Changes in Blastogenic Response of Bovine Lymphocytes during Acute Clinical Mastitis

Hajime NAGAHATA, Hiroshi NODA, and Tekehiko ABE¹⁾

Department of Animal Health, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069, and

¹⁾Hokkaido Agricultural Mutual Relief Association, Higashi Chitose, Hokkaido 069-11, Japan

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It is well known that immediate postpartum cows are highly susceptible to peracute mastitis compared with those of mid and late lactational period [3, 4]. One explanation is that the host defense mechanisms may be depressed during the immediate postpartum period [3]. The lymphocyte functions in relation to the pathogenesis of bovine mastitis are still poorly understood [10]. The blastogenic response of lymphocytes is commonly used as a measure of the immune response capability [6]. There seem to be few studies on the blastogenic response of lymphocytes from cows during the course of naturally occurring clinical mastitis. To clarify the characteristics of lymphocyte functions in mastitis, the changes of the blastogenic response of lymphocytes from mastitic cows during the course of inflammation were examined.

A total of 10 lactating Holstein cows in 1-2 postpartum months of lactation and with naturally occurring clinical mastitis were obtained from local dairy farms in Ebetsu, Hokkaido, and used. The most consistent clinical features were loss of appetite, pyrexia and depression. Blood was obtained from the jugular vein and placed in a tube containing heparin. Lymphocytes isolated from blood [8], were washed twice in PBS and finally resuspended in culture medium to give 1×10^6 cells/ml. Lymphocyte blastogenic responses to mitogens were measured according to the method as described previously [8]. Five hundred μ l aliquots of lymphocyte suspension (5×10^5 cells/well in 25 mM Hepes buffered-RPMI 1640 containing 20% fetal calf serum, 200 μ g/ml streptomycin and 200 units/ml penicillin) were plated into each well of 24 flat-bottomed tissue culture plates (Coaster 3424) and 500 μ l of each of the following mitogens were added: concanavalin A (Con A) 5 μ g/ml, phytohemagglutinin-P (PHA) 1 μ l/ml and pokeweed mitogen (PWM) 10 μ g/ml. The cultures were incubated at 37°C in humidified 5% CO₂ for 72 hr. Fluorometric microassay of DNA in cultured lymphocytes was carried out according to the method as described previously [8]. Results were expressed as stimulation index (SI). SI was calculated using the following formula: $SI = (A - C) / (B - C)$, where A is mean fluorescence intensity (FI) with mitogen; B is mean FI without mitogen; C is background FI [Ethidium bromide (EB) and sodium dodecyl sulfate (SDS) solutions were mixed].

In this study, the criteria of clinical mastitis were

based on typical systemic signs, the nature of the secretions obtained from the infected quarters, the degree of udder swelling, results of the California Mastitis Test and N-acetyl-B-D-glucosaminidase (NAGase) activity. These findings were all positive, and the animals were treated with fluid and antibiotics. The first few squirts of milk were discarded and 2-3 ml samples of quarter fore-milk were collected aseptically in sterile plastic tubes before treatment and submitted to the clinical laboratory for bacteriological examinations. For monitoring the mastitic milk, NAGase activity in milk was measured according to the method of Kitchen [5] with slight modifications [9]. NAGase activity was measured by a fluorometric procedure using 4-methylumbelliferyl-N-acetyl-B-D-glucosaminide as substrate. The enzymic reaction was started by adding 30 μ l of a milk sample to a tube containing 200 μ l of 2 mM 4-methylumbelliferyl-N-acetyl-B-D-glucosaminide in 0.25 M citrate buffer (pH4.4), and incubated for 5 min at 37°C. The reaction was terminated by the addition of 5.5 ml of 0.1 M carbonate buffer (pH10), and the amount of released 4-methylumbelliferone was measured at an excitation wavelength of 365 nm and an emission wavelength of 450 nm by a spectrofluorophotometer. The enzyme activity was expressed as nanomoles of liberated 4-methylumbelliferone per minute in 1 ml of milk (nM/min/ml). The plasma cortisol was determined by using an enzyme immunoassay kit (ENDAB, Immunotech, Corp.).

Ten mastitic cows were divided into two groups according to the results of blastogenic responses: group A consisted of 5 cows which showed severe depression of blastogenesis at the time of initial phase of mastitis, and group B consisted of the other 5 cows which showed no characteristic changes of blastogenesis (Fig. 1). Changes in NAGase activity in milk from 10 mastitic cows during the course of clinical mastitis were monitored (Fig. 1). For group A the NAGase activity was 212.8 ± 122.1 (mean $\pm$ SD) nM/min/ml on the first day, and this activity was 2.3-fold higher than that of group B. The activity decreased rapidly after 3-5 days, however, the activity of milk at 9-18 days after the onset of mastitis was retained (37.4 ± 27.0 nM/min/ml) at mastitic levels after adequate antibacterial therapy and treatments. For group B, the general changes of NAGase activity were similar to those of NAGase profile of group A. At 10-23 days after the onset of mastitis, the NAGase activity was restored (14.8 ± 10.7

Fig. 1. Changes in blastogenic response and N-acetyl-B-D-glucosaminidase activity during the course of mastitis. Days after the onset of mastitis as follows: I (onset), II (3 days), III (5–9 days) and IV (10–23 days). A: group A (n=5), B: group B (n=5). Con A (○), PHA (△), PWM (□). The shaded area represents the range of the normal milk. Results were expressed as mean $\pm$ SD. *Significantly different from the values of stages III and IV at $P<0.05$.

nM/min/ml) to almost normal levels (1.9–14.3 nM/min/ml). The results of blastogenic responses of peripheral blood lymphocytes from 10 mastitic cows during the course of acute clinical mastitis were shown in Fig. 1. In group A, blastogenic responses of lymphocytes from cows with the stages I and II against Con A and PHA were significantly decreased ($P<0.05$) compared with those of stages III and IV. The blastogenic responses in group A returned gradually to the normal levels associated with recovering mastitis, as evidenced by NAGase activity. No such changes were observed in group B. Our results were similar to those of the blastogenic responses of blood lymphocytes from cows with experimentally induced staphylococcal mastitis [10]. Changes in the concentration of plasma cortisol in groups A and B during the course of mastitis are shown in Fig. 2. The levels of plasma cortisol at the onset of mastitis in cows of groups A and B were 57.0 ± 22.5 and 30.4 ± 21.1 (mean $\pm$ SD, $n=3$) ng/ml, respectively. Higher cortisol concentration in blood plasma from mastitic cows in groups A and B were recorded within 5–9 days after the onset of mastitis. Mean plasma level of cortisol from cows in group A was 1.9-fold higher than that of group B. Micro-organisms isolated from mastitic milk were *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli*, and cows showing severe mastitis seems to be caused by *Klebsiella pneumoniae* in our present study. The difference of

Fig. 2. Changes in cortisol concentration during the course of mastitis. Figs. 1, II, III and IV see Fig. 1. ●: group A (n=3), ○: group B (n=3). Results were expressed as mean $\pm$ SD.

clinical features between groups A and B may be due to the severity of mastitis, virulence of bacteria, lactational stages and host defense capability. It is well known that some viral, bacterial and metabolic diseases are associated with reduced lymphocyte reactivity to mitogens [6]. The major cause of the suppressed blastogenesis in mastitic cows appears to be related to the elevated level of cortisol concentrations, known as suppressive effect to immune system [11], and other attributable factors may related to chemical mediators such as prostaglandins [2, 7] and acute phase reactants associated with acute inflammation [1]. Reduced blastogenic response of lymphocytes from mastitic cows might indicate a general impairment of lymphocyte function due to severe acute inflammation [10]. This condition might be linked with the pathogenic aspects of mastitis. Further studies are necessary to elucidate the lymphocyte functions in relation to the progress of bovine mastitis.

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要 約

急性臨床型乳房炎経過中におけるリンパ球幼若化反応の推移（短報）：永幡 肇・野田 寛・安倍健彦¹⁾（酪農学園大学家畜衛生学教室¹⁾北海道石狩地区農共家畜診療所）——臨床型乳房炎牛10頭中5頭において、炎症初期にリンパ球幼若化反応の著明な抑制がみられ、乳汁中NAGase活性の低下（回復期）に伴いリンパ球幼若化反応の回復が認められた。また、血漿コルチゾール値は炎症初期に上昇して $57.0 \pm 22.5 \text{ ng/ml}$ (mean $\pm$ SD, n=3) となり、その後漸次減少し10～23日後では $9.9 \pm 3.7 \text{ ng/ml}$ であった。幼若化反応抑制の一因として炎症に関連したコルチゾール濃度の上昇が考えられた。