

Development of Peripheral Blood Mononuclear Cell Responses to Mitogens in Japanese Black Newborn Calves

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ABSTRACT. Development of peripheral blood mononuclear cells (PBMC) responses to phytohemagglutinin (PHA) and concanavalin A (Con A) in 5 Japanese Black newborn calves was examined by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay until the day 28 after birth. The PHA responses of PBMC from calves aged from 0 to 7 days were less than those of PBMC from control adult cattle. While the difference of the Con A response of PBMC between newborn calves and adults was not significant statistically. These results suggest that the response of T cell to PHA may be insufficient at early stage after birth in Japanese Black.—**KEY WORDS:** blastogenic response, calf (Japanese Black), development.

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Newborn calves often suffer from some infectious diseases such as diarrhea [2] during the perinatal periods because of their insufficient immune system [11]. The defensive immunity of newborn calves mainly depends on the passive immunity, immunoglobulin-rich colostrum from dams [6]. The active immunity of calves is thought to be developed after certain periods after birth.

The responsiveness of peripheral blood mononuclear cells (PBMC) to mitogens is one of indicators for the immune function of individuals. Low responsiveness of PBMC represents often a high risk condition to infectious diseases or immune deficiency in general [10]. There are some reports examined calf PBMC responses to mitogens [3, 4, 9, 12, 13], however Holstein, a dairy breed, are used in most of them. In the present study, we reports the development of calf PBMC responses to T cell mitogens in Japanese Black, a popular beef breed in Japan.

Five newborn calves of Japanese Black, which were kept in the farm of Yamaguchi University, were used in this study. No calf had developed clinical problems such as diarrhea or pneumonia during the period of the study. PBMC were separated from them at within 8 hr (Day 0), 24 to 32 hr (Day 1), 72 to 80 hr (Day 3), 7 days (Day 7), 14 days (Day 14) and 28 days (Day 28) after birth according to the method described by Inokuma *et al.* [1]. Another 6 cattle aged 2 to 4 years were also used for adult control. PBMC were suspended at 4×10^6 cells/ml in complete RPMI1640 medium (ICN Biomedical Japan), which contained 10% heat-inactivated fetal calf serum (ICN Biomedical Japan), penicillin (100 IU/ml) and streptomycin (20 μ g/ml). Observation of PBMC stained by Giemsa revealed that the cell suspensions contained 95.2 to 98.7% of mononuclear cells. In addition, 91.0 to 97.9% PBMC were judged viable by trypan blue dye exclusion. There was no significant difference in the purity and viability of PBMC in each sample. Fifty μ l of PBMC suspension were added in triplicate to wells in a 96 well microculture plate (Flow Laboratories Inc., U.S.A.) along with 50 μ l of an optimal concentration of phytohemagglutinin (PHA, EY Laboratories Inc., U.S.A., final concentration 1.25 μ g/ml)

or concanavalin A (Con A, EY Laboratories Inc., final concentration 5.0 μ g/ml). The optimal concentration was determined in preliminary experiments using PBMC from 3 healthy adult cattle. The cell cultures were incubated for 72 hr at 37°C in a humidified atmosphere of 5% CO₂. The blastogenic activities of cells in cultures were evaluated by a MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) assay [7, 8]. Fifty μ l of the supernatant were replaced with 10 μ l of MTT (Dojindo, Japan, 5 mg/ml in phosphate buffered saline) for all wells of an assay and the plates were incubated for 4 hr at 37°C. Acid isopropanol (100 μ l of 0.04 N HCl isopropanol) was then added to all wells and mixed thoroughly to dissolve dark blue crystals. The plates were read by a ELISA reader (CORONA, Japan), using a test wave length at 570 nm and a reference wave length at 630 nm. The results are expressed as the stimulation index (SI) according to the following formula from the results of triplicate assays, mentioned by Iwata and Inoue [5].

$$SI = (\text{O.D. (570/630 nm) of stimulated cells}) / (\text{O.D. (570/630 nm) of unstimulated cells})$$

The results were presented as the mean and standard deviations. The differences from the values of the control adult cattle were analyzed by Student's *t*-test.

The responses of PBMC to PHA and Con A are shown in Fig. 1. The PHA responses of PBMC from newborn calves on Day 0 to 7 were significantly lower than those of PBMC from control adult cattle ($p < 0.05$). On the other hand, the difference of the Con A response of PBMC between newborn calves and adults was not significant statistically.

There are some previous studies about the development of PBMC or lymphocyte responses to T cell mitogens, PHA or Con A in newborn calves of Holstein. Whitebread and Rowan [12] reported that whole blood from Holstein calves aged 3 to 23 days after birth could respond to Con A and PHA. Nagahata *et al.* [9] and Ishikawa [3, 4] also found that the PHA-induced blastogenic response of lymphocytes isolated from newborn calves was satisfactory in Holstein. Limited information is available about the Japanese Black calves. Takagi *et al.* [12] showed that the PHA response of

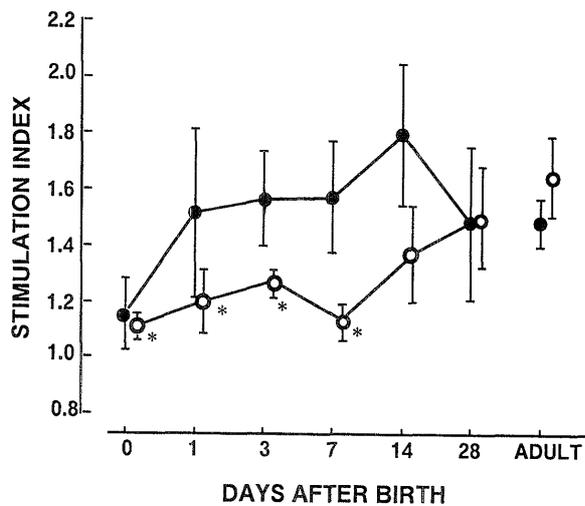


Fig. 1. Mitogenic responses of PBMC to PHA and Con A in Japanese Black calves in age ranging from 0 to 28 days and adult cattle. PBMC were cultured with the optical concentrations of PHA (1.25 $\mu\text{g}/\text{ml}$) and Con A (5 $\mu\text{g}/\text{ml}$) for 72 hr. Each point and bar represents the mean \pm standard error of stimulation index in the mitogenic responses of PBMC to PHA (open circle) and Con A (closed circle). *: significantly different ($p < 0.05$) from the control according to the Student's *t*-test.

lymphocyte from a healthy Japanese Black calf aged less than 7 days after birth was extremely low and another calf was sufficient. In our study, we found that the low PBMC response to PHA in calves at early stage after birth, but not to Con A. Those findings suggest that the function of T

cells, especially a responsiveness to PHA, in Japanese Black calves may be insufficient at early stage after birth. Furthermore different breeds of cattle may have the different ways of development in the immune system.

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