NOTE *Internal Medicine*

Effects of Active Egg White Product/*Clostridium butyricum Miyairi 588* Additive on Peripheral Leukocyte Populations in Periparturient Dairy Cows

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ABSTRACT. The leukocyte populations of periparturient dairy cows were analyzed after administration of active egg white/*Clostridium butyricum Miyairi* additive. Sixty-eight Holstein milking cows were divided into 3 groups. Group A was administered active egg white product (AEWP)/*Clostridium butyricum Miyairi 588* (Miyairi 588) additive (n=23). Group B was administered Miyairi 588 only (n=23), and Group C was the control group (n=22). The challenged groups were administered 100 g of AEWP + Miyairi 588, or Miyairi 588 alone, daily for 60 days from 1 month before until 1 month after paturition. Blood samples were collected from all groups three times (1 month before, 1 week after and 1 month after parturition) for analysis of the peripheral leukocyte population. The results showed significantly higher numbers of $CD4^+$ cells in Group A compared with Group C 1 week after paturition. AEWP/Miyairi 588 additive may enhance the number of $CD4^+$ T cells in periparturient dairy cows.

KEY WORDS: AEWP, $CD4^+$ T cell, Dairy cow.

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Inflammatory diseases, such as mastitis and puerperal metritis, develop frequently in periparturient dairy cows, and the consequent economic damage is substantial. In dairy cows, undernutrition from the dry period makes it easier for them to contract periparturient diseases. Inflammatory diseases, decreased cellular immunity and significantly low peripheral blood $CD4^+$ T cell numbers have all been reported following parturition [6]. In recent years, various additives have been used to maintain and improve the immunological function of cows, but their efficacies and the changes induced in immunological functions are mostly unknown. Oral administration of active egg white product (AEWP) improves macrophage functions in mice [1] and promotes activation of neutrophilic functions in calves [3]. Sato *et al.* [9] observed enhancement of the NBT (nitroblue tetrazolium) reducibility of peripheral blood neutrophils and lymphocyte blastogenesis following oral administration of AEWP in healthy dairy cows, suggesting that AEWP has an immunostimulatory effect. *Clostridium butyricum Miyairi 588* (Miyairi 588) has been reported to inhibit proliferation of enterohemorrhagic Escherichia coli O157:H7 and the production of Shiga-like toxin in infected mice [11]. Oral administration of Miyairi 588 has been reported to inhibit *Helicobacter pylori*, the causal agent of human gastric ulcers, from colonizing the mucous membrane in mice [12]. Addition of Miyairi 588 to Peyer's patch cell culture tends to improve the production of IgA, IgM and IgG, and oral administration of Miyairi 588 improves immunological functions locally at the intestinal mucous membrane [2]. These findings suggest that administration of AEWP/Miyairi 588 additive may effectively prevent reduction of immunological functions in periparturient daily cows.

The objective of this study was to investigate the effect of oral administration of AEWP/Miyairi 588 additive or Miyairi 588 alone to the peripheral leukocyte populations of dairy cows during the parturitient period.

Sixty-eight Holstein milking cows raised at 6 dairy farms were used as the subjects of this study. They were divided into three groups. Group A (mean age of 4.46 ± 0.40 years old, n=23) was administered AEWP/Miyairi 588 additive, Rich-On-Rich, (Odajima Shoji Co., Inc., Iwate, Japan); Group B (mean age of 4.06 ± 0.35 years old, n=23) was administered Miyairi 588 alone, Miya Rich, (Odajima Shoji Co., Inc.); and Group C (mean age of 4.06 ± 0.26 years old, n=22) was the control group. Rich-On-Rich contains 0.5 g/100 g of active egg white product and 20 g/100 g of 1.0×10^8 CFU/g Miyairi 588 live bacterial preparation. Miya Rich contains 20 g/100 g of 1.0×10^8 CFU/g Miyairi 588 live bacterial preparation. Groups A and B were administered 100 g/day of Rich-On-Rich and Miya Rich, respectively, for 60 days, starting 30 days before and ending 30 days after parturition. The control group was arranged such that there ware no differences among the groups with respect to the numbers of calves and cows. This research was conducted during summer. Blood samples were collected 1 month before, 1 week after and 1 month after parturition. The subpopulation of peripheral blood white blood cells (WBCs) and lymphocyte blastogenesis were examined. Flow cytometric analysis was performed on leukocytes incubated with lineage-specific monoclonal antibodies: VMRD (Pullman, WA, U.S.A.), CACT183A (bovine CD4-specific monoclonal antibody), BAT82A (bovine CD8-specific monoclonal antibody), TH14B (bovine MHC class II-specific monoclonal antibody) and MY-4 (bovine CD14-specific monoclonal antibody; Coulter

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Immunology, Hialeah, FL, U.S.A.). Cell surface markers were visualized with goat anti-mouse IgG-fluorescein isothiocyanate (Cappel, Durham, NC, U.S.A.) using a staining procedure described previously [5]. The samples were analyzed using a FACScan flow cytometer and Lysis II software (BD Biosciences Immunocytometry Systems, San Jose, CA, U.S.A.).

Lymphocyte blastogenesis was evaluated using the MTT assay. Briefly, 106 of peripheral blood mononuclear cells in a total volume of 1 mL of 10% FCS-RPMI were added to a 96-well plate and stimulated with phytohemagglutinin (PHA Chemical, St. Louis, MO, U.S.A.; 5 μg/mL) for 72 hr at 37°C. After incubation, cell numbers were determined by colorimetric assay using 2,2,5-diphenyltetrazolium bromide (MTT). The incidences of periparturient diseases and treatment times around paturition were also investigated.

Mean values and standard deviations associated with the clinical and laboratory data were calculated. The data were evaluated by ANOVA for variance. Values of $p<0.05$ were regarded as significant.

The incidences of metabolic diseases, including postparturient hypocalcemia, retention of the placenta and displacement of the abomasums, were 21.7%, 30.4% and 31.8% for groups A, B and C, respectively, and the incidences of inflammatory disease, including mastitis and pneumonia, were 0.0%, 13.0%, and 4.5%, respectively; treatment was given on average 2.4 times, 3.2 times and 3.0 times in groups A, B and C, respectively (Table 1). Group A had lower incidences of metabolic diseases and inflammatory disease and was administered fewer treatments compared with the other groups. No differences were observed amongst the groups with respect to types of diseases.

In regard to the subpopulation of peripheral blood WBCs, the number of $CD4^+$ cells were significantly higher in Group A compared with those in Group C at 1 week after paturition (Table 2). Additionally, there was no difference in the numbers of MHC class II^+ $CD14^-$ cells and $CD14^+$ cells amongst the groups. The results for the number of $CD4^+$ cells suggest that the number of $CD4^+$ T cells was higher in Group A (only at 1 week after parturition, Table 2). The PHA lymphocyte blastogenesis test revealed that Group A had higher values 1 week after parturition compared with those on groups B and C, but no significant differences were observed (Table 2).

Cellular immune functions are likely to decrease in periparturient dairy cows, and the number of T cells of the dairy cows that develop postparturient mastitis and/or puerperal fever is prone to decrease from the day of parturition until 1 week after [4]. In this study, administration of the AEWP/Miyairi 588 additive resulted in a significant increase in the number of $CD4^+$ cell at 1 week after paturition. The $CD4^+$ T cell expresses the helper T cell, and it assumes an important role in the cellular immune function. Therefore, we suggest administration of AEWP/Miyairi 588 additive before and after parturition in order to prevent infectious diseases that dairy cows are susceptive to. No differences were observed

Table 1. Rates of occurrence of metabolic and inflammatory disease in the calving cows of each group

	Group A (n=23)	Group B (n=23)	Group C (n=22)
peripaturient disease (%)	21.7	30.4	31.8
inflammatory disease (%)	0.0	13.0	4.5
treatment (time)	2.4	3.2	3.0

The rates are the mean rates of occurrence of peripaturient disease in calving cows that required veterinary treatment during the peripaturient period.

Table 2. Leukocyte populations and PHA lymphocyte blastogenesis for each group

		Group A (n=23)	Group B (n=23)	Group C (n=22)
$CD4^+$ T cells	−1 month	731.5 ± 385.3	834.3 ± 691.5	747.2 ± 568.1
(cell/μl)	1 week	676.3 ± 328.5*	629.7 ± 363.4	531.0 ± 207.3
	1 month	617.6 ± 286.1	697.0 ± 304.6	672.5 ± 332.4
$CD8^+$ T cells	−1 month	337.1 ± 127.6	307.1 ± 118.6	412.9 ± 225.3
(cell/μl)	1 week	306.8 ± 176.9	276.3 ± 137.1	385.9 ± 376.1
	1 month	271.1 ± 144.6	300.3 ± 123.5	338.3 ± 133.9
MHC class II^+	−1 month	1331 ± 645.8	1291 ± 758.3	1258 ± 597.4
$CD14^-$cells(cell/μ)	1 week	959 ± 402.0	1089 ± 770.9	1043 ± 505.2
	1 month	1075 ± 715.0	1061 ± 502.1	1148 ± 827.7
$CD14^+$	−1 month	480 ± 226.1	531 ± 486.1	592 ± 254.8
(cell/μl)	1 week	740 ± 536.0	787 ± 446.7	888 ± 641.1
	1 month	613 ± 327.4	602 ± 196.6	642 ± 240.4
PHA	−1 month	328 ± 114.7	306 ± 160.3	351 ± 154.3
(SI)	1 week	344 ± 169.9	301 ± 155.6	289 ± 139.0
	1 month	316 ± 93.5	410 ± 160.9	359 ± 136.9

Values are expressed as means ± SD.
*: Significantly different from Groupe C ($P<0.05$).

in the numbers of MHC class II$^+$ CD14$^-$ cells or CD14$^+$ cells. It is reported that activities of neutrophils and lymphocytes are depressed in periparturient dairy cows, though administration of AEWP encourages PHA lymphocyte blastogenesis, indicating that administration of AEWP prevents suppression of the immune function [9]. Group C had lower CD4$^+$ cell numbers and PHA lymphocyte blastogenesis levels compared with Groups A and B at 1 week after parturition. This suggestes that administration of AEWP/Miyairi 588 additive or Miyairi 588 alone prevent the decrease of CD4$^+$ T cell numbers and maintained PHA lymphocyte blastogenesis levels during the periparturient period. However, the mechanism through which AEWP acted on the immune system was not clarified in this study.

Sato *et al.* [10] demonstrated enhancement of T and B lymphocyte activity in healthy dairy cows administered an oral *Bacillus subtilis* product and suggested that it be used for long-term prevention of infectious diseases such as mastitis. Similar probiotics, such as *Bifidobacterium thermophilum* and *Lactobacillus casei* and their components, are known to enhance resistance against experimental bacterial infection and tumor cells in mice [7, 8, 13]. However, CD4$^+$ cell numbers were not significantly different Group B cows, indicating that the effect of Miyairi 588 alone on the immunological system of parturient dairy cows was not very strong. Further studies on Miyairi 588 dosage levels and administration methods may be necessary in relation to dairy cows.

Since nutrition and immunological functions are closely correlated in periparturient dairy cows, continuous use of products that improve immunological functions and probiotics is considered effective, if given in addition to appropriate nutrition management. Considering the importance of safe livestock production using the minimum amount of antibiotic agents, the efficacies of probiotic additives known to improve immunological functions need further investigation.

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