Preinfection In Vitro Chemotaxis, Phagocytosis, Oxidative Burst, and Expression of CD11/CD18 Receptors and Their Predictive Capacity on the Outcome of Mastitis Induced in Dairy Cows with *Escherichia coli*

T. VAN WERVEN,* E. N. NOORDHUIZEN-STASSEN,* A.J.J.M. DAEMEN,* Y. H. SCHUKKEN,* A. BRAND,* and C. BURVENICH†

*Department of Herd Health and Reproduction, Utrecht University, Yalelaan 7, 3584 CL Utrecht, The Netherlands

†Department of Veterinary Physiology, Biochemistry and Biometrics, University of Ghent, Salisburylaan 113, B-9820 Merelbeke, Belgium

ABSTRACT

Four to 6 wk after parturition, 12 cows in second, fourth, or fifth lactation were experimentally infected in one gland with *Escherichia coli*. The capacity of chemotaxis, phagocytosis, oxidative burst, and expression of CD11/CD18 receptors to predict the severity of IMI was measured. Bacterial counts in the infected quarter, expressed as area under the curve, and residual milk production in the uninfected quarters were compared to determine severity of the infection. Although these two outcome parameters were highly negatively correlated, regression models with preinfection tests for leukocyte function fitted best with bacterial counts as an outcome parameter. Of the preinfection tests for leukocyte function, chemotaxis best predicted the outcome of the IMI that had been experimentally induced by *E. coli*.

The number of circulating peripheral leukocytes just prior to inoculation was used to predict 52 and 45% of the severity of IMI for bacterial counts and residual milk production, respectively. As a categorical variable, parity predicted 75 and 56% of the severity of IMI expressed as bacterial counts and residual milk production, respectively. Because of the strong effect of parity on the outcome of the experimentally induced mastitis, analysis was performed to discriminate between second parity cows and older cows. Significant differences were found for the number of circulating peripheral leukocytes and for the expression of CD11b/CD18 and CD11c/CD18 receptors between younger and older cows.

(**Key words**: bovine, *Escherichia coli*, mastitis, leukocyte function test)

Abbreviation key: **AUC** = area under the curve, **PMN** = polymorphonuclear neutrophil, **RMP** = residual milk production.

INTRODUCTION

Mastitis remains an important disease despite an increasing body of knowledge on risk factors for herds and individual cows. Even in well-managed herds, incidence of clinical mastitis varied from 0 to 80 cases per 100 cow-years (21). In herds with a high risk profile, some cows never developed mastitis but, in herds with a low risk profile, some cows did develop mastitis. Therefore, cow factors play an important role in the susceptibility of individual cows to mastitis. An understanding of the mechanisms involved in susceptibility would make intervention possible and might lead to an even lower number of cases of mastitis. Immune function tests are able to differentiate between cows that are highly susceptible and cows that are less susceptible to mastitis. Measurement of these immunological markers might permit discrimination between these two groups of cows. The preinfection under agarose chemotaxis assay was able to predict fairly accurately the outcome of mastitis induced experimentally with *Escherichia coli* (14). Other components of cellular immunity, such as phagocytosis and killing, are expected to contribute also to the susceptibility of an animal (9). However, currently little is known on the quantitative decomposition of preventive immune function.

Although diapedesis and migration of leukocytes are considered to be two of the most important immunological mechanisms against *E. coli* mastitis (3, 12, 18), those processes are only a part of the total defense mechanism. After chemotaxis has been completed, phagocytosis and intracellular killing are the next steps in the defense process. Although chemotaxis and phagocytosis are not completely understood, expression of receptors on the polymorphonuclear

Received November 9, 1995.
Accepted May 3, 1996.

neutrophils (**PMN**) and macrophages are important regulators of the intensity of adherence to epithelial cells, diapedesis into the udder, and attachment of microorganisms to phagocytes (2). The leukocyte CD11/CD18 integrins or β2 integrins are crucial in this regard (17, 24). Because the severity of IMI caused by coliforms is at least partly explained by an excessive reaction of immune function, it is currently unknown whether the severity of the IMI can be predicted by a high or low expression of CD11/CD18 receptors. Inhibition of neutrophil adhesive interactions with an antibody against CD11b/CD18 reduced myocardial reperfusion injury (23) and reduced neutrophil accumulation and plasma leakage in vivo in response to an injection of a complement activation product, C5a (1).

Experimentally, *E. coli* IMI has often been used to study the importance of the components of cellular immunity. In most of those studies, the parameter used to measure severity of the IMI was the area under the curve (**AUC**) of the bacterial growth in the infected quarter. Another outcome parameter used was the residual milk production (**RMP**) in the uninfected quarters (9). Both outcome parameters essentially give information about two subsequent steps of the inflammation process. The correlation between these parameters has not yet been quantified, but correlation is important when studies using either of the two parameters are to be compared.

The objectives of this study were 1) to quantify the importance of preinfection immunological in vitro tests of cell function, such as chemotaxis, phagocytosis, oxidative burst, and expression of adhesion receptors, in the observed variation in severity of an *E. coli* IMI and 2) to estimate the relationship between parameters for severity of IMI.

MATERIALS AND METHODS

Cows

Twelve crossbred Holstein-Friesian × Friesian-Holstein cows in their second, fourth, or fifth lactation and 4 to 6 wk postpartum were used. Foremilk samples from the inoculated quarters (right rear) were bacteriologically negative for major pathogens, and the SCC in each quarter was <150,000 cells/ml. The concentration of BHBA in peripheral blood before inoculation was <1.4 mmol/L. Cows were housed in tie stalls and fed grass silage and corn silage for ad libitum intake and concentrates according to milk production. Cows were milked twice daily at 0100 and 1300 h.

Milk Samples

Twice daily, quarter milk production was measured from 3 d before to 7 d after inoculation. The SCC was measured by the Fossomatic device (Foss Electric, Hillerød, Denmark). Determination of SCC was carried out at d –3, –2, and –1 and immediately prior to inoculation. After inoculation, milk samples for SCC were taken every 3 h over a 24-h period. Bacteriological examination of the quarter samples of foremilk was conducted at d –5, d –2, and immediately prior to inoculation. Bacterial counts of *E. coli* were determined using a spiral plater (Lameris Laboratory, Breukelen, The Netherlands) every 3 h during the 1st d and then at 30, 42, 54, 66, 78, 114, and 126 h postinoculation.

Bacterial Suspension and Experimental IMI

An encapsulated strain of *E. coli* O:157 that was previously isolated from a cow with clinical mastitis was used for intramammary infusion (15). The strain was maintained on brain-heart infusion agar with glycerol and stored at –70°C. The strain was subcultured and adjusted to a concentration of approximately 50 cfu/ml as described previously (14). Cows were infected with approximately 10^3 cfu of *E. coli* diluted in 20 ml of pyrogen-free saline on d 0. The suspension was aseptically infused into the right rear quarter immediately after milking. The subsequent milking at 0100 h was shipped.

Sampling for Cell Function Tests

Blood samples for chemotaxis assay were obtained by puncture of the jugular vein; blood was collected in vacutainer tubes (Venoject®; Terumo Corp., Tokyo, Japan) containing EDTA as anticoagulant. Blood samples were taken every morning at 0730 h for 7 consecutive d from d –6 to d –1, and immediately prior to inoculation. Blood samples for phagocytosis were taken at d –5, –4, –2, and –1 and immediately prior to inoculation. Samples for oxidative burst and adhesion receptors were taken at d –6, –4, –2, and –1 and immediately prior to inoculation. Samples for these three tests were collected in tubes containing Alsever solution (Gibco, Life Technologies, Gent, Belgium).

Blood samples for differential leukocyte counts were obtained by puncture of the jugular vein and collected in vacutainer tubes (Venoject®) containing EDTA as the anticoagulant. Samples were collected from d –6 to d –1 and immediately prior to inocula-

tion. After inoculation, samples were taken every 3 h over the first 24 h.

Isolation of White Blood Cells

Immediately after venipuncture, blood was put in polypropylene tubes and centrifuged for 20 min at 1000 × *g* at 4°C. White blood cells were isolated as described previously (14). Cells were resuspended in Eagle's Minimal Essential Medium and were adjusted to a concentration of 5×10^7 cells/ml by an automatic cell counter (Sysmex K-1000; Goffin, IJsselstein, The Netherlands).

Chemotaxis Assay

The under agarose technique was used to determine the in vitro chemotactic response of white blood cells as described by Kremer et al. (14) with a few modifications. Briefly, six series of two 2.7-mm wells were cut from a 0.8% agarose plate using a template. Five microliters of white blood cell suspension (5×10^7 cells/ml) were placed in one well. Untreated, pooled bovine serum (5 μl) with proven chemotactic activity (5) was added to the opposite well as a chemoattractant. Serum was obtained from 10 healthy cows and pooled. In each experiment, the same batch of pooled serum, stored at –80°C, was used. Plates were incubated at 37°C in a humified atmosphere of 5% CO_2 and 95% air for 4 h. Migration was arrested by methanol, and cells were fixed with formalin. Agarose was removed, and cells were stained with Diff-Quick® (Merz and Dade AG, Düdingen, Switzerland). Migration distance was measured using an ocular micrometer in a stereo microscope at 24× magnification. The distal limit of the migration pattern was defined as the farthest three cells parallel to the margin of the central well (6). Distance toward the chemoattractant was defined as directed migration, and distance migrated away from the chemoattractant was defined as random migration. Chemotactic activity was expressed as the chemotactic differential, the difference between directed and random migration. Each sample was measured in triplicate, and results were averaged.

Phagocytosis

The *E. coli* strain O:157 of the inoculum was labeled with fluorescin isothiocyanate (FITC isomer 1; Sigma Chemical Co., St. Louis, MO) as described previously (7). Briefly, to the bacterial suspension of 10^9 cfu/ml, carbonate-bicarbonate buffer was added (1:5, vol/vol). Twice this volume of a 0.03% fluorescein isothiocyanate in carbonate-bicarbonate buffer solution was added and followed by incubation for 2 h at room temperature (18°C) in the dark. The suspension was washed three times with Hanks balanced salt solution, and the pellet was resuspended to a final concentration of 10 cfu/ml. The suspension was stored in 1-ml aliquots at –80°C after addition of one droplet of sterile glycerol. Phagocytosis was tested according to a method described by Saad and Hageltorn (20). Thawed bacterial suspension and PMN suspension were added into test tubes at a 10:1 (vol/vol) ratio of bacteria to PMN. Pooled bovine serum was added to a final serum concentration of 5%. After incubation for 15 min at 37°C in a shaking water bath, phagocytosis was arrested by addition of 500 μl of ice-cold EDTA (0.3%). Fluorescein isothiocyanate fluorescence was measured by flow cytometry (EPICS 741; Coulter Electronics Ltd., Luton, England) using a 530- to 560-nm filter. Trypan blue was used for quenching extracellular fluorescence. In the green fluorescence, phagocytosis was measured as a percentage of fluorescent neutrophils on a logarithmic scale from 1 to 1000.

Respiratory Burst Activity

Oxygen-dependent killing of invading pathogens by neutrophil leukocytes generates superoxide, H_2O_2, and hydrophile radicals. These respiratory burst activities were quantified after stimulation of the cells with phorbol myristate acetate (100 ng/ml) (9). The release of H_2O_2 from cells was monitored fluorometrically by means of peroxidase-catalyzed oxidation of scopoletin. Production of the superoxide anions was quantified continuously using phorbol myristate acetate as an activator, followed by the spectrophotometric reduction of cytochrome *c*, which was able to be inhibited by superoxide dismutase. Production of H_2O_2 was measured in nanomoles of H_2O_2/min per 10^6 cells.

Adhesion Receptors

One hundred microliters of a 10^7 leukocytes/ml suspension were centrifuged for 10 min at 200 × *g* at 4°C. After removal of the supernatant, 100 μl of staining reagent (RPMI 1640; Gibco Life Technologies) supplemented with 1% BSA, 0.2% NaN_2, and 100 μl of antibovine monoclonal antibodies IL-A99 (anti-CD11a), IL-A15 (anti-CD11b), and IL-A46 (anti-CD11C) (25) were added. After incubation in ice in the dark for 30 min, the suspension was centrifuged for 10 min at 200 × *g* and was followed by two washings with 240 μl of staining reagent. Then the

pellet was incubated with 100 ml of conjugate (goat anti-mouse IgG-fluorescein isothiocyanate; Sigma Chemical Co.) for 30 min in ice in the dark. After centrifugation at 200 × *g* for 10 min, the pellet was washed two times with 240 μl of PBS, and finally the pellet was resuspended in 2 ml of PBS supplemented with 1% paraformaldehyde. Samples were stored at 4°C in the dark until fluorescence analysis. Fluorescence was measured with an EPICS 741 flow cytometer equipped with a 5-W argon laser (Coherent, Palo Alto, CA). Excitation wavelength was 488 nm, and emitted fluorescence was measured between 530 and 560 nm. Expression of cell adhesion molecules was quantified as mean fluorescence intensity of the positive fluorescent cells expressed as mean channel number and plotted on a logarithmic scale.

Severity of Infection

Two parameters were used to measure the severity of IMI: RMP in the uninfected quarters and bacterial counts in the inoculated rear quarter. Mean milk production of the three uninfected quarters 3 d before inoculation served as the baseline. The RMP of the uninfected quarters on the 2nd d after inoculation (mean of 52 and 64 h after inoculation) was expressed as a percentage of the baseline. Bacterial counts of the infected right rear quarters were expressed as AUC calculated for 126 h postinoculation (15). The AUC ($\log_{10}$ colony-forming units per hour) was calculated using the following equation:

$$AUC = [(t_i - t_{i-1})\ f_{i-1}] + [0.5\ (t_i - t_{i-1})(f_i - f_{i-1})]$$

where

t_i = time of observation,
t_{i-1} = previous time of observation,
f_i = $\log_{10}$ bacterial count at time i, and
f_{i-1} = $\log_{10}$ bacterial count at time t_{i-1}.

Statistical Analyses

Associations of immune functions were estimated; correlations used results from d 0. Regression analysis was used to determine the effect of preinfection immunological parameters on the severity of IMI. The general model for analysis of variance was

$$Y = \mu + Z + P + Int + e$$

where

Y = severity of infection,
μ = overall mean,
Z = preinfection parameters,
P = parity category,
Int = interaction terms (Z × P), and
e = error term.

Cows were divided into two categories based on parity. Second parity cows were designated as 0 (n = 5), and cows in parity ≥4 were designated as 1 (n = 7). Statistical significance was determined at $P \leq 0.05$. To minimize the random fluctuation of the in vitro chemotaxis assay, a Markov model of the first order was used to calculate adjusted least squares means over 7 consecutive d as has been described (26). The Markov model was

$$Y_{ij} = a + bX_{i-1,j} + cow_j + e_{ij}$$

where

Y_{ij} = chemotaxis of cow j on day i,
a = intercept,
b = estimated auto correlation term,
$X_{i-1,j}$ = chemotaxis of cow j on day i – 1,
cow_j = cow j, and
e_{ij} = random error term of cow j on day i.

The influx of somatic cells into the udder after experimental IMI was evaluated graphically. Statistical testing of observed differences in speed of influx was done using a *t* test on log-transformed SCC.

RESULTS

The two parameters for severity of IMI, AUC of bacterial count and RMP, differentiated between the more diseased and less diseased cows. The AUC ranged from 151 to 872 cfu/h, and RMP ranged from 6 to 105% of baseline. The AUC of colony-forming units in the milk and RMP showed a high correlation (r = –0.94; $P = 0.001$), and no outliers were observed (Figure 1).

Results of preinfection leukocyte function tests are presented in Table 1. Assuming that measurements on d 0 should have the highest predictive value because they were determined on the day of inoculation, correlation coefficients between the days before inoculation and d 0 are shown in Table 2. For each function test, d –6 to d –1 was positively correlated with d 0. Chemotaxis showed the lowest correlation, and phagocytosis showed the highest correlation. Because of the large random fluctuation of the under agarose chemotaxis assay, adjusted least squares means were calculated with the Markov model of the first order over 7 consecutive d (26). Chemotactic values used in

TABLE 1. Means and standard deviations of chemotaxis, phagocytosis, oxidative burst, and expression of CD11/CD18 receptors from d –6 to d 0 (n = 12).

	Preinfection leukocyte function test											
Day	Chemotaxis		Phagocytosis		Oxidative burst		CD11a Expression		CD11b Expression		CD11c Expression	
	(mm)		(% of neutrophils)		(nmol of H_2O_2)		(mean fluorescence intensity)					
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
–6	3.7	0.9	ND[1]	ND	4.2	0.9	9.3	1.3	12.3	1.8	4.3	1.7
–5	2.5	0.9	62.6	7.3	ND	ND	ND	ND	ND	ND	ND	ND
–4	4.7	0.7	66.6	8.1	3.8	0.8	8.9	1.3	12.8	2.5	4.1	1.4
–3	3.7	0.9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
–2	3.9	0.7	60.1	6.6	3.0	0.7	8.6	1.6	12.8	1.9	4.0	1.1
–1	3.4	0.8	66.2	6.0	3.4	0.7	9.3	2.1	13.3	1.4	4.0	0.8
0	3.1	0.7	64.1	7.2	4.0	0.8	9.5	1.6	14.9	3.3	4.0	0.8

[1]Not determined.

the regression model were adjusted least squares means.

The number of peripheral circulating leukocytes from d –6 to just prior to inoculation and correlation coefficients between the days prior to inoculation and d 0 are presented in Table 3. Days –5 to d –1 were highly and positively correlated with d 0. Regression an-alyses with the two different outcome variables and the preinfection leukocyte function tests were executed. We started with the simple model in which parity category was the only independent variable (Table 4). Parity category accounted for 75 and 56% of the variation in AUC and RMP, respectively. The model was expanded by adding the preinfection parameters, individually and per day. Oxidative burst and expression of CD11/CD18 receptors did not improve the predictive capacity with either AUC or RMP as the outcome variable. Expansion of the AUC model with adjusted least squares means of chemotaxis as a single indicator slightly increased R^2 to 0.79 (Figure 2), and, with chemotaxis as an interaction term, R^2 increased to 0.80. The RMP model with chemotaxis as a single indicator increased R^2 to 0.63, and, with chemotaxis as an interaction term, R^2 was 0.65. The predictive value of phagocytosis and killing on the severity of IMI was small and not statistically significant. As a single indicator, the number of circulating peripheral leukocytes before inoculation predicted the

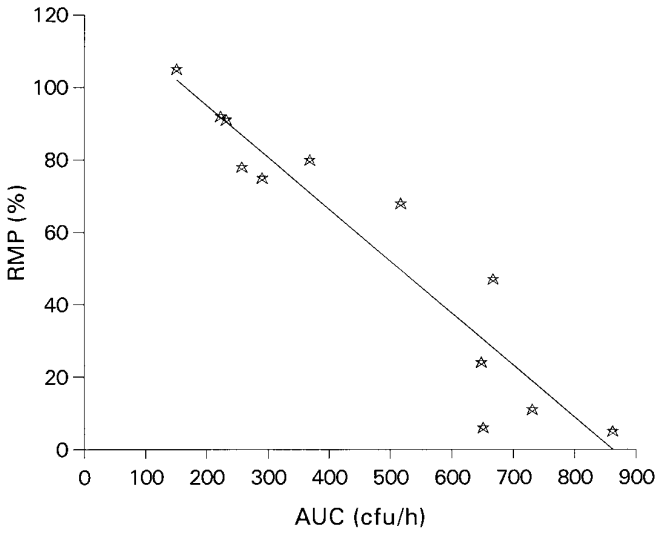

Figure 1. Relationship between area under the curve (AUC) of bacterial count and residual milk production (RMP).

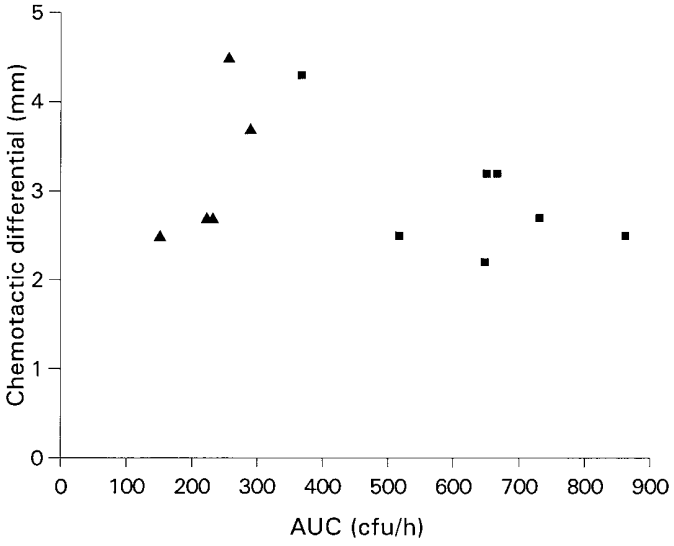

Figure 2. Relationship between adjusted least squares means of chemotactic differential and area under the curve (AUC) of bacterial count for second parity cows (▲; n = 5) and cows in parity ≥4 (■; n = 7).

TABLE 2. Correlation coefficients between d 0 and the days prior to inoculation of chemotaxis, phagocytosis, oxidative burst, and expression of CD11/CD18 receptors (n = 12).

	Preinfection leukocyte function test					
Day	Chemotaxis	Phagocytosis	Oxidative burst	CD11a Expression	CD11b Expression	CD11c Expression
–6	0.53	ND[1]	0.30	0.73	0.69	0.24
–5	0.75	0.50	ND	ND	ND	ND
–4	0.66	0.86	0.70	0.78	0.89	0.33
–3	0.59	ND	ND	ND	ND	ND
–2	0.35	0.84	0.85	0.47	0.80	0.68
–1	0.05	0.77	0.84	0.46	0.31	0.92

[1]Not determined.

AUC for 52% of the IMI and the RMP for 45% of the IMI ($P < 0.001$). Expansion of the model with parity category increased R^2 to 0.78 and 0.62 for AUC and RMP, respectively. Because of the strong effect of parity on the severity of IMI, we analyzed differences of the preinfection parameters between second parity cows and older cows (Table 5). In this experiment, second parity cows had more circulating leukocytes just prior to inoculation (7.8×10^9/L) than did the older cows (6.1×10^9/L) ($P < 0.05$). Figure 3 shows the rapidity of the increase in SCC in the infected quarter and the decrease of peripheral leukocytes for the first 24 h after inoculation for second parity cows and for older cows. For both parity categories, maximum values of SCC were reached 12 h after inoculation. Although growth of *E. coli* was slower (Figure 4) and IMI was less severe in second parity cows than in cows in parity ≥4, severity was not correlated with a more rapid increase in SCC ($P > 0.05$). The drainage of number of peripheral leukocytes was calculated by subtracting the lowest number of leukocytes of each cow from the number of leukocytes just prior to inoculation. This drainage was higher for second parity cows than for older cows, 6.4×10^9 and 4.9×10^9 leukocytes/L, respectively ($P < 0.05$). Expression of adhesion receptors CD11b/CD18 and CD11c/CD18 was different for second parity cows and cows in parity ≥4. Expression of CD11b/CD18 was lower in second parity cows, and expression of CD11c/CD18 was higher in second parity cows than in cows in parity ≥4 ($P < 0.05$).

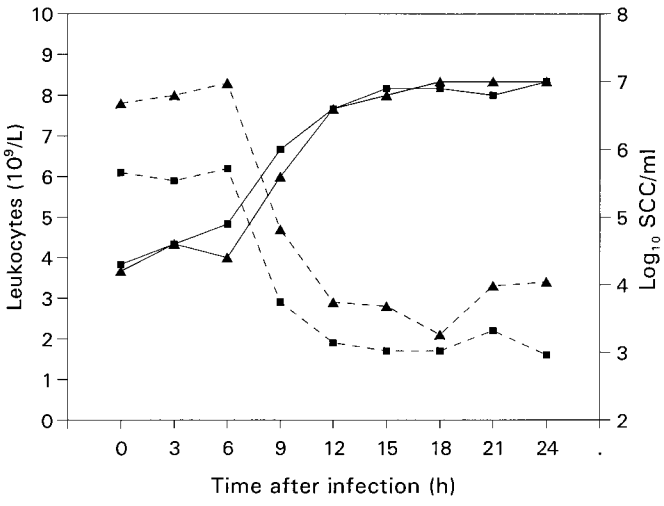

Figure 3. Course of SCC (—) in the infected quarter and peripheral leukocytes (- - -) for second parity cows (▲; n = 5) and cows in parity ≥4 (■; n = 7) during the first 24 h after infection.

DISCUSSION

In this experiment, both RMP of the uninfected quarters and AUC of colony-forming units in the milk of the infected quarter were evaluated as parameters for the severity of IMI. Both parameters were highly correlated; more colony-forming units of *E. coli* in the infected quarter resulted in a greater decrease in milk production in the uninfected quarters. However, results from the regression models with the two outcome parameters showed some differences. The

TABLE 3. Means and standard deviations of circulating peripheral leukocytes from d –6 to just prior to inoculation and correlation coefficients between d 0 and the days before inoculation (n = 12).

Day	Peripheral circulating leukocytes		r
	— (10^9 cells/L) —		
	$\bar{X}$	SD	
–6	6.5	1.3	0.47
–5	6.6	1.1	0.74
–4	7.2	1.2	0.81
–3	7.1	1.3	0.68
–2	7.4	1.5	0.83
–1	7.0	1.2	0.81
0	6.8	1.2	1.00

TABLE 4. Results of the regression of the area under the curve (AUC) and the residual milk production (RMP) against parity category.

	AUC[1]			RMP[2]		
Parameter	Coefficient	SE	*P*	Coefficient	SE	*P*
Intercept	230.6	57.0	0.002	88.6	11.2	0.000
Parity	405.7	74.7	0.000	–54.1	14.7	0.004

[1]R^2 = 0.75.

[2]R^2 = 0.56.

predictive capacity of the leukocyte function tests were higher in the AUC models than in the RMP models. The AUC of colony-forming units in the infected quarter might be closely related to influx of neutrophils into milk (19), but the loss of milk production in the uninfected quarters might be caused by suppression of milk secretion because of general illness and toxin resorption (22).

The importance of the number of circulating leukocytes just prior to IMI is in accordance with results of Kremer et al. (14). A larger pool of circulating leukocytes did not mean a faster migration of leukocytes into the mammary gland in this experiment. Although fast migration of leukocytes is considered to be the most important step in the defense cascade against *E. coli* infections and determines the outcome of the IMI (10, 12), no correlation was found between the severity of IMI and the rapidity of SCC increase.

Of all preinfection leukocyte function tests, chemotaxis had the best predictive capacity. The effect of parity on the outcome of the IMI was very strong. Increasing risk for developing mastitis with each subsequent parity has been shown before. Gilbert et al. (8) reported that the degree of periparturient impairment in neutrophil function was more severe for cows in parity ≥4 than for younger cows. In our data, the only significant differences between second parity cows and older cows were number of peripheral circulating leukocytes and expression of CD11b/CD18 and CD11c/CD18 receptors. The effect of parity appeared to be rather pathogen-specific. Older cows have been shown to be more susceptible to coliform mastitis, and heifers typically have a relatively high incidence of staphylococcal mastitis (11, 16).

Both CD11b/CD18 and CD11c/CD18 contribute to leukocyte adherence reactions, which are active in inflammation and host defense (13, 24), but the precise relationship of these two β2 integrins to the severity of coliform IMI is not clearly understood. Emigration of neutrophils into extravascular inflammatory sites requires binding of β2 integrins to neutrophil surfaces with the intracellular adhesion molecule-1 expressed on vascular endothelium (17, 24). In our data, expression of adhesion receptors as a single indicator was not correlated with severity of IMI. After data were analyzed for the two parity categories, cows that had higher expression of CD11c/

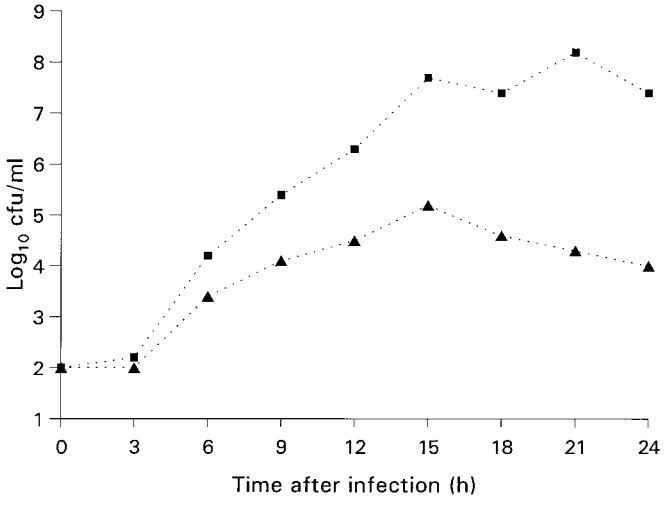

Figure 4. Course of bacterial count in the infected quarter for second parity cows (▲; n = 5) and cows in parity ≥4 (■; n = 7) during the first 24 h after infection.

TABLE 5. Differences between second parity cows (n = 5) and cows in parity ≥4 (n = 7) on d 0 for peripheral circulating leukocytes and mean fluorescence intensity (MFI) of adhesion receptor expression.

	Parity				
Parameter	2		≥4		*P*
	$\bar{X}$	SD	$\bar{X}$	SD	
Leukocytes, 10^9 cells/L	7.8	1.38	6.1	0.51	0.02
CD11b/CD18 Expression, MFI	13.0	1.51	16.3	3.56	0.05
CD11c/CD18 Expression, MFI	4.6	0.71	3.6	0.90	0.05

CD18 but a lower expression of CD11b/CD18 were typically younger cows that showed less severe disease. The impact of these findings requires further study and might be biased by parity.

Apart from aspects that inform about the quality of leukocyte function in our data, quantitative aspects of leukocyte presence in the peripheral bloodstream or drainage into the mammary gland appeared to be important in the prediction of severity of experimental *E. coli* IMI. In retrospect, these findings might be in agreement with those of previous experiments (9, 14, 15) in which leukocyte count was evaluated but not always statistically modeled in the final prediction equations. A retrospective analysis of previously published data might be of great interest.

From this study, we concluded that chemotaxis and parity have a great influence on the outcome of the severity of an experimental *E. coli* IMI. The importance of the number of peripheral leukocytes requires further study, but promises to be of great value, especially because leukocyte counts may be obtained in large quantities under field conditions. Longitudinal studies of dairy farms with a high incidence of clinical mastitis (4) would become feasible using such parameters and would also allow a more in-depth analysis of the role of aging in the susceptibility for clinical coliform mastitis.

REFERENCES

1 Arfors, K., C. Lundberg, L. Lindbom, K. Lundberg, P. G. Beatty, and J. M. Harlan. 1987. A monoclonal antibody to membrane glycoprotein complex CD18 inhibits polymorphonuclear leukocyte accumulation and plasma leakage in vivo. Blood 69:338.

2 Burton, J. L., M. E. Kehrli, S. Kapil, and R. L. Horst. 1995. Regulation of L-selectin and CD18 on bovine neutrophils by glucocorticoids: effect of cortisol and dexamethasone. J. Leukocyte Biol. 57:317.

3 Burvenich, C., A. J. Guidry, and M. J. Paape. 1995. Natural defense mechanisms of the lactating and dry mammary gland. Page 3 *in* Proc. 3rd Int. Mastitis Sem., Tel Aviv, Israel. A. Saran and S. Soback, ed. M. Lachmann Printers Ltd., Haifa, Israel.

4 Cai, T. Q., P. G. Weston, L. A. Lund, B. Brodie, D. I. Mckenna, and W. C. Wagner. 1994. Association between neutrophil functions and periparturient disorders in cows. Am. J. Vet. Res. 55: 934.

5 Caroll, E. J., R. Mueller, and L. Panico. 1982. Chemotactic factors for bovine leukocytes. Am. J. Vet. Res. 43:1661.

6 Chenoweth, D. E., J. G. Rove, and T. E. Hugli. 1979. A modified method for chemotaxis under agarose. J. Immunol. Methods 24: 337.

7 Gelfand, J. A., A. S. Fauci, I. Green, and M. M. Frank. 1976. A simple method for the determination of complement receptor-bearing mononuclear cells. J. Immunol. 116:595.

8 Gilbert, R. O., Y. T. Gröhn, P. M. Miller, and D. J. Hoffman. 1993. Effect of parity on periparturient neutrophil function in dairy cows. Vet. Immunol. Immunopathol. 36:75.

9 Heyneman, R., C. Burvenich, and R. Vercauteren. 1990. Interaction between the respiratory burst activity of neutrophil leukocytes and experimentally induced *Escherichia coli* mastitis in cows. J. Dairy Sci. 73:985.

10 Hill, A. W. 1981. Factors influencing the outcome of *Escherichia coli* mastitis in the dairy cow. Res. Vet. Sci. 31:107.

11 Houben, E.H.P. 1995. Economic optimization of decisions with respect to dairy cow health management. Ph.D. Diss., Univ. Wageningen, Wageningen, The Netherlands.

12 Kehrli, M. E., B. J. Nonnecke, and J. A. Roth. 1989. Alterations in bovine peripheral blood neutrophil function during the periparturient period. Am. J. Vet. Res. 50:207.

13 Kehrli, M. E., and D. E. Shuster. 1994. Factors affecting milk somatic cells and their role in health of the bovine mammary gland. J. Dairy Sci. 77:619.

14 Kremer, W.D.J., E. N. Noordhuizen-Stassen, F. J. Grommers, A.J.J.M. Daemen, P.A.J. Henricks, C. Burvenich, and A. Brand. 1993. Preinfection chemotactic response of blood polymorphonuclear leukocytes to predict severity of *Escherichia coli* bovine mastitis. J. Dairy Sci. 76:2414.

15 Lohuis, J.A.C.M., Y. H. Schukken, P.A.J. Henricks, R. Heyneman, C. Burvenich, J.H.M. Verheyden, A.S.J.P.A.M. van Miert, and A. Brand. 1990. Preinfection functions of blood polymorphonuclear leucocytes and the outcome of experimental *Escherichia coli* mastitis in the cow. J. Dairy Sci. 73:342.

16 Myllys, V., T. Honkananen-Buzalski, S. Pyörälä, and H. Rautala. 1995. Clinical heifer mastitis. Page 18 *in* Proc. 3rd Int. Mastitis Sem., Tel Aviv, Israel. A. Saran and S. Soback, ed. M. Lachmann Printers Ltd., Haifa, Israel.

17 Nagahata, H., H. Nochi, K. Tamoto, H. Noda, and G. J. Kociba. 1995. Expression and role of adhesion molecule CD18 on bovine neutrophils. Can. J. Vet. Res. 59:1.

18 Paape, M. J., A. J. Guidry, N. C. Jain, and R. H. Miller. 1991. Leukocytic defence mechanisms in the udder. Flem. Vet. J. 62(Suppl. 1):95.

19 Rainard, P. 1985. Assessment of attachment, ingestion, and killing of *Escherichia coli* by bovine polymorphonuclear cells with combined micromethods. Vet. Immunol. Immunopathol. 10:155.

20 Saad, A. M., and M. Hageltorn. 1985. Flow cytometric characterization of bovine blood neutrophil phagocytosis of fluorescent bacteria and zymozan particles. Acta Vet. Scand. 26:289.

21 Schukken, Y. H., F. J. Grommers, D. van de Geer, and A. Brand. 1989. Incidence of clinical mastitis on farms with low somatic cell counts in bulk milk. Vet. Rec. 124:60.

22 Shuster, D. E., R. J. Harmon, J. A. Jackson, and R. W. Hemken. 1991. Suppression of milk production during endotoxin-induced mastitis. J. Dairy Sci. 74:3763.

23 Simpson, P. J., I.R.F. Todd, J. C. Fantone, J. K. Mickelson, J. D. Griffin, and B. R. Lucchesi. 1988. Reduction of experimental canine myocardial reperfusion injury by a monoclonal antibody (anti-Mo1, anti-CD11b) that inhibits leukocyte adhesion. J. Clin. Invest. 81:624.

24 Smith, C. W., S. D. Marlin, R. Rothlein, C. Toman, and D. C. Anderson. 1989. Cooperative interactions of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence and transendothelial migration of human neutrophils in vitro. J. Clin. Invest. 83:2008.

25 Splitter, G., and W. I. Morrison. 1991. Antigens expressed predominantly on monocytes and granulocytes: identification of bovine CD11b and CD11c. Vet. Immunol. Immunopathol. 27:87.

26 van Werven, T., J. van den Broek, E. N. Noordhuizen-Stassen, A.J.J.M. Daemen, and A. Brand. 1995. Estimating the immune state of cows based on results of sequential chemotaxis assays. Page 71 *in* Proc. 3rd Int. Mastitis Sem., Tel Aviv, Israel. A. Saran and S. Soback, ed. M. Lachmann Printers Ltd., Haifa, Israel.