Transformation of Bovine Peripheral Blood Lymphocytes in the Perinatal Period

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ABSTRACT. Transformation of apparently healthy bovine lymphocytes with phytohemagglutinin (PHA), concanavalin A (Con A) and pokeweed mitogen (PWM) from peripheral blood in the perinatal period was observed. In multiparous dairy cows, lymphocyte incorporation of 3H-thymidine (3H-TdR) decreased twice within 40 days after calving: the first one occurred within 10 days, and the second one was seen around 30 days after calving, respectively. This trend of decreasing was almost same in the transformation with PHA, ConA and PWM, respectively. On the other hand, the marked decreasing was seen once around 20 days after parturition in primiparous cows.—KEY WORDS: bovine lymphocyte, lymphocyte transformation.

It is well known that a relatively high incidence of various infections, such as mastitis, occur in dairy cattle during the perinatal period. On the other hand, various stress and aging factors may lead to an immunocompromised condition, and consequently, the onset of opportunistic infections might be seen.

Recently, lymphocyte transformation has been utilized commonly as one of the tools to analyse the mechanisms of immunoreponsiveness and there are many reports concerning the influence of stress, hormones, drugs and so forth on lymphocyte transformation [1–3, 5, 6]. There are few reports, however, on the function of lymphocytes in bovine peripheral blood during the perinatal period [7]. Thus, we conducted an experiment on bovine lymphocyte transformation of the peripheral blood in that period.

Nine female Holstein-Friesian cows, reared on the farm of Hokkaido University and a local dairy farm were employed. Of these, four cows were primiparous, and the remaining five were multiparous, i.e., two were secundipara, one was tripara, one was quadripara, and one was hexaparous cows, respectively. All animals were apparently in good health throughout the experimental period.

Blood samples were collected from each of the cows 10 days before the expected term, and at one, two, three, four, five and six weeks after calving respectively.

Blood was collected from the jugular vein and placed into tubes containing preservative-free heparin. The lymphocytes were purified according to the Ficoll (Pharmacia, Sweden) and Conray (Daiichi Pharm., Japan) technique previously described [4, 6]. Briefly, a Ficoll-Conray solution with a specific gravity of 1.087 was prepared. Heparinized blood was mixed with an equal volume of phosphate buffer saline (PBS), and approximately 8 ml of the diluted blood were subsequently layered over 6 ml of the Ficoll-Conray solution in 16 ml tubes fitted with a plastic closure using a pasteur pipette. All samples were centrifuged at 2500 rpm for 30 minutes at 20°C. After centrifugation, each mononuclear cell-layer was removed carefully and transferred.
to 10 ml tubes. The lymphocyte suspensions were washed twice with PBS to reduce the number of platelets and once with RPMI 1640 medium. Lymphocyte populations collected in this manner had greater than 90% pure lymphocytes with a viability of more than 95%. The cell concentrations were adjusted to 10^6 cells/ml with RPMI 1640 medium.

Phytohemagglutinin (PHA, Difco, U.S.A.), concanavalin A (ConA, Difco, U.S.A.) and pockweed mitogen (PWM, Gibco, U.S.A.) were reconstituted with sterile PBS and tested at concentrations in preliminary experiments to determine the optimal dose. Finally, PHA, ConA and PWM were used at a concentration of 2.5 μl/ml and 10 μl/ml of RPMI 1640 medium, respectively, in which bovine fetal serum (10%), penicillin (100 unit/ml), streptomycin (100 μl/ml) and fungison (2.5 μg/ml) were added beforehand.

Two hundred μl of cells (10^6/ml) in triplicate cultures were incubated in 96 flat bottom well microtiter plates with 20 μl of mitogen in PBS alone for 54 hours at 37°C in 5% humidified CO₂ in the air.

The cultures were subsequently pulsed with 0.25 μCi of ³H-TdR (specific activity 21.5 Ci/m mole) in 20 μl PBS for an additional 18 hours before being harvested by an automatic cell harvester on glass fiber filter using distilled water. The filter was then transferred to a scintillation solution (PPO 5 g plus 0.1 g/l of toluene). All samples were counted for 1 minute (cpm) on a scintillation spectrometer.

The results of lymphocyte transformation with PHA, ConA and PWM in the perinatal period were shown in Fig. 1 (multiparous cows) and Fig. 2 (primiparous cows) respectively. Though the blastogenetic cpm with the mitogens were relatively the same in the lymphocytes of both the primiparous and the multiparous cows in the period around ten days before parturition, significant differences were seen after calving between these cows. In the multiparous cows, the cpm showed a clear decrease soon after the calving, followed by a gradual increase until about 30 days after calving, and then it decreased again between 30 and 40 days after calving. However, unlike the blastogenesis of multiparous cows, the trend of decreasing of cpm of the lymphocyte with PHA and
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Fig. 3. Changes of bovine blood lymphocyte counts during the perinatal period. Each value represents the mean±S.D. of 4 primiparous cows (●) and of 5 multiparous cows (○).

ConA in primiparous cows was very moderate soon after the calving, while no such trend was seen in the lymphocytes with PWM of the same cows. But anyhow, the lowest cpm in cases with PHA, ConA and PWM were seen around 20 days in the primary calving cows. The number of lymphocytes in the peripheral blood of the cows employed showed no remarkable changes during the experimental period, although it showed slight decrease at parturition in the primiparous cows (Fig. 3).

The results of slight decreasing of 3H-TdR incorporated count of lymphocytes (PHA and ConA) in the primiparous cows were clearly different from those in the multiparous cows within 10 days after calving. One explanation of this is that primiparous cows are younger and have had no milking period throughout the first year; thus they have not been exposed to the same intense stressful conditions as have multiparous cows. However, further studies should be needed to clarify the reason concerning the difference among the primiparous and the multiparous cows.

From the present study, it seems likely that dairy cows in the perinatal period may be remarkably susceptible to various kinds of stress, and consequently, depression of lymphocyte activity may occur. This facts indicate that dairy cows are highly susceptible to certain infectious disease such as mastitis within 40 days after calving.

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REFERENCES

要約

初産期における乳牛の末梢血リンパ球幼若化反応（短報）: 柏崎俊人、前出吉光、波岡茂朗（北海道大学獣医学部家畜内科学講座）——初産及び経産牛の分娩前後における末梢血リンパ球の PHA, ConA 及び PWM に対する幼若化反応を検討した。経産牛では、いずれのマイトジェンに対しても分娩後10日以内及び同30日前後に著しく低下したのに対し、初産牛のそれは分娩後20日前後にのみ著明な低下を示した。