ASSESSMENT OF THE PROTECTIVE ACTION OF DIPHOSPHONATE COMPOUNDS AGAINST DAMAGE TO T-LYMPHOCYTES BY ANTILYMPHOCYTIC SERUM

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Rabbit antilymphocytic serum (ALS) and complement were taken in doses causing death of 50% of lymphocytes (stained with 1% trypan blue solution). The number of rosette-forming and transformed cells in the rosette-formation and blast-transformation tests was 60 and 24% of the number in the control respectively. After addition of 0.1 ml of 10 mM solution of the potassium salt of hydroxyethylenediphosphonic acid, belonging to the group of synthetic diphosphonates, a protective effect was observed: The number of rosette-forming and transformed lymphocytes did not differ from the control, in which the test was carried out with intact cells.

KEY WORDS: T-lymphocytes; antilymphocytic serum; immune injury; calcium ions.

Synthetic diphosphonates belong to a group of complex-forming compounds which bind ions of the alkaline earth metals, including Ca ions, which are utilized in complement-dependent immune reactions. The choice of this complexone was determined by the fact that, unlike the widely used ethylenediaminetetraacetate (EDTA), it has no marked toxic action [1].

The object of this investigation was to study in vitro the protective action of diphosphonate compounds and, in particular, the potassium salt of hydroxyethylenediphosphonic acid (HEDPA), using a model of immune injury to human lymphocytes by antilymphocytic serum (ALS).

EXPERIMENTAL METHOD

Lymphocytes were isolated from the peripheral blood in a Ficoll–Verografin density gradient (density 1.077). The suspension contained 0.2×10^6 cells/ml. The dilutions of rabbit ALS and the quantity of complement necessary to cause death of 50% of human lymphocytes, revealed by staining with 1% trypan blue solution, were determined in preliminary experiments. The potassium salt of HEDPA was used in a concentration of 10 mM.

The functional state of the T-lymphocytes was determined from their ability to take part in the rosette-formation test by the method of Mendes et al. [5] in the modification of Petrov et al. [4] and Kochergin [3], and in the blast-transformation test (BTT) of lymphocytes with nonspecific mitogen by the method of Zaretskaya et al. [2]. In 25 investigations each of these reactions was carried out simultaneously in three parallel tests: 1) a control with intact lymphocytes; 2) reaction with washed lymphocytes after injury by ALS and complement in a dose causing death of 50% of the cells; 3) a reaction with washed lymphocytes incubated with ALS and complement for 30 min at 37°C in the presence of the potassium salts of HEDPA in a dose of 0.1 ml of 10 mM solution to 1 ml of lymphocyte suspension (final concentration 1 mM).

EXPERIMENTAL RESULTS

It was noted during analysis of the results that the number of rosette-forming T-lymphocytes in the case of preliminary treatment with ALS and complement was 60% compared with the control (intact cells), whereas the number of transformed cells in the BTT was only 24%. The difference between the results of these tests

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can be explained by the fact that the membrane of some lymphocytes shown by staining with 1% trypan blue solution to have died still remained capable of fixing sheep's red cells for some time. This fact was verified by performing the rosette-formation test with completely dead cells obtained after treatment with large doses of ALS and complement. Some of these cells were found to fix 1, 2, or in some cases even 3 red cells to themselves. In experiments with lymphocytes treated with ALS and complement in the presence of HEDPA, the percentages of rosette-forming cells and of cells transformed under the influence of phytohemagglutinin were virtually indistinguishable from the control. Statistical analysis of the data showed that the effect of HEDPA is significant ($P < 0.05$).

It can thus be concluded from the results of these investigations that the potassium salt of HEDPA protects T-lymphocytes against immune injury caused by ALS and complement, possibly as a result of binding of Ca ions. Some injured lymphocytes, considered to be dead on the basis of staining with 1% trypan blue solution, could still form rosettes with sheep's red cells. Finally, the blast-transformation test was shown to be more sensitive than the rosette-formation test for assessment of the viability of T-lymphocytes.

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DEPENDENCE OF THE FUNCTIONAL ACTIVITY OF IMMUNOCOMPETENT MOUSE SPLEEN CELLS ON THEIR HEMATOPOIETIC MICROENVIRONMENT

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Accumulation of erythroid cells and granulocytes, changes in the relative and absolute numbers of T-, B-, and "null" lymphocytes, and also sharp inhibition of the primary immune response were found in the spleen of CBA mice treated with specific antierythrocytic serum (AES). The inductive phase of immunogenesis proved to be sensitive to the action of AES. Injection of syngeneic macrophages and splenocytes did not overcome the immunodepression. The results can be regarded as evidence of the existence of functional dependence between the immunocompetent cells of the spleen and their nonlymphoid hematopoietic microenvironment.

KEY WORDS: immune response; T- and B-cells; microenvironment.

The mouse spleen is an important peripheral organ of immunity. In the plasma cells of its red pulp, most of the humoral antibodies of the organism are synthesized in response to intraperitoneal or intravenous injection of an antigen [1]. Meanwhile marked hematopoiesis takes place in the spleen. Cells of the erythro-, myelo-, and megakaryocytic type coexist with lymphocytes. It may be that a hematopoietic microenvironment is essential for the normal functioning of immunocompetent cells. If this is true, a change in the ratio between hematopoietic nonlymphoid components of the red pulp or injury to them may be reflected in the intensity of the immune response. There are as yet only solitary communications that suggest the existence of a functional

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