

Blood Polymorphonuclear Leukocyte Chemotaxis During Experimental *Escherichia coli* Bovine Mastitis

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ABSTRACT

The relationship between the severity of experimental *Escherichia coli* mastitis and the chemotactic response of blood polymorphonuclear leukocytes was investigated before and during mastitis. Experimental *E. coli* mastitis was induced in 10 healthy cows by inoculation of the rear right quarters with 10^3 cfu of *E. coli*. Cows were classified into two groups based on the severity of the mastitis. Bacterial growth in the inoculated quarter was used as parameter that indicated severity.

Before and during experimental mastitis, the chemotactic response and the number of circulating polymorphonuclear leukocytes were greater for the moderately diseased cows than for the severely diseased cows. During the first 24 h of the experimental mastitis, the chemotactic response of polymorphonuclear leukocytes decreased in both groups. Recovery of the chemotactic response of white blood cells was more rapid in moderately diseased cows than in severely diseased cows. Possibly, the larger proportion of band neutrophils (the less chemotactically active band neutrophils) partially accounts for the lower chemotactic response of the circulating

polymorphonuclear leukocytes during experimental mastitis in the severely diseased cows.

(Key words: polymorphonuclear leukocytes, chemotactic response, *Escherichia coli* mastitis)

Abbreviation key: AUC = area under the curve, PMNL = polymorphonuclear leukocytes, WBC = white blood cells.

INTRODUCTION

Clinical signs of *Escherichia coli* mastitis show great variability among cows. Variation in functional activity of circulating polymorphonuclear leukocytes (PMNL) has been associated with differences in mastitis susceptibility among individual cows or herds (4, 6, 10, 11, 13, 14, 16). Only a few studies exist concerning the relationship between in vitro functional activity of bovine blood PMNL before and during experimental mastitis and the severity of the disease (5, 11). The correlation was negative between the capacity of blood neutrophils to generate reactive oxygen species before and during experimental *E. coli* mastitis and the severity of the subsequent mastitis (5). Recently, a relationship has been described between the chemotactic response of circulating PMNL before experimental mastitis and the severity of the disease (11).

Migration of PMNL from the blood into the udder is thought to be of major importance in host defense against *E. coli* intramammary infections in cows (2, 5, 6, 7, 9, 18). During bacterial and viral infections, in vitro

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chemostatic response of circulating PMNL can be depressed (1, 3, 8, 13, 15). It is not known whether the in vitro chemostatic response of PMNL during experimental *E. coli* mastitis is depressed or whether the chemotactic response is related to the severity of disease. The objective of this study was to investigate the relationship between the severity of experimental *E. coli* mastitis and the chemotactic response of blood PMNL in vitro during the disease.

MATERIALS AND METHODS

Cows

Ten clinically healthy dairy cows of the Dutch Friesian breed or crossbreeds (Dutch Friesian × Holstein-Friesian) in their third to sixth lactation and wk 3 to 6 of lactation were used. All cows had calved normally, and, from parturition until inoculation, no clinical signs of periparturient disease or mastitis existed. Foremilk samples from the inoculated (right rear) quarters and the control (left rear) quarters were bacteriologically negative for major pathogens, coagulase-negative staphylococci, or *Corynebacterium bovis* at 2 wk before inoculation and at inoculation. Before inoculation, mean total daily milk production was 26.7 ± 5.6 (SE) kg. The SCC of these quarters were below 250,000 cell/ml. Cows were housed in a tie-stall barn and fed wilted grass silage and concentrates according to milk production. Water was provided for ad libitum intake. Milkings were at 0100 and 1400 h.

Experimental Procedures

Experimental *E. coli* mastitis was induced by aseptic infusion of the rear right quarters with 10^3 cfu of *E. coli* O:157, suspended in 20 ml of pyrogen-free saline at 0700 h (14). The infused quarters were not milked at the first milking postinoculation (1400 h). The *E. coli* colony-forming units were counted in foremilk samples from the inoculated quarter; samples were collected aseptically at 8, 15, 32, 46, 54, 72, 104, and 120 h postinoculation. Bacterial counts were determined using a spiral plater (Lameris Laboratory, Breukelen, The Netherlands) on violet red bile glucose agar CM 484 (Oxoid, Basingstoke, Hampshire, England).

Rectal temperature, heart rate, and quarter milk production were recorded as described

(11). At d 6, 7, and 8 postinoculation, all cows were treated (11).

Bacterial growth in the inoculated quarter was used to classify the severity of the disease because this bacterial growth was directly proportional to the variation in clinical signs of the experimental mastitis (6, 7, 11, 14). To relate the chemotactic response of blood PMNL to the severity of the experimental mastitis, cows were divided into two artificial groups: severely or moderately diseased cows. The 5 cows with the highest bacterial growth in the inoculated quarter were considered to be severely diseased. The 5 cows with the lowest bacterial growth were considered to be moderately diseased.

Blood Sampling Schedule and Analysis

Jugular vein puncture blood samples for chemotaxis assay cell isolation were collected as described (12) at 48 and 24 h prior to inoculation; immediately prior to inoculation; and at 24, 48, and 120 h postinoculation. Chemotaxis of PMNL was measured in white blood cell (WBC) suspensions and in purified PMNL suspensions (consisting of immature band and segmented neutrophils and eosinophils). For total and differential leukocyte counts, coccygeal vein puncture blood samples were collected in vacutainer tubes (Venoject® Terumu Corp., Tokyo, Japan) containing heparin as anticoagulant. Samples were collected at 120, 48, 24, 12, and 3 h before inoculation; immediately prior to inoculation; and at 3, 6, 9, 12, 18, 24, 27, 32, 36, 48, and 120 h postinoculation. Leukocyte counts were determined by use of an automatic cell counter (Sysmex K-1000; Goffin, IJsselstein, The Netherlands). Smears were prepared from whole blood and stained by using the May-Grünwald-Giemsa method. Differential counts were determined by counting 100 cells under a microscope.

WBC Isolation

The WBC suspensions and purified PMNL suspensions were obtained as described previously (12). Blood samples were centrifuged for 20 min at $1000 \times g$ (4°C). The plasma layer was discarded during WBC isolation. Plasma and the buffy coat layer were discarded during isolation of purified PMNL suspensions.

Erythrocytes were lysed in two steps by hypotonic lysis (12). After being washed twice in Eagle's minimal essential medium (Flow Laboratories, Irvine, England), the cells were resuspended in Eagle's minimal essential medium and adjusted to 5×10^7 cells/ml.

Chemotaxis Assay

The *in vitro* chemotactic response of WBC suspensions or purified PMNL suspension was determined using the under agarose technique (17) as previously described (11). Results were expressed as chemotactic differential or chemotactic index (17). Results of five observations per sample are averaged.

Statistical Analysis

Baseline values of the chemotactic response were defined as the mean chemotactic response at 48 and 24 h prior to inoculation and immediately prior to inoculation.

Bacterial growth in the infected right quarters was expressed as the area under the curve (AUC) (\log_{10} *E. coli* colony-forming units per hour during the first 120 h postinoculation). The AUC colony-forming units per hour was calculated for each cow using the following equation:

$$\text{AUC} = [(t_i - t_{i-1})f_{i-1}] + [.5 (t_i - t_{i-1})(f_i - f_{i-1})]$$

where

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

Means and standard deviations were computed, and means were compared by paired *t* tests or two sample *t* tests using a microcomputer package (Statistix[®] NH Analytical Software, Roseville, MN).

RESULTS

Course of Experimentally Induced *E. coli* Mastitis

The AUC was used to classify the severity of the experimental mastitis. The AUC ranged

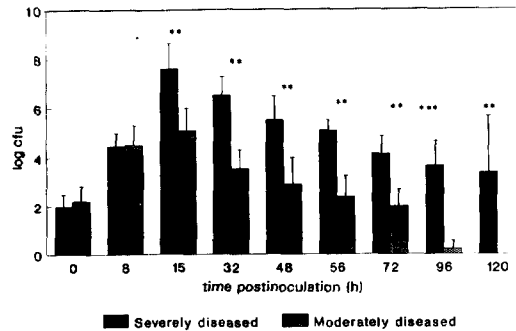


Figure 1. Mean number (\pm SE) of colony-forming units during experimental *Escherichia coli* mastitis in moderately and severely diseased cows. * $P < .05$, ** $P < .01$, and *** $P < .001$.

from 199 to 690 cfu/h ($n = 10$). The mean AUC of the 5 cows in the severely diseased group and of the 5 cows in the moderately diseased group were 607 ± 71 and 285 ± 57 \log_{10} cfu/h, respectively. Bacterial growth and clearance in both groups are presented in Figure 1. The peak for \log_{10} *E. coli* colony-forming units was significantly higher ($P < .001$) for foremilk samples from the inoculated quarter of cows in the severely diseased group than for foremilk samples from the moderately diseased cows ($7.93 \pm .95$ and $5.88 \pm .91$ \log_{10} cfu, respectively). At 120 h postinoculation, the number of *E. coli* in foremilk samples taken from the inoculated quarters of the severely diseased cows ranged from 1.4×10^3 to 3.8×10^8 cfu/ml. In contrast, all foremilk samples from the inoculated quarters of moderately diseased cows were bacteriologically negative at 120 h postinoculation. At d 5 postinoculation, 1 cow in the severely diseased group developed acute necrotic *Staphylococcus aureus* mastitis in the right front quarter and was euthanized. All data obtained from this cow until her death were used.

Leukocyte Counts in the Peripheral Blood

The mean number of circulating leukocytes and neutrophils in severely and moderately diseased cows before inoculation and during experimental mastitis is presented in Figures 2 and 3. Before inoculation, the mean number of

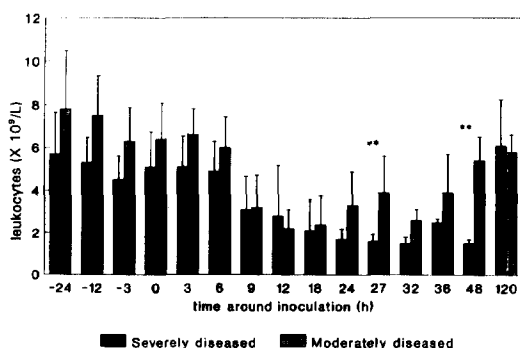


Figure 2. Mean number (\pm SE) of circulating leukocytes before and during experimental *Escherichia coli* mastitis in moderately and severely diseased cows. $**P < .01$.

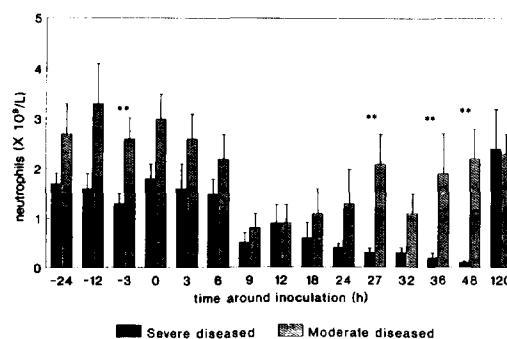


Figure 3. Mean number (\pm SE) of circulating neutrophils (band and segmented) before and during experimental *Escherichia coli* mastitis in moderately and severely diseased cows. $**P < .01$.

circulating neutrophils was higher in the moderately diseased cows than in the severely diseased cows (Figure 3). In both groups, the number of circulating leukocytes and neutrophils decreased significantly ($P < .01$) during the first 9 h of infection. From 12 to 48 h postinoculation, the mean number of circulating leukocytes and neutrophils increased significantly ($P < .01$) in the moderately diseased cows. In the severely diseased cows, the mean number of circulating leukocytes and neutrophils remained low. At 120 h postinoculation, the mean number of circulating leukocytes and neutrophils reached preinoculation concentrations in both groups. The mean number of band neutrophils was not significantly different in either group up to 18 h postinoculation. At 24 and 120 h postinoculation, the percentage of band neutrophils in the severely diseased cows was significantly higher ($P < .05$) than that in the moderately diseased cows (Figure 4). The number of circulating eosinophils and lymphocytes was not different in either group during the experimental mastitis.

Course of Chemotactic Response During Experimental Mastitis

Changes in the chemotactic differential, in the chemotactic index of WBC suspensions, and in purified PMNL suspensions before and during experimental mastitis were in the same range. Therefore, only the changes of the

chemotactic differential of WBC suspension are presented (Figure 5). Chemotactic response of WBC prior to inoculation was higher in the moderately diseased cows than in the severely diseased cows (Figure 5). In both groups, the chemotactic response of WBC suspension decreased within the first 24 h of the experimental mastitis. In the moderately diseased cows, the chemotactic response recovered to a mean that was 34 and 83% of baseline values at 48 and 120 h postinoculation, respectively. In the severely diseased cows, the chemotactic response of WBC suspension recovered slightly but remained relatively low up to 120 h postinoculation (average of 24% of baseline values at 120 h postinoculation).

DISCUSSION

Before and during the experimental *E. coli* mastitis, the chemotactic responsiveness of circulating PMNL was higher for the moderately diseased cows than for the severely diseased cows. The pattern was similar for the respiratory burst activity of circulating PMNL before and during experimental mastitis (5).

During the first stages of the experimental mastitis, the chemotactic response of WBC decreased in both groups (Figure 5), which is in accord with results from other studies (3, 8, 13, 15). In the present study, the chemotactic response of WBC recovered more rapidly in moderately diseased cows than in severely diseased cows (Figure 5). Because immature band neutrophils were less chemotactically active

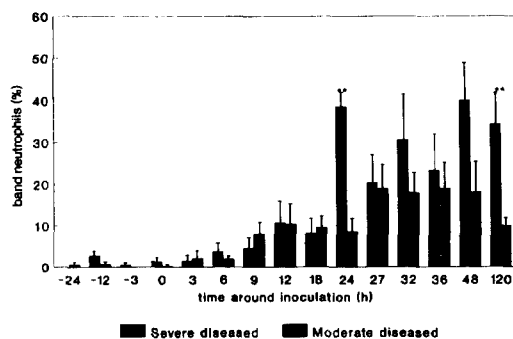


Figure 4. Mean number (\pm SE) of circulating band neutrophils (expressed as a percentage of total number of circulating neutrophils) before and during experimental *Escherichia coli* mastitis in moderately and severely diseased cows. $^{***}P < .01$.

(8), the larger proportion of circulating band neutrophils in the severely diseased cows could partially account for the lower chemotactic response of the circulating PMNL during the experimental mastitis in the severely diseased cows.

The number of circulating neutrophils was higher in the moderately diseased cows than in the severely diseased cows before and during experimental mastitis (Figure 3). In another study, a relationship was also demonstrated between the number of circulating PMNL and increased incidence of postpartum disease in cows (19). However, neutrophil functions were not investigated in the latter study.

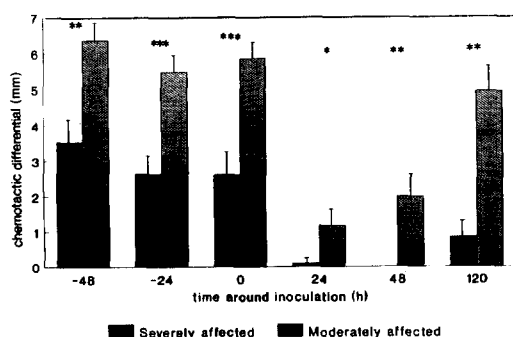


Figure 5. Mean of chemotactic differential of white blood cells (\pm SE) before and during experimental *Escherichia coli* mastitis in moderately and severely diseased cows. $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$.

CONCLUSIONS

Before induction of the experimental *E. coli* mastitis, the number of circulating PMNL and the chemotactic response of these cells were greater for the moderately diseased cows than for the severely diseased cows. During the first stages of the experimental mastitis, the chemotactic response of WBC decreased in both groups. Recovery of the chemotactic response of WBC was more rapid in moderately diseased cows than in severely diseased cows.

REFERENCES

- 1 Brown, G. B., S. R. Bolin, D. E. Frank, and J. A. Roth. 1991. Defective function of leukocytes from cattle persistently infected with bovine viral diarrhoea virus, and the influence of recombinant cytokines. *Am. J. Vet. Res.* 52:381.
- 2 Burvenich, C., R. Heyneman, G. VandePutte-vanMessom, A. M. Massart-Leën, and E. Roets. 1989. Differences in local and systemic reactions during severe and moderate cases of *Escherichia coli* mastitis in cattle soon after parturition. Page 20 in *Proc. Int. Conf. Mastitis*, Vienna, Austria, May 29–June 2.
- 3 Ellis, M., S. Gupta, S. Galant, S. Hakim, C. VandeVen, C. Toy, and M. S. Cairo. 1988. Impaired neutrophil function in patients with AIDS or AIDS-related complex: comprehensive evaluation. *J. Infect. Dis.* 158:1268.
- 4 Grommers, F. J., D. van de Geer, H. van der Vliet, P.A.J. Henricks, and F. P. Nijkamp. 1989. Polymorphonuclear leucocyte function: relationship between induced migration into the bovine mammary gland and in vitro cell activity. *Vet. Immunol. Immunopathol.* 23:75.
- 5 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.
- 6 Hill, A.W., 1981. Factors influencing the outcome of *Escherichia coli* mastitis in the dairy cow. *Res. Vet. Sci.* 31:107.
- 7 Hill, A.W., A. L. Shears, and K. G. Hibbit, 1979. The pathogenesis of experimental *Escherichia coli* mastitis in newly calved cows. *Res. Vet. Sci.* 26:97.
- 8 Jain, N. C., M. J. Paape, L. Berning, S. K. Salgar, and M. Worku. 1991. Functional competence and monoclonal antibody reactivity of neutrophils from cows injected with *Escherichia coli* endotoxin. *Comp. Hematol. Int.* 1:10.
- 9 Jain, N. C., O. W. Schalm, and E. J. Carroll. 1968. Experimental mastitis in leukopenic cows: immunologically induced neutropenia and response to intramammary inoculation of *Aerobacter aerogenes*. *Am. J. Vet. Res.* 29:2089.
- 10 Kehrli, M. E., B. J. Nonnecke, and J. A. Roth. 1989. Alterations in bovine neutrophil function during the periparturient period. *Am. J. Vet. Res.* 50:207.
- 11 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 as a predictor of the severity of experimental *Escherichia coli* bovine mastitis. *J. Dairy Sci.* 76: 2514.
- 12 Kremer, W.D.J., E. N. Noordhuizen-Stassen, P.A.J. Henricks, and H. VanderVliet. 1992. A procedure for parallel isolation of white blood cells and purified neutrophil suspensions from the peripheral blood of cattle. *Vet. Immunol. Immunopathol.* 31:189.
- 13 Löfstadt, J., J. A. Roth, R. F. Ross, and W. C. Wagner. 1983. Depression of polymorphonuclear leukocyte function associated with experimentally induced *Escherichia coli* mastitis in sows. *Am. J. Vet. Res.* 44:1224.
- 14 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 leukocytes and the outcome of experimental *Escherichia coli* mastitis in the cow. *J. Dairy Sci.* 73:342.
- 15 Mowat, A. G., and J. Baum. 1971. Polymorphonuclear leukocyte chemotaxis in patients with bacterial infections. *Br. Med. J.* 3:617.
- 16 Nagahata, H., S. Makino, S. Takeda, H. Takahashi, and H. Noda. 1988. Assessment of neutrophil function in the dairy cow during the perinatal period. *J. Vet. Med.* B35:747.
- 17 Nelson, R. D., P. G. Quie, and R. L. Simmons. 1975. Chemotaxis under agarose: a new and simple method for measuring chemotaxis of and spontaneous migration of human polymorphonuclear leukocytes and monocytes. *J. Immunol.* 115:1650.
- 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 Reid, I. M., A. M. Dew, and L. A. Williams. 1984. Haematology of subclinical fatty liver in dairy cows. *Res. Vet. Sci.* 37:63.