NOTE *Internal Medicine*

Acute Phase Response in Naturally Occurring Coliform Mastitis

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ABSTRACT. Changes in the activities of serum cytokines and in acute phase response were observed in dairy cows with naturally occurring coliform mastitis. Seven cows with severe mastitis showed systemic and mammary inflammatory response throughout the observation period, and 11 cows with mild mastitis recovered and were able to be milked within 3 days of onset of mastitis. Serum interleukin (IL)-1 and tumor necrosis factor (TNF) activities were higher in the severe group than in the mild group at the first appearance of symptoms. Elevated IL-1 activity was evident in the severe group throughout the observation period. Serum α-1-acidglycoprotein (α1AG) concentration began to rise with the beginning of mastitis in the severe group, and peaked at 9 days. Serum haptoglobin (Hp) concentrations peaked at 3 days, and decreased gradually after 3 days in the severe group. These results showed that there are dynamic changes in serum IL-1 activity and in serum α1AG and Hp concentrations in cows with severe coliform mastitis.

KEY WORDS: acute phase protein, coliform mastitis, cytokine.

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Acute phase response is observed in cows with inflammatory disease such as pneumonia, endometritis, and mastitis. This response is characterized by a number of physiological changes, including fever, leukocytosis, changes in hematologic levels, changes in plasma metal levels, and changes in the levels of hepatocyte-derived plasma proteins, and acute-phase protein (APP) [3, 7, 13].

Cytokines are known to be the pivotal mediators of acute phase response in infectious diseases [2]. Previous experimental and clinical studies of coliform mastitis have characterised the roles of IL-1, IL-6, and TNF in cows [11, 12, 14]. However, the immunological response has not yet been elucidated in a clinical study. The purpose of this study was to examine the serum cytokines activities and acute phase proteins in cows in naturally occurring cases of coliform mastitis.

Eighteen cows showing clinical signs of coliform mastitis were used in this study. All of the cows had been treated with antibiotic drugs such as aminoglycoside and fluid therapy. The cows were divided into two groups: a severe mastitis group (Group 1, n=7), and a mild mastitis group (Group 2, n=11). Gram-negative bacteria were isolated in cultures of milk collected from all of the cows at the onset of mastitis. *Esherichia coli* (*E. coli*) was found in samples from all cows in Group 1. In Group 2, *E. coli* was found in samples from 4 cows, and *Krebsiella spp*. was found in samples from 7 cows.

In the Group 1 cows, clinical signs of systemic inflammatory response, including anorexia, severe depression, fever, and increase in heart rate were observed during the observation period. Prolonged and marked signs, including decrease of milk production, abnormal milk, swelling, fever, and pain were observed in the infected mammary glands. In the Group 2 cows, clinical signs of mild inflammatory responses, including anorexia, depression, fever, and increased heart rate were observed. Most inflammatory signs in the mammary gland were not observed and had returned to normal levels at 3 days after initial onset of mastitis in Group 2.

Blood samples were collected for hematological and immunological analysis. The blood samples were centrifuged at 3,000 rpm for 15 min, and then the serum was aspirated. Each sample was stored at –80°C until use. Blood samples were collected on the day of onset and at 3, 6, and 9 days after onset of mastitis to measure changes in the levels of serum cytokines, acute phase proteins, A/G ratio, and the numbers of white blood cells (WBC) and platelets (PLT) during the course of coliform mastitis.

The activities of cytokines were measured with a cellular bioassay, as described previously [5, 14]. IL-1 activities were measured by a growth inhibition assay using A375 cells. Recombinant human IL-1-β (R&D System, MN, U.S.A.) was used as the standard for calculating bovine IL-1 activity. For the IL-6 assay, serum was incubated at 56°C for 30 min and then assayed for proliferation activity on IL-6-dependent MH60 cells, and recombinant human IL-6 (Gibco, BRL, U.S.A.) was used as the standard. A cellular cytotoxicity assay using the WEHI-164 murine sarcoma cell line was used for the determination of TNF activity. Recombinant human TNF-α (Amersham, U.K.) was used as the standard control in each experiment. The specificity of cytokines was confirmed by inhibition or reduction of activity by polyclonal antibodies.

The concentrations of serum α1AG and haptoglobin were

Table 1. Clinical and laboratory data in coliform mastitis

	(day)	Group1 (n=7)	Group 2 (n=11)
Temperature (°C)	0	39.5 ± 0.2	39.3 ± 0.2
	3	39.2 ± 0.1	39.1 ± 0.2
	6	39.9 ± 0.3	39.0 ± 0.2*
	9	40.1 ± 0.3	38.6 ± 0.1*
Pulse (breats/min)	0	106.2 ± 4.1	89.6 ± 3.4*
	3	100.9 ± 5.8	87.4 ± 3.3*
	6	96.3 ± 6.5	81.5 ± 4.6*
	9	93.7 ± 8.8	75.5 ± 4.0*
Respiration (breath/min)	0	40.6 ± 4.9	43.4 ± 4.7
	3	45.9 ± 4.6	42.6 ± 5.7
	6	47.5 ± 6.0	51.7 ± 6.3
	9	55.3 ± 7.4	45.0 ± 15.1
WBC ($\times 10^2/\mu l$)	0	13.9 ± 0.9	34.5 ± 5.0*
	3	54.8 ± 10.5	93.4 ± 8.7*
	6	84.9 ± 22.0	124.5 ± 18.1
	9	78.2 ± 5.7	87.8 ± 9.2
PLT ($\times 10^4/\mu l$)	0	13.9 ± 2.8	31.7 ± 10.6
	3	20.5 ± 3.6	33.4 ± 2.2*
	6	19.2 ± 4.6	42.2 ± 2.4*
	9	20.9 ± 5.8	38.1 ± 8.5*
A/G rate	0	1.10 ± 0.09	0.92 ± 0.07
	3	0.76 ± 0.07	0.99 ± 0.10*
	6	0.65 ± 0.03	0.80 ± 0.09
	9	0.59 ± 0.04	0.73 ± 0.06*
Number of treatment times		7.29 ± 3.01	2.89 ± 1.14*

Values are expressed as the mean ± S.E.
* Means significantly different between two groups ($P<0.05$). 0 day means onset day.

measured by the single radial immunodiffusion method [13].

The data were evaluated by Student's *t*-test, and values of $p<0.05$ were regarded as significant. Statistical analysis was performed using Microsoft Excel Version 6.0.

In the Group 1 cows, rectal temperature increased significantly from 6 to 9 days after onset of mastitis, where as the rectal temperature decreased gradually from 3 to 9 days after onset of mastitis in Group 2. The pulse rate peaked on the day of onset in all cows. A significant difference in pulse rate was found between Group 1 and Group 2 cows during the observation period. The pulse rate in group 2 cows decreased gradually after onset. The numbers of WBC and PLT were decreased on the day of onset. The number of WBC was decreased on the day of onset and then increased at 6 days. A significant decrease of WBC was found between onset to 3 day in Group 1 compared with Group 2. The number of PLT was significantly lower in Group 1 than in Group 2 when suffering from mastitis, and the level remained low in Group 1 during the whole observation period. The A/G ratio decreased gradually after onset of mastitis in Group 1 and was significantly lower than that in Group 2 from day 3 to day 9. The number of treatment times was significantly higher in Group 1 than in Group 2 (Table 1).

Serum TNF activity in Group 1 was significantly higher than that of Group 2 at the onset. Serum IL-1 activity was significantly higher in Group 1 than in Group 2 on the day of onset. Elevated IL-1 activity was evident in the severe group throughout the observation period. Serum IL-1 activity in Group 1 decreased gradually from day 3 to day 9. No significant difference of serum IL-6 activity was found between Group 1 and Group 2 from day 3 to day 9 (Fig. 1).

Serum α1AG concentration increased between 6 and 9 days after onset of mastitis in Group 1. A significant increase in serum α1AG concentration in Group 1 was detected at 9 days compared to that in Group 2. Serum Hp concentration in Group 1 peaked at 3 days after onset, and decreased between days 6 and 9. A significant increase in serum Hp concentration in Group 1 was found at 3 days. Serum Hp concentration in Group 2 decreased during the observation periods (Fig. 2).

This study describes the progress of acute phase proteins and inflammatory cytokines in coliform mastitis in naturally

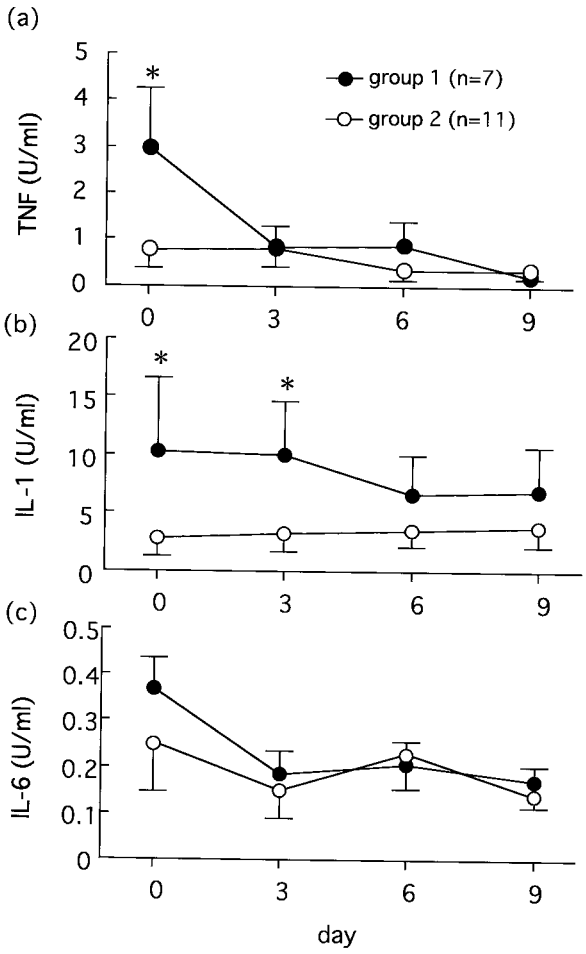

Fig. 1. (a) Changes in serum TNF activity, (b) serum IL-1 activity, and (c) serum IL-6 activity during infection of coliform mastitis. Data shown as mean values ± SE. Asterisks denote significant differences.

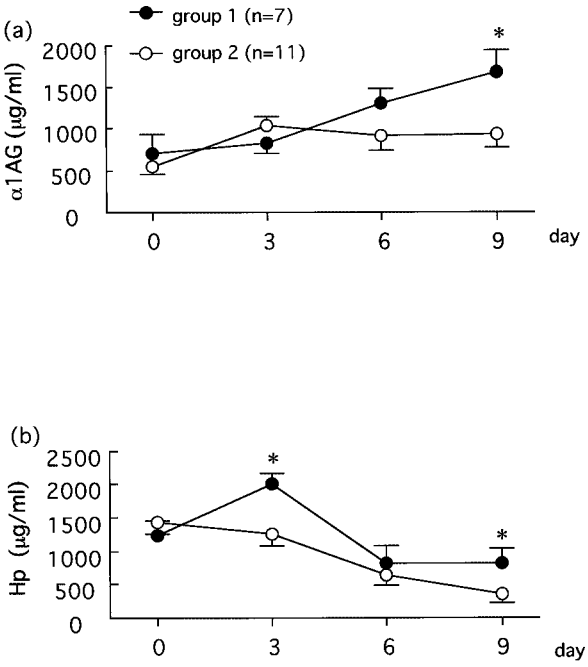

Fig. 2 (a) Changes in concentrations of serum α1AG, and (b) serum haptoglobin during infection of coliform mastitis. Data shown as mean values ± SE. Asterisks denote significant differences.

occurring cases. Previous experimental studies reported that intramammary injection of coliform bacteria or coliform bacterial endotoxin induced production of inflammatory cytokines [11, 12]. Our study also indicated that the systemic inflammatory response was caused by intramammary infection on coliform mastitis. Depending on the amount and location of inflammatory cytokines such as TNF and IL-1, the severity of the inflammatory response may extend to the systemic circulatory response due to bacterial endotoxin in severe coliform mastitis. The changes in clinical signs, serum proteins and leukocytes that occur after tissue damage represent a part of the systemic response of the injured mammary gland.

Increased serum α1AG concentration was delayed compared to that of serum Hp concentration, thus showing that several APPs were regulated differently between the two groups of coliform mastitis. A previous study showed that serum Hp in cattle with acute inflammation was significantly higher than that in cattle with chronic inflammation [6]. On the other hand, increased concentration of serum α1AG was observed in mastitic cattle [10]. In this study, it was observed that the A/G ratio decreased gradually in severely mastitic cows after onset. These findings suggest that severe mastitic cows develop systemic and chronic inflammation.

IL-6 is a major regulator of acute phase protein synthesis in hepatocytes [1]. Plasma levels of IL-6 correlated with disease severity in primary septic shock in humans [15]. However, in spite of significant changes in acute phase protein in the severe group, it was serum IL-1 activity which was one of the inflammatory cytokines in which a significant difference was detected between the two groups. In a previous report of clinical coliform mastitis cases, serum IL-6 levels were higher in the surviving cattle than those in the cattle that died [9]. In an experimental study, injection of rBo-IL-1 to cattle induced increases of serum Hp and a1AG concentrations [4]. Although, serum Hp concentration increased, no change in serum α1AG concentration was observed after administration of rh-IL-6 in calves [8]. In cows, IL-1 might be the pivotal cytokine for induction of acute phase protein rather than IL-6.

There were different patterns of acute phase proteins between the two groups of coliform mastitis. This finding may indicate that the activity of serum inflammatory cytokines does not depend on the severity of the inflammatory response. In this study, we used the serum samples col-

lected from the jugular vein to evaluate the circulated activities of serum cytokine. A previous report showed that serum TNF concentration is lower than milk TNF concentration in experimental coliform mastitis [11]. Cytokines' activity may decline after absorption into the circulation or after interaction with other cells. Although inflammatory cytokine is producted in great volume of in the mammary gland, this does not necessarily increase the cytokine activities of jugular blood. It was suggested that many factors including concentration of cytokine protein, cytokine activity, and a combination of several cytokines might effect the pathophysiological condition in coliform mastitis. Further study is needed on the changes of the relationship between the cytokine network and pathophysiology in coliform mastitis.

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