Changes of peripheral T lymphocytes in the young calves

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Abstract

The changes of peripheral T lymphocytes (T cells) in 32 calves were examined from birth to the age of 12 weeks using rosette formation against AET (2-aminoethyl isothiouronium bromide) treated sheep erythrocytes (SRBC). They had approximately 50% of rosette forming cells (REC) in their peripheral lymphocytes soon after birth, although wide variations were observed. These percentages increased gradually with the proceeding of age until the observation period ended. The mean RFC percentage was 73.25±5.36% at 12 weeks almost the same level as adult cows (70.34±8.69%). From the results of this study, it was concluded that neonatal calves had considerable amounts of circulating T celle, the proportion of T cells reaching the adult level in a few weeks.

Introduction

It has been well recognized that in humans and some animals, thymus derived lymphocytes (T cells) can be distinguished from other lymphocytes or mononuclear cells with the ability to bind heterogenous erythrocytes through forming rosette (Kaplan & Clark 1974, Paul et al. 1979, Belden & Strelkauskas 1981). This phenomenon is not immunological but often used as a convenient marker of T cells.

In cattle, few successful reports had been available on the rosette formation of bovine lymphocytes because of the low ability to bind SRBC. So, many attempts have been made to enhance this rosette formation by treating SRBC with enzymes or some chemical reagents (Grewal et al. 1976, Higgins et al. 1977, Grewal & Babiuk 1978). In 1979, Paul et al. reported that AET (2-aminoethyl isothiouronium bromide) treated SRBC enhanced and stabilized the rosette formation of bovine peripheral lymphocytes.

There are few reports dealing with the T cell alteration of the neonatal calf. Outteridge & Dufty (1981) noted that the RFC of the newborn calf was low and the percentage of RFC increased with maturation. Binns (1978) studied the tissue distribution of RFC in calves and reported that the proportion of RFC in the lymphocytes recovered from the thymus and the lymph nodes was high but a lower percentage was observed in the peripheral blood.

The purpose of this study is to determine the percentage of RFC against AET-treated SRBC in the peripheral blood of neonatal calves and observe the ontogeny of circulating T cells during the first 12 weeks after birth.

Materials and Methods

Animals: Thirty two Holstein calves were obtained from the University farm. They were separated from their dams soon after birth and kept individually in outdoor hutches, where they were fed with colostrum twice a day. Blood samples were collected by juglar venipuncture immediately after birth, and at weekly or biweekly intervals thereafter until they reached 12 weeks of age. Daily records of each calf were kept during the experimental period. Nineteen healthy cows in the same farm, 4 to 6 years old, were bled for the adult control.

The blood samples were examined RBC, WBC, Ht and the hemograms, and lymphocyte preparation was carried out as soon as possible. The absolute lymphocyte count was estimated from the WBC and the hemogram of each calf.

Lymphocyte preparation: The lymphocytes were separated from the heparinized blood (20 IU/m*l* of blood) by Ficoll Pacque (Pharmacia, Sweden) gradient. They were washed three times with Hanks' balanced salt solution (HBSS) and once with the rosetting medium (RPMI 1640 containing 10% of fetal calf serum absorbed with SRBC). The lymphocytes were resuspended in the rosetting medium and cell concentrations were adjusted 1×10^7 cells per m*l*. The cell preparation contained about 95% lymphocytes and 96% or more of the cells were judged viable by trypan blue exclusion in this experiment.

AET treatment of SRBC: Four volumes of 0.1 M AET (2-amino ethyl isothiouronium bromide, Sigma, U. S. A.) were added to one volume of packed SRBC which had been washed three times with phosphate buffered saline (PBS, pH 7.2) and incubated at 37°C for 20 minutes. Then the mixture was washed three or four times with cold PBS, twice with the rosetting medium. The AET treated SRBC was resuspended in the medium to be 1% of cell suspension.

Rosetting assay: One hundred microliters of the lymphocyte suspension were mixed with 200 microliters of 1% SRBC and incubated at 37°C for 10 minutes, thereafter centrifuged at

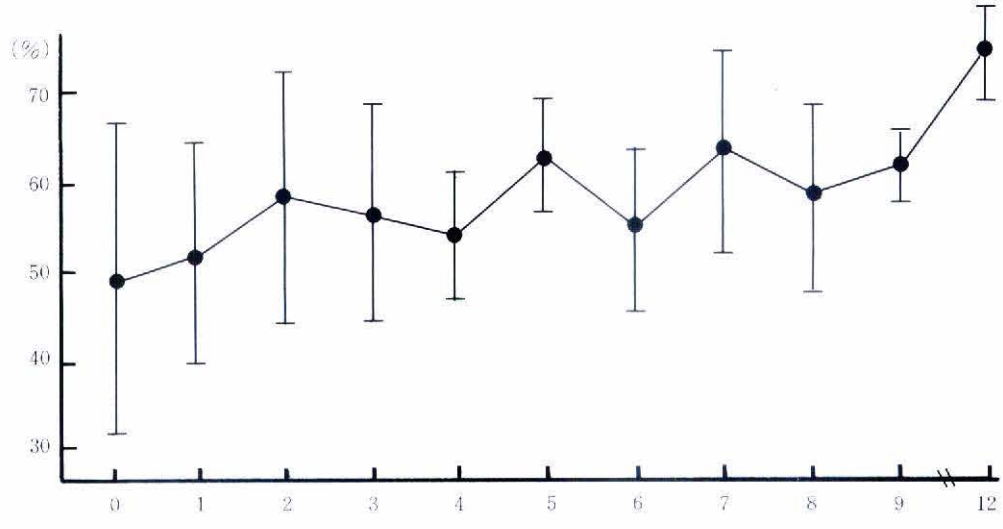

Fig 1 Changes of rosette forming cells (RFC) in the peripheral blood of the calves. Each value is expressed by mean±S.D.

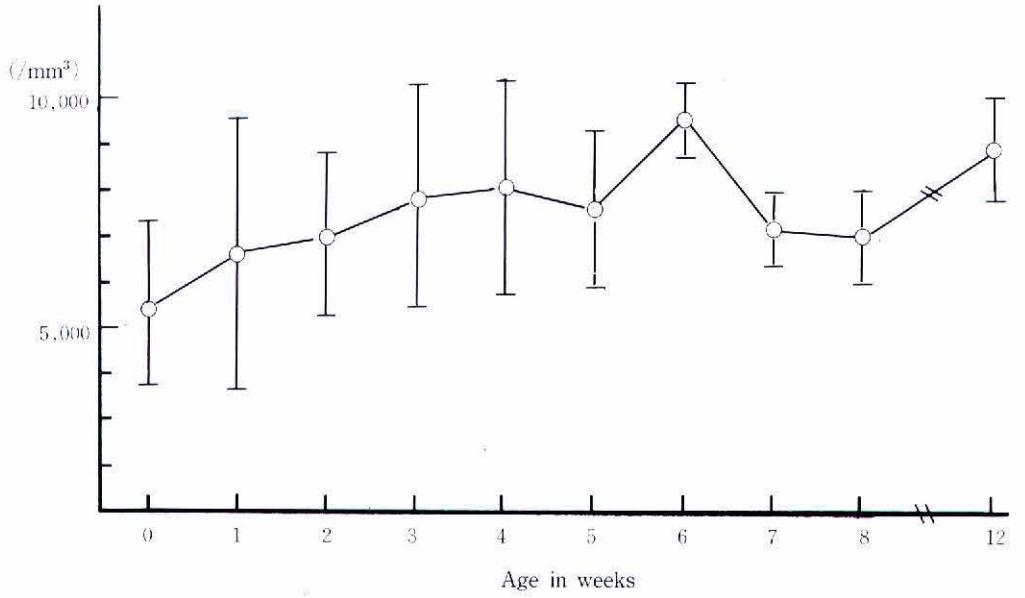

Fig 2 Absolute lymphocyte numbers in the peripheral blood of the calves. Each value is expressed by mean±S. D.

200*g* for 5 min. After overnight incubation at 4°C, a drop of 0.2% brilliant cresyl blue (BCB) was added to the cell suspension and gently shaked. A drop of cell mixture was placed in a haemocyte meter and 400 cells were counted. A lymphocyte with three or more adherent SRBC was judged as a rosette forming cell (RFC). The results were expressed as a mean of two determinations.

These methods and conditions were based on the methods of Paul et al.(1979).

Results

The percentages of RFC in the peripheral blood of 19 healthy adult cows ranged from 60.0 to 80.5% with a mean of 70.34±8.69%, higher than the data of paul et al. (62.80±9.95%, 1979).

As shown ir Fig 1, it was observed that the neonatal calves had 48.10±18.0% of RFC soon after birth in their peripheral blood although the variations were wide. These levels of RFC increased gradually with the proceeding of age and by the time the calves were 12 weeks old, it was the same as in adult animals (73.25±5.36%) and the variations became rather small.

The changes of absolute lymphocyte numbers ara given in Fig. 2. The lymphocyte count increased steadily from 5,000 at birth to 8,000 per microliter at 12 weeks of age, when it seemed to remain constant until the calves matured. These changes paralleled RFC fluctuations.

Discussion

Lymphocytes possessing the ability to bind SRBC and form rosette have been classified as T lymphocytes were reported to develop in the thymus and enter the circulation. Then they recirculate or settle in lymphoid organs such as thymus or lymph nodes.

There are few studies of neonatal T lymphocytes in cattle. Binns (1978) found the lower percentages of RFC in the peripheral blood of young calves (23%) although in the lymphoid tissues such as thymus and lymph nodes, the higher percentage of RFC was observed. Outteridge and Dufty (1973) reported that in

younger cattle, the percentage of RFC was lower than in mature cattle and this level increased with maturation. They also stated that very young calves contained a population of unmarked or 'null' cells. In their studies, dextran was used in the rosetting medium to enhance the rosette formation but SRBC used was non-treated.

In this study, improved rosetting method of using AET treated SRBC was employed. This treatment was confirmed to enhance and stabilize rosette formation of T lymphocytes (Kaplan & Clark, 1974, Grewal & Babiuk, 1978, Paul et al., 1979). From the result of this study, it was concluded that calves had about 70% of adult level even soon after birth, although great variations expressed by large S. D. were observed among the calves. It is suggested that calves had aquired circulating T lymphocytes when they were in the womb. Senogles et al. (1979) reported that the proportion of peripheral T lymphocytes in fetal calves was only 1% in the first trimester but at the end of the gestational period it increased to 45%. It is assumed that neonatal calves had immune competence on cellular immunity even after birth and in the following weeks, it reached adult level.

In contrast the percentage of immunoglobulin bearing lymphocytes (B cells) in the peripheral blood of neonatal calves had been found to be only 5% (Senogless et al., 1978).

In contrast, the percentage of immunoglobulin to infection is due to the immaturity of B lymphocytes and sequent hypogammaglobulinaemia. Previous studies concluded that B cell maturity was a T cell dependent phenomenon and T cells obtained from newborn children had suppressive activity on B cell maturity (Hayward & Lawton 1977, Durandy et al. 1979).

Further studies on the function of calf lymphocytes, especially in the T cells and subsets, are desired to clarify the immunopotency of neonatal calves.

References

Belden, E. L., Mccroskey, R. M. K. & Strelkauskas, A. T. (1981) *Veterinary Immunology and Immunopathology* **2**: 467–474.

Binns, R. M. (1978) *Journal of Immunological Methods* **21**: 197–210.

Durandy, A., Fisher, A. & Griscelli, C. (1979) *The Journal of Immunology* **123**: 2644–2650.

Grewal, A. S., Rouse, B. T. & Babiuk, L. A. (1976) *Canadian Journal of Comparative Medicine* **40**: 298–305.

Grewal, A. S. & Babiuk, L. A. (1978) *Journal of Immunological Methods* **24**: 355–361.

Hayward, A. R. & Lawton, A. R. (1977) *The Journal of Immunology* **119**: 1213–1217.

Higgins, D. A. & Stack, M. J. (1977) *Clinical and Experimental Immunology* **27**: 348–356.

Kaplan, M. E. & Clark, C. (1974) *Journal of Immunological Methods* **5**: 131–135.

Outteridge, P. M. & Dufty, J. H. (1973) *Research in Veterinary Science* **14**: 389–391.

Paul, P. S., Senogles, D. R., Muscoplat, C. C. & Johnson, D. W. (1979) *Clinical and Experimental Immunology* **35**: 306–316.

Senogles, D. R., Muscoplat, C. C., Paul, P. S. & Johnson, D. W. (1978) *Research in Veterinary Science* **25**: 34–36.

Senogles, D. R., Paul, P. S., Johnson, D. W. & Muscoplat, C. C. (1979) *Clinical and Experimental Immunology* **36**: 299–303.

初生期における仔牛の末梢血Tリンパ球の変動について

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摘　　要

初生期における仔牛の免疫機能検討の一環として，仔牛の末梢血中のTリンパ球の生理的変動を，出生直後より一週間毎に12週齢まで検査した。Tリンパ球の検出は，PAULらの方法によるAET処理を施したヒツジ赤血球に対するロゼット形成能を指標とし，成績は分離リンパ球に対する百分率で表わした。この結果，仔牛は出生直後に，かなりの個体差はあるもののすでに平均50%近いTリンパ球を保有しており，加齢と共に多少の変動を伴ないながら徐々に増加し，12週齢では成牛レベルにまで達した。また，リンパ球総数もTリンパ球と平行した推移を示した。この様に仔牛は，出生直後に，すでに数の上では成牛に近いTリンパ球を保有しており，胎仔期に細胞性免疫能はある程度まで完成しているものと思われた。

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