Suppression of Lymphocyte Blastogenesis in Cows with Puerperal Metritis and Mastitis

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ABSTRACT. Mitogenic responses of peripheral blood lymphocytes in naturally occurring clinical puerperal metritis and mastitis were investigated. Glucose consumption index (GCI) values for phytohaemagglutinin (PHA), concanavalin A (Con A) and pokeweed mitogen (PWM) in the puerperal metritic cows and mastitic cows were significantly lower than those in the healthy cows. Suppression of lymphocyte blastogenesis was correlated to an increased concentration of serum ammonia in the puerperal metritic cows, and of α_1-acid glycoprotein (α_1AG) in the mastitic cows. Lymphocyte blastogenesis in the mastitic cows was also correlated to the serum concentration of vitamin E. These findings indicate that the puerperal metritic and mastitic cows are associated with impaired lymphocyte blastogenesis.—KEY WORDS: lymphocyte blastogenesis, mastitis, puerperal metritis.

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The incidence of bovine infectious diseases such as puerperal metritis and mastitis is high during the periparturient period. This may be due to suppression of neutrophil and lymphocyte functions after calving, compared with the values observed during the dry period [7, 8, 10, 14]. Mitogenic responses of lymphocytes were impaired within the first 10 days after calving [6], and bovine lymphocyte responses to phytohaemagglutinin (PHA) were severely suppressed within 24 hr of parturition [18]. Impaired lymphocyte function may therefore contribute to increased susceptibility to infectious diseases during the periparturient period. In the present study, to know the immunosuppressive condition in the cows with puerperal metritis and mastitis, mitogenic responses of peripheral blood lymphocytes in the naturally occurring clinical cases, and the relationships between lymphocyte blastogenesis and serum concentrations of ketone bodies, ammonia, cortisol, vitamin E, selenium, and α_1-acid glycoprotein (α_1AG) were investigated.

Blood samples were obtained from 14 Holstein cows with naturally occurring puerperal metritis, and from 12 Holstein cows with mastitis. The cows were within 30 days after parturition. The puerperal metritic cows were diagnosed by clinical symptoms such as retained placenta, decreased appetite, pyrexia (over 39.5°C), and positive urine ketone bodies (5 among 14 cows). Similarly, the mastitic cows were diagnosed by clinical features such as decreased appetite and milk production, mammary fever symptoms including swelling and pain in the mamma, positive results of the California mastitis test, and pyrexia (6 among 12 cows). Blood samples were also obtained from 26 healthy lactating Holstein cows which were within 30 days after parturition. Serum concentrations of total ketone, acetoacetic acid and β-hydroxybutyric acid (Ketone test; Sanwa Chemical, Nagoya), ammonia (Monotest; Boehringer Mannheim Yamanouchi, Tokyo), cortisol (EIA test; Boehringer Mannheim Yamanouchi), vitamin E (HPLC with a Zorbax ODS column), selenium (Fluorometric method), and α_1AG (Bovine α_1AG plate; Saikin Kagaku, Sendai) were determined. Serum concentrations of total cholesterol, and activities of aspartate aminotransferase (AST) and γ-glutamyltranspeptidase (γGTP) were also evaluated with a clinicochemical analyzer (Super Z 818; Nittec, Tokyo) by using commercial kits for enzyme, UV rate, and rate method, respectively. Blastogenic responses of lymphocytes to mitogens were evaluated by the glucose consumption test described by Ishikawa and Shirahata [4]. Phytohaemagglutinin-P (10.0 μl/ml) (PHA; Difco, Detroit, Michigan), concanavalin A (25.0 μg/ml) (Con A; Pharmacia, Sweden) and pokeweed mitogen (10.0 μl/ml) (PWM; Gibco, Grand Island, N.Y.) were used as mitogens, and cultures without mitogen were used as a control. Lymphocyte blastogenic activity was expressed by the glucose consumption index (GCI) on the basis of the glucose concentration [G] as follows:

$$GCI = \frac{[G]\ \text{medium} - [G]\ \text{mitogen stimulated culture}}{[G]\ \text{medium} - [G]\ \text{control culture}} \times 100$$

Peripheral blood leukocytes counted with a hemocytometer (Celltac; Nihon Koden, Tokyo) in the puerperal metritic cows, mastitis cows, and healthy cows were 5,750±2,030 (mean ± standard deviation)/μl, 6,880±2,490/μl, and 6,950±1,780/μl, respectively. Lymphocyte counts determined by means of May-Giemsa-stained blood smears in the puerperal metritic cows, mastitic cows, and healthy cows were 3,950±1,300/μl, 3,020±870/μl and 3,420±1,010/μl, respectively. No significant difference was observed in the peripheral blood leukocyte and lymphocyte counts of the puerperal metritic cows, mastitic cows and the healthy cows. The mean concentrations of total ketone and β-hydroxybutyric acid in the puerperal metritic cows were significantly higher than in the mastitic cows and healthy cows (Table 1). The ammonia concentration in the mastitic cows was significantly higher than in the puerperal metritic cows and healthy cows. The vitamin E concentration in the puerperal metritic cows was significantly lower than in the mastitic cows and healthy cows. Serum concentrations of α_1AG in the diseased cows were significantly higher than in the healthy cows, but no significant difference was observed in the values for cortisol, selenium, AST, γGTP, and total cholesterol in the puerperal metritic cows, mastitic cows and healthy cows.

Mean GCI values in the puerperal metritic cows and mastitic cows were significantly lower than in the

Table 1. Values for ketone bodies, ammonia, cortisol, vitamin E, selenium, α_1-acid glycoprotein (α_1AG), aspartate aminotransferase (AST), γ-glutamyltranspeptidase (γGTP), and total cholesterol in the puerperal metritic cows, mastitic cows, and healthy cows

Measurement	(Units)	Serum concentrations		
		Metritic cows (n=14)	Mastitic cows (n=12)	Healthy cows (n=26)
Total ketone	(mM)	1.22± 0.47[a)d)]	0.76± 0.24	0.93± 0.41
Acetoacetic acid	(mM)	0.05± 0.04[b)]	0.03± 0.01	0.03± 0.01
β-hydroxybutyric acid	(mM)	1.17± 0.55[a)d)]	0.72± 0.24	0.90± 0.41
Ammonia	(μg/d*l*)	220± 32[d)]	293± 70[b)]	196± 49
Cortisol	(μg/d*l*)	0.41± 0.52	0.36± 0.37	0.51± 0.71
Vitamin E	(μg/m*l*)	1.40± 0.46[b)c)]	2.18± 1.04	2.46± 0.82
Selenium	(*n*g/m*l*)	61.8± 15.9	74.9± 16.0	62.7± 12.4
α_1AG	(μg/m*l*)	1,061± 486[b)]	1,202± 464[b)]	421± 118
AST	(IU/*l*)	92± 38	96± 41	86± 29
γGTP	(IU/*l*)	22± 5	20± 7	23± 6
Total cholesterol	(mg/d*l*)	92± 38	101± 38	116± 35

Data are expressed as the mean±standard deviation. Statistical analyses are carried out using the Student's and Welch's *t*-test.
Significant difference [a): $p<0.05$, b): $p<0.01$] was observed between the healthy cows and the diseased cows.
Significant difference [c): $p<0.05$, d): $p<0.01$] was observed between the metritic cows and the mastitic cows.

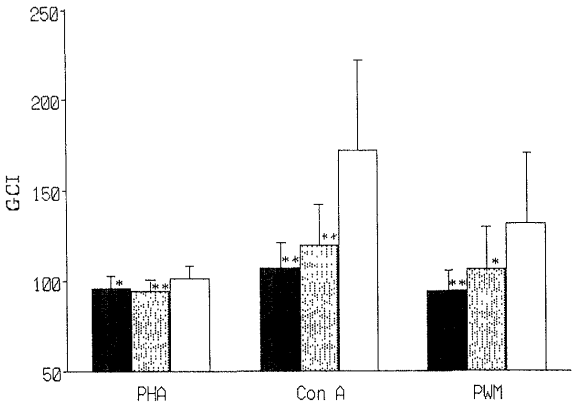

Fig. 1. Glucose consumption index (GCI) values in the puerperal metritic cows (■; n=14), mastitic cows (▤; n=12), and healthy cows (□; n=26). Peripheral blood lymphocytes were stimulated by phytohaemagglutinin (PHA), concanavalin A (Con A) and pokeweed mitogen (PWM). Data are expressed as the mean ± standard deviation of GCI values from triplicate results in the cows indicated. Significant difference compared with the healthy cows is indicated by single (*: $p<0.05$) and double asterisks (**: $p<0.01$).

healthy cows (Fig. 1). The GCI value for PHA in the puerperal metritic cows and mastitic cows was significantly lower than in the healthy cows. The pattern of the GCI values for Con A and PWM was similar to that for PHA. The GCI values for Con A and PWM in the puerperal metritic cows and mastitic cows were significantly lower than in the healthy cows. A negative correlation was observed between the GCI values and concentrations of total ketone, acetoacetic acid and β-hydroxybutyric acid, but the correlations were not statistically significant. In the puerperal metritic cows, a significant ($p<0.05$) negative correlation was observed between the GCI value for PHA and the ammonia concentration (coefficient of correlation: -0.724). In the mastitic cows, a significant ($p<0.05$) positive correlation was also observed between the GCI values for Con A and PWM and concentration of vitamin E (coefficient of correlation: 0.604 and 0.655, respectively), and the GCI value for PHA and concentration of α_1AG negatively (coefficient of correlation: -0.579). No significant correlation was observed between the GCI values and cortisol, selenium, AST, γGTP and total cholesterol values in the puerperal metritic cows and mastitic cows.

In the present study, impaired lymphocyte blastogenesis was confirmed in the mastitic cows, as reported previously [5, 11]. Suppression of neutrophil functions has been recognized in cows with retained placenta [3], but the findings obtained in the present study indicate that puerperal metritic cows with retained placenta also tend to have impaired lymphocyte blastogenesis. Mean serum concentrations of ketone bodies and cortisol in the puerperal metritic cows and mastitic cows investigated in the present study were at low levels compared with those in previous reports [2, 11], so that no significant correlation was observed between the GCI values and serum concentrations of ketone bodies and cortisol, whereas, the normal concentration of ammonia in bovine blood (from 80 to 250 μg/d*l*) influenced the viability and function of lymphocytes [17]. Furthermore, suppression of lymphocyte blastogenesis was correlated with the serum

α_1AG concentration [9]. Impaired lymphocyte blastogenesis in the puerperal metritic cows might be related to an increased concentration of serum ammonia, and the serum concentration of α_1AG might be related to the suppression of lymphocyte blastogenesis. On the other hand, mean concentrations of selenium in the puerperal metritic cows and mastitic cows investigated in the present study were not deficient levels. Selenium deficiency was associated with depressed lymphocyte blastogenesis, reduced non-specific immunity dependent on neutrophil and macrophage functions, and with lowered antibody production [15, 16]. In addition, physiological levels of selenium selectively stimulated B-lymphocyte proliferation [15], and vitamin E and selenium had interactive effects on lymphocyte responses to Con A and PWM [12]. The serum concentrations of vitamin E and selenium might therefore be related to impaired lymphocyte blastogenesis in the cows with puerperal metritis and mastitis. A supplement of vitamin E and selenium given to cows might be effective in the prevention of puerperal metritis and mastitis [1, 13].

Lymphocyte blastogenesis is influenced by many factors such as stress, hormonal changes, age, diseases, and drug therapy. Furthermore, the roles of peripheral blood lymphocytes in local immunity including the uterus and mammary gland have not been established. Further study is therefore needed to clarify the mechanism of suppression of lymphocyte blastogenesis in cows with puerperal metritis and mastitis.

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