Nonspecific esterase and E-rosette formation of bottle-nosed dolphin lymphocytes

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Staining of bottle-nosed dolphin lymphocytes by nonspecific esterase stain and E-rosette test were performed. 35.4 % of lymphocytes were simultaneously E-rosetted and NE positive. They are apparently, mature T-cells. On the size and shape of esterase-positive granules at least 2 subpopulations of lymphocytes are distinguishable.

Key words: Nonspecific esterase, lymphocytes, E-rosettes, bottle-nosed dolphin.

Introduction

For the first time Müller has offered to use nonspecific esterase (NE) as a marker of mouse T lymphocytes [3]. Later numerous investigations of various mammal species lymphocytes and, first of all, man with cytochemical esterase technique appeared. However till now it is not clear, whether higher activity of this enzyme in T cells is the feature of some mammal species or wether it is general attribute of all animals with an advanced lymphoid system.

Properties and functions of marine mammal lymphocytes are investigated very poorly. And at the same time these animals are a valuable objects for study of immunodeficient states, as the evolution in clean environment could resulted in preservation of primitive features of lymphoid system. Moreover, metabolic changes, induced by hypoxia in diving, influence the functions of immunocompetent cells. Question posed long ago [3] about existence in cetaceans and pinnipeds immune system features, ensuring low immunological resistance in modern environmental conditions, is not cleared up to a sufficient degree. Cytochemical researches could allow to consider this question from another point of view and also to add ideas about metabolic immunodeficiencies. In this paper we have attempted to define possibilities of dolphin lymphocyte distinction on activity of nonspecific esterase. Spontaneous rosette formation test with sheep erythrocytes (E-rosettes) was also applied for marking T-lymphocytes.

Materials and methods

The venous blood was obtained from 14 adult dolphins. Blood is taken from veins of a tail fin in test-tubes with heparin. Centrifugation on ficoll-verografin density gradient (1.077 g/ml) was applied to separate lymphocytes. The smears of the whole blood and lymphocyte concentrate were fixed by a neutral formalin for 10 minutes at 4°C and in formalin vapours for 5 minutes at a room temperature. Then they were stained in the mixture, containing alpha-naphthylacetate and hexazotized fuchsine at pH 5.8. All procedures of rosette formation test, subsequent fixation and staining of rosette-forming cells were carried out according to the method, described by Obrist [4],

except for use Hank's balanced salt solution instead of the minimal essential medium.

Results and discussion

At the staining of smears by Müller (fixation for 10 minutes in 10 percent neutral formalin at 4°C and incubation for 21 hours at room temperature) 73.3±5.5% of lymphocytes were esterase-positive. The coloured product of reaction is concentrated in 1-3 rounded or oval granules. After fixation in formalin vapours and staining during 2, 4, 6, 12, 24 hours at room temperature relative number of esterase-positive lymphocytes was 59.5±4.9; 74.3±4.4; 81.2±3.9; 98.6±1.2 and 100%, respectively. In this case the morphological picture of reaction is more clear, than after water solution of formaldehyde. The peculiarities of the reaction product intracellular distribution are seen better. The number of esterase-positive granules is higher: up to 20. According to the size and shape of esterase-positive area opposite to a cavity in the nucleus two groups of lymphocytes are distinguishable, called in the literature "granular" or "dot-like" (small granules of rounded shape) and "large granular", "globular" or "paranuclear" (large granules like cap). The first type of reaction is displayed by T helper cells and, at least, part of the circulating human B lymphocytes. The second type of reaction is characteristic of T supressor cells, killers and null lymphocytes [2]. Indirect proof that lymphocytes of dolphins with the last type of esterase reaction could have similar immunological • properties we have got, studying blood cells of pregnant bottle-nosed dolphins. The average number of lymphocytes with paranuclear reaction was during pregnancy (12 months) 64.6±1.4 and 51.3±1.6% and in a month up to pregnancy - 31.0±4.6 and 33.0±4.7%, respectively. Two nonpregnant females, observed together with pregnant females during 16 months had in this period a lower level of this parameter: 21.1 ±1.2 and 27.8±1.4% . Pregnancy, as the implantation of genetically foreign material (genes of the father in the genotype of a fetus) in an organism of a mother, is accompanied by an immunodepression, increase in the number of immature lymphocytes. Therefore the stated assumption is quite plausible.

The picture of esterase reaction after the test of rosette formatoin and fixation by glutaraldehyde is similar to the observed after fixation in formaldehyde solution. But, together with esterase-positive granules, diffuse staining is present sometimes. Esterse-positive E-rosetted lymphocytes demonstrate a dot-like NE reaction. The data about the ratio of various groups of lymphocytes at direct staining of E-rosette forming cells are shown in the table.

The numbers of E-rosetted and NE positive lymphocytes are close: 58.7 and 55.1 percent, respectively. At the same time, E-rosette positive and E-rosette negative lymphocytes include almost similar percentages of NE positive cells. Nevertheless, among esterase-positive cells the

number of E-rosettes is higher, than among NE negative cells. It is the evidence that lymphocytes of dolphins with different cell surface receptors and, probably, immunological functions, differ also in nonspecific esterase activity.

Table. Percentages of E-rosetted and NE active lymphocytes (mean±standard error / range)

Esterase activity	E-rosettes	
	Positive	Negative
Esterase positive	35.4±2.7 30.2-39.8	19.7±5.4 4.2-27.8
Esterase negative	23.3±7.3 12.2-44.5	21.6±5.3 11.5-31.9

The functional significance of dolphin lymphocyte subpopulations, distinguishing by size and shaped of esterase-positive granules, can be defined with the help of some of the sparse immunological tests, used in marine mammal researches, such as stimulation by polyclonal mitogens. Nonspecific esterase is lysosomal enzyme, therefore heterogeneity of lymphocytes reflects distinctions between differential types of cells, degree of differentiation and the level of antigene stimulation. Changes in cellular organells at differentiatioin and preparation of cells to division are similar in various species of the animals. It could be a basis for application of nonspecific esterase in comparative immunobiological studies of marine and terrestrial mammals.

References

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