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BREED-RELATED DIFFERENCES IN THE LYMPHOCYTE TRANSFORMATION IN RAMS

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ABSTRACT

The development of cell-mediated immunity in rams was analyzed by the breed-dependent patterns of the responsiveness of peripheral blood lymphocytes to common mitogens (phytohemagglutinin, PHA; concanavalin A, ConA; poke-weed mitogen, PWM), in a mixed lymphocyte culture (MLC), in order to establish the basic immune parameters in clinically normal rams.

Investigations were carried out on 26 rams from the breeds Karakachan and Copper-Red Shoumen. It was established that there are close differences between prosliferative activity of lymphocytes of the two groups of animals. The immune parameters obtained for the two local sheep breeds may serve as a basis for further research on lamb systemic immune responsiveness regarding differences among breeds.

Key words: rams native breeds of sheep, Karakachan breed, Copper-Red Shoumen breed, mitogens, cell-mediated immunity, lymphocytes.

Introduction

For many years, genetic selection in sheep production has targeted the improvement of growth rate, feed efficiency and sheep meat quality. Adaptation to housing conditions among the breed was evaluated of animal neuroendocrine and immune system (4, 5).

There is clear evidence in rodents, poultry, pigs and cattle that it is possible to selectively breeding for high (H), average (A) or low (L) – immune responsiveness, and that H-responders can positively influence resistance to infectious disease (3)

Cell-mediated immunity is a primary component in the defense against most pathogens, but we didn't find information about responsiveness of peripheral blood lymphocytes to common mitogens (phytohemagglutinin, PHA; concanavalin A, ConA; poke-weed mitogen, PWM) in a mixed lymphocyte culture (MLC) of rams.

The purpose of the study reported here was to determine the effect of the breed on the function of rams lymphocytes using the blast transformation test.

Materials and methods

Animals. The study was conducted on 18 rams from the breed Karakachan and 8 rams from the breed Copper Red Shoumen.

Blood collection. From each healthy animal venous blood was collected with Vacutainer tubes containing heparin as an anticoagulant.

Lymphocyte isolation. Peripheral blood mononuclear cells were isolated from heparinized blood samples by density gradient centrifugation, washed in Hank's Balanced salts (HBSS) culture medium and resuspended at a concentration of 2.10^6 cells/ml in RPMI 1640 supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin and 1% L-glutamine. (2). The numbers of viable cells were evaluated during time courses by counting trypan blue-excluding cells.

Test setting. The resulting lymphocytes from the two group of rams were cultivated in a final concentration of 1.10^6 cells/ml. Three test tubes of each sample were prepared, the first of which was inoculated with PHA (10µg/ml, Sigma-Aldrich), the second – with Con A (5 µg/ml, Sigma-Aldrich), and the third - with 5µg/ml PWM. The cultures were placed in a thermostat at 37 °C for 5 days. After cultivation the tubes were centrifuge at 300 x g for 10 minutes. Smears were prepared from the sediment and were stained with Romanovsky-Giemsa dye. Two hundred lymphocytes were counted in each sample. The blast cells were expressed in percent. Large size, presence of numerous nuclei, appearance of vacuoles in the cytoplasm, the so called "sponge cytoplasm" were the criteria to distinguish between the blast and normal lymphocytes.

Statistical analysis. The significance of differences for all parameters was estimated by Student's T-criterion.

Results and Discussion

Blood lymphocyte proliferation was affected by the presence of mitogens in cell cultures (Fig. 1).

Lymphocyte blastogenic responses to the PHA, Con A and PWM in rams were observed. Lymphocyte proliferation was greater ($p<0.01$) in rams from Cooper-Red Shoumen breed than rats from Karakachan breed when PWM was used as a mitogen. However, when PHA was used, lymphocyte proliferation was greatest ($p<0.05$) in rams of the same breed compared to the other breed. Con A showed low stimulation in the rams of both breeds.

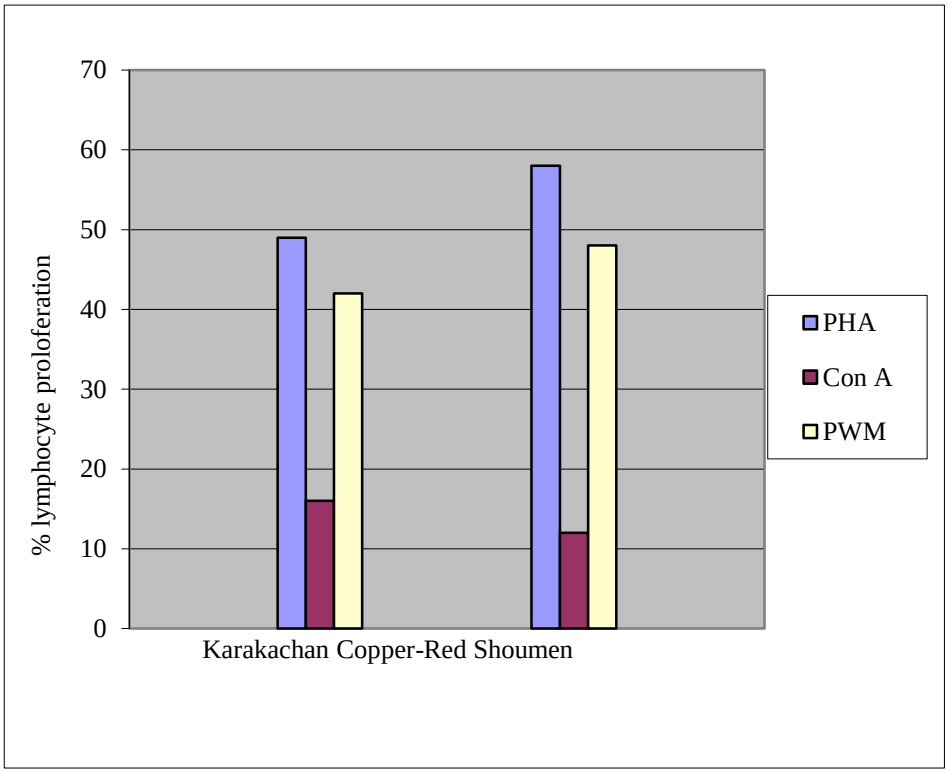

Figure 1: Blast transformation of lymphocytes of rams from two different local breeds.

In vitro cellular immunity was measured by a lymphocyte blastogenesis assay.

Studies with purified lymphocyte populations have shown that the presence of monocytes or macrophages is essential for maximal response of lymphocytes to mitogen stimulation (1). In our work we didn't deplete them by absorption because macrophage and monocytes had an essential role in the modulation of lymphocyte activity.

Lymphocyte purification by Ficoll–Paque as a density gradient is satisfactory. It is used in goats too (7). In preliminary studies we determined the optimal concentrations of lectins and they were given a good stimulation of rams lymphocyte proliferation. Only Con A at the concentration of 5 μg per ml showed low values. Ram et al. (1987) also reported a low efficiency of Con A in stimulating goat lymphocytes. Pokeweed mitogen is a T-cell-dependent B-cell mitogen, whereas PHA is a T-cell mitogen.

Stimulation of different cell population may explain the differences in immune response in rams from the breeds Karakachan and Copper-Red Shoumen.

Conclusion

Our present findings may serve as a basic for further research on ram cell-mediated immunity in relation to differences among breeds, differences in age, development and physiological status.

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