$) with the iodination results. All 3 of these assays depend on the oxidative burst of neutrophil metabolism.

Effects of normalizing principals' immune cell function data—The grand mean for each immune cell function assay over the study's duration (Table 5) indicated the controls' immune cells were virtually identical to the overall average of the principals; therefore, the use of the steers' cells to normalize the data for day-to-day variation did not impart any effect of scale on the normalized data from principals. Plots of the controls' raw data values (Fig 3) for the ingestion and iodination values of neutrophil function illustrate a negative slope for the daily values obtained over the course of the experiment.

A components of variance analysis of the raw data and normalized data of the principals was performed to evaluate the benefit of normalizing data to block out daily variations in assay results. Raw data variation from one day to the next averaged approximately 4 times greater than variation between principals in 5 neutrophil function assays (Table 5). Conversion of the principals' data into percentage of controls reduced the day effect to the same fraction (0.1) of variance attributed to the animal differences for these neutrophil function assays. On average, normalization reduced the fraction of variance attributed to the day effect for all functions and, therefore, improved the validity of these assays for use in long-term, longitudinal studies. During the 67-day experiment, there was more variation in the principals' cell function than in the controls'. Dramatic changes in immune cell function that developed around calving accounted for much of this difference in variation.

Discussion

Primiparous Holstein cows were determined to have impaired neutrophil function after parturition, although peak values of certain neutrophil functions occurred 2 to

3 weeks before parturition. There also was an association of IMI with suppressed neutrophil function in periparturient principals. The association of IMI with impaired neutrophil function is largely based on our findings of immunosuppression and their correlation to a time when mastitis has a high incidence.[1-5] The zenith and nadir of neutrophil function values observed around parturition are relative to the average values observed during the study. It is possible that the decreased values may reflect a return to normal values. However, based on clinical disease problems after parturition, such as coliform mastitis, we believe the decrease of neutrophil function after parturition represents an immunosuppressed condition in primiparous dairy cows. Neutrophils of 2 principals with coliform mastitis clearly functioned differently than those of the other principals. The coliform infections were detected after impairment of neutrophil function had been observed. However, we cannot rule out the possibility that coliform mastitis contributed in part to the duration of immunosuppression observed in these 2 principals. Immunosuppression associated with gestation and parturition has been reported in many species.[24]

Recruitment of neutrophils to an infection site is one of the first steps in the inflammatory response. Early and rapid neutrophil influx is important in the eradication of IMI in mice.[25] Defective neutrophil chemotaxis in periparturient cows and diverse abilities of cows to initiate an inflammatory response of neutrophils in the udder during early stages of lactation have been reported.[13,14,26] We observed in vitro alterations in neutrophil chemokinesis after calving; the functional significance of which was unclear. Increases in chemokinesis are associated with a loss of neutrophil "stickiness" and reduced adherence to capillary endothelia.[27-29] Adherence is an important initial step when neutrophils egress from the blood stream. Conversely, a dramatic decrease in normal chemokinesis can be interpreted as an impediment to migration through tissues.[27,28] A delay in neutrophil diapedisis into the udder may result in severe disease.[14]

Ingestion of infective agents is the next step in the phagocytic process. Periparturient changes in yeast and bacterial ingestion by bovine neutrophils have been reported.[10,11] These studies found either a prepartum increase in the number of yeast cells ingested per neutrophil followed by a rapid decrease at parturition (which was believed to be compensated for by an increase in the percentage of neutrophils phagocytically active), or an increase in the number of phagocytically active neutrophils in blood to a maximum 2 weeks before calving, which then decreased to a minimum during the first week after parturition. Results of our ingestion assay indicated that the ingestion capacity of the neutrophil population isolated from blood is high during the periparturient period, but this capacity may decrease slightly after calving. Our data regarding prepartum peaks in total leukocyte numbers, the percentage of neutrophils in blood on the day of calving, and the rapid decrease of neutrophils after calving were in agreement with that reported by Newbould.[10] Increased circulating neutrophils have been associated with an increase in blood plasma corticosteroid concentration at parturition.[11] Increased serum cortisol concentration adversely affects bovine neutrophil function and has been implicated as an initiating factor in the pathogenesis of certain bovine infectious diseases.[9,29,30]

There have been few reports on changes in later stages of the phagocytic process of neutrophils from periparturient cows. A biphasic response in luminol-enhanced neutrophil chemiluminescence, which closely paralleled our iodination results, has been reported.[f] Both of these assays reflect the activity of myeloperoxidase-catalyzed reactions in the phagolysosome. The native stimulated chemiluminescence assay is a general measure of neutrophil oxidative metabolism, which detects dismutation of superoxide anion into H_2O_2, myeloperoxidase-catalyzed reactions, and oxidation of membrane phospholipids and arachidonic acid metabolites.[31-33] That neutrophil functions associated with the oxidative burst of metabolism accompanying phagocytosis are impaired after calving is clearly supported by the correlated depression of stimulated native chemiluminescence and iodination reactions from maximal activity about 2 weeks before parturition to minimal activity the first week after calving. Enhanced stimulated neutrophil chemiluminescence has been reported in pregnant women, which could not be explained by different amounts of myeloperoxidase in neutrophil granules when compared with controls.[34-36]

Our study found that neutrophil chemokinesis was significantly impaired the first week after calving when compared with activity during week -2. Production of superoxide anion, antibody-dependent, cell-mediated cytotoxicity, and bacterial ingestion also decreased during week 1 after parturition, although not significantly.

Many neuroendocrine changes develop in cows during the periparturient period. In our study, the earliest changes in neutrophil function developed 2 to 3 weeks before parturition. Enhanced activities of neutrophils before parturition could be attributed to the effects of increases in estrogens, prolactin, growth hormone, and/or insulin before parturition.[37-41]

Periparturient hormone fluxes also may adversely affect immune cell function. Suppression of human neutrophil chemotaxis by prolactin has been reported in patients with prolactin-secreting tumors.[42] There is no effect of estrogen on bovine neutrophil function either during the follicular phase of the estrous cycle in cows or after administration of high doses of estradiol to steers.[43,44] However, supraphysiologic concentrations of estradiol have been reported to suppress human neutrophil oxidative metabolism and the neutrophil myeloperoxidase-catalyzed halogenation reaction.[45,46] These high concentrations of estrogens may be germane to the onset of impaired neutrophil function in the cow before parturition. Before calving, total plasma estrogen concentrations increase dramatically in the cow (at least 10 times greater than during estrus).[41]

Many of the hormonal and metabolic changes that prepare the mammary gland for lactation take place during the 3 weeks preceding parturition. During this critical period, the dairy cow's body metabolism shifts from the demands of pregnancy to those of lactation, with increased body demands for energy and protein. Negative energy and protein balances that exist during early lactation may contribute to impaired neutrophil function and, thus, account for a portion of the periparturient immunosuppression we and others have observed.

[f] Gustafson B, Schwyzer M, Wyler R, et al. The leukocyte function in the periparturient cow (abstr), in *Proceedings*. 67th Conf Res Workers Anim Dis 1986;67:20.

In our study, a group of steers was used to normalize the data from the principals. It was not our objective to use these steers as biologic controls for comparison with the principals. Trends and large day-to-day variations are typical of neutrophil function assays and indicate the need for a control group of cattle to counteract the large day-to-day variation with these assays. A few discrepancies between raw data and normalized data may arise, as pointed out with the neutrophil iodination values 2 weeks after parturition. This discrepancy points out how use of raw data from these neutrophil assays can be misleading and erroneous. Significant time trends (sometimes associated with the half-life of radioisotopes used) exist for these cell assays that are in part, independent of changes in the animals being studied (Fig 3).

In summary, we detected impairment of neutrophil microbicidal mechanisms, and the membrane-associated activities of chemokinesis and ingestion, which may be manifested as a cumulative deficit in the native defense system afforded by neutrophils. Native defenses of the bovine mammary gland are continually challenged by environmental exposure to bacteria, and many factors affect the outcome of this challenge. Once the teat canal barrier is penetrated by bacteria, the local host defenses in lacteal secretions (lactoperoxidase, complement, lactoferrin, and resident immune cells) determine the outcome of bacterial presence in the udder (eradication, subclinical infection, or clinical disease). In lacteal secretions, neutrophils ingest milk fat and casein, resulting in degranulation and a loss of pseudopodia, which dramatically reduces the neutrophils' microbicidal potential.[47,48] If neutrophils in milk are derived from blood neutrophils that already are defective, then the balance between bacterial clearance and IMI may favor the bacteria establishing an IMI or the ultimate progression of an IMI into clinical mastitis.

The results of our study cannot attribute the alterations in the immune status of the periparturient cow to the effect of any specific hormone, combination of hormones, or stress, because these were not evaluated. Because mastitis in well-managed dairy herds is usually caused by opportunistic bacteria,[49] alterations in nonspecific host defenses (eg, neutrophil function) may be more relevent to new infection rates and development of clinical diseases during the periparturient period. Our results cannot ascribe, however, a cause and effect relationship between a faltering immune system and the development of IMI.

Immunomodulators that might abrogate the development of periparturient immunosuppression currently are not available. Characterization of defective immune cell function during periods of peak IMI rates with opportunistic bacteria should guide efforts to evaluate immunotherapeutic agents for defective bovine neutrophil function.

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