ANTIGENIC DIFFERENCES IN THYMOCYTES AND LYMPHOCYTES

N. V. Medunitsin

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The use of the indirect immunofluorescence method has shown that lymphocytes of the thymus and lymphocytes of extrathymic origin contain common antigens, while thymocytes in addition possess antigens absent from other lymphocytes.

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Thymocytes and lymphocytes of extrathymic origin are indistinguishable morphologically but they react differently to physical, chemical, and biological agents: x rays, phytohemagglutinin, antigens, and so on. In their antigenic properties, thymocytes and lymphocytes are very similar. Antithymocyte serum, like antilymphocyte serum, reduces the number of circulating lymphocytes in vivo and causes agglutination of lymphocytes in vitro [1, 3]. On the other hand, there is experimental evidence that antigenic differences between thymocytes and lymphocytes may exist. Experiments on rats [2] have shown that antiserum against the microsomal fraction of thymocytes, if carefully absorbed by other lymphoid cells, can react specifically only with thymocytes.

Antigenic differences between thymocytes and lymphocytes were studied in guinea pigs. The indirect immunofluorescence method was chosen, using two labeled antisera: rabbit antiserum against guinea pig thymocytes and goat antirabbit serum.

EXPERIMENTAL METHOD

Rabbits were immunized three times with thymus cells from guinea pig embryos aged 7-8 weeks. Cells were isolated from the thymus with glass needles and washed three times with Dulbecco's solution containing 10% dextran solution. The cells were injected intravenously into a rabbit at intervals of two weeks in doses of 1.2×10^8, 1×10^8, and 1.5×10^8 cells. Blood serum was obtained from the animals two weeks after the last injection of cells and inactivated at 56° for 30 min.

To remove antibodies against serum proteins and erythrocytes, 5 ml of the inactivated serum was treated with 1 ml washed erythrocytes and 0.2 ml blood serum of a normal guinea pig. The mixture was incubated at 37° for 1 h. The agglutinated cells were removed by centrifugation and absorption of the serum by the erythrocytes was repeated 3 or 4 times until the hemagglutinating power of the serum had completely disappeared. Serum was exhausted with liver tissue in the same way. The absorption was carried out once and 10 ml serum was used with the washed homogenate obtained from the liver of one adult guinea pig.

To determine the activity of the antithymocyte serum, the agglutination reaction with cells from the thymus and lymph glands of a guinea pig was used. A series of 1:2 dilutions of serum was prepared in Dulbecco's solution. To 0.5 ml diluted serum, 0.05 ml of a suspension containing about 2×10^7 thymus cells or lymph gland cells per ml was added. After incubation of the mixture for 1 h at 37°, one drop of acridine orange in a dilution of 1:5000 was added, vital preparations were made, and these were examined under the luminescence microscope. A series of tubes with various dilutions of normal rabbit serum and tubes containing no serum whatsoever were used as controls. Normal serum, like the antiserum, was first treated with guinea pig erythrocytes, blood serum, and liver tissue.

To obtain antiserum against rabbit γ-globulin, a goat was immunized by intravenous injection of 12 ml blood serum of a normal rabbit. Reimmunization was carried out two months later by the same method, the serum being injected in fractional doses to avoid an anaphylactic reaction. The antiserum was obtained two weeks after reimmunization.

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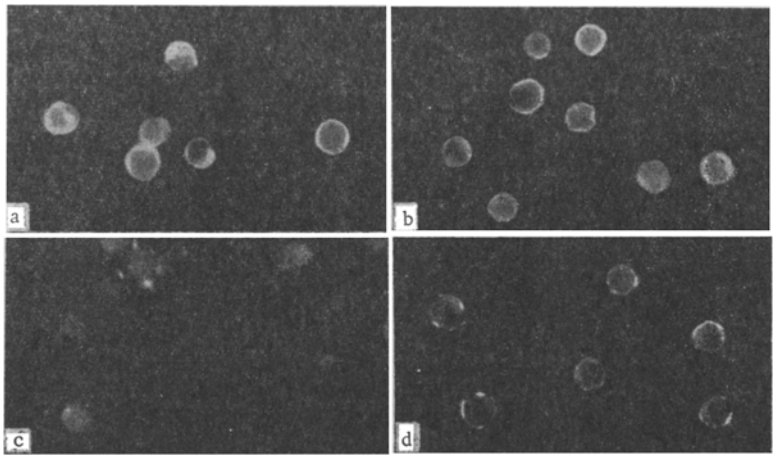

Fig. 1. Antigenic differences between thymocytes and lymphocytes. Cells were treated with two labeled sera in succession: rabbit antiserum against guinea pig thymocytes and goat antirabbit serum; lymphocytes (a) and thymocytes (b) before absorption of antithymocyte serum by lymph gland and spleen cells of a guinea pig; lymphocytes (c) and thymocytes (d) after absorption of serum. 450×.

The globulin fraction was precipitated from the goat and rabbit sera with a semisaturated solution of ammonium sulfate. After dialysis for 6 days the 2% protein fraction was labeled with fluorescein isothiocyanate and passed through a column containing Sephadex G-50. Activity of the labeled antirabbit serum was tested by the microdiffusion reaction in agar. Before the experiment began, labeled serum diluted 8-16 times was treated with guinea pig liver powder at the rate of 50 mg powder to 1 ml liquid.

A suspension of cells containing about 2 million lymphocytes/ml was prepared separately from the spleen, lymph glands, and thymus of guinea pig. To 0.5 ml of the washed cells, 0.5 ml of labeled absorbed antithymocyte serum in a dilution of 1:16 was added and the mixture was incubated for 30 min at 37°. The cells were washed 3 times and treated in the same manner with labeled goat antirabbit serum in a dilution of 1:8. They were then again washed, a vital preparation made from their residue, and this was examined under the luminescense microscope. Preliminary washing of the cells, dilution of the serum, and washing the cells to remove serum were performed with Dulbecco's solution containing 10% dextran. Control tests were carried out at the same time with cells treated with labeled antirabbit serum only and with the antithymocyte serum replaced by labeled rabbit antiserum against human γ-globulin. Altogether 17 experiments were carried out.

EXPERIMENTAL RESULTS

According to the agglutination reaction both with thymus cells and with lymph gland cells, the antibody titer of the antithymocyte serum was 1:256. The microdiffusion reaction in agar with extracts of lymphoid cells fragmented by repeated freezing and thawing was negative.

The indirect immunofluorescence method showed that antibodies of the antithymocyte serum, unabsorbed by spleen and lymph gland cells, can be fixed both on thymocytes and on lymphocytes from other lymphoid organs. During treatment of the cells with the two labeled sera in succession, both types of cells acquired bright fluorescence. The cells fluoresced in the form of rings, because of fixation of antibodies on their surface (Fig. 1a, b). These results, like the cross agglutination results, demonstrate the presence of common antigens in thymocytes and lymphocytes.

In the absence of complement, the antibodies probably could not produce a powerful cytotoxic effect, because the nuclei of most cells remained unstained.

To detect the specific antigenic properties of the thymocytes, antithymocyte serum was carefully absorbed with cells from the lymph glands and spleen of a guinea pig. Cells of the spleen and the popliteal, inguinal, and cervical lymph glands, together with connective-tissue fragments of these organs from one

guinea pig were washed 6 times with Dulbecco's solution. The residue was treated with 4 ml labeled antithymocyte serum diluted 1:16 and the mixture was incubated at 37° for 1 h. After centrifugation, a fresh batch of lymphoid cells was added to the supernatant. The absorption procedure was repeated twice or three times.

The indirect Coons' test was carried out with the absorbed serum. In the course of repeated absorption the intensity of fluorescence of both lymphocytes and thymocytes was reduced. However, whereas cells from the lymph glands and spleen, with a few exceptions, gradually lost their power of fluorescence, the thymus cells retained a thin, yet clear ring of fluorescence (Fig. 1c, d). The intensity of fluorescence of the cells decreased when the labeled antithymocyte serum was replaced by unlabeled. In the direct immunofluorescence method (treatment of the cells with labeled antithymocyte serum only) weak fluorescence of the cells was observed.

In control preparations of thymocytes treated with labeled antirabbit serum only or preliminarily incubated with normal rabbit serum, no fluorescent cells were present. Fluorescence of the thymocytes also disappeared if antithymocyte serum was preliminarily absorbed by guinea pig thymus cells.

The results show that lymphocytes of the thymus differ in antigenic composition from lymphocytes of other lymphoid organs. Besides antigens common to all lymphocytes, they also contain specific antigens characteristic of this type of cell. The presence of these antigens in thymocytes is probably due to their distinctive nature. In the modern view, thymocytes are epithelial in origin, whereas lymphocytes of the spleen and lymph glands are mesenchymal. On the other hand, the possibility cannot be completely ruled out that the antigenic differences discovered are due to quantitative rather than qualitative differences in the antigenic composition of these cells. For example, thymus cells are richer in nucleic acids, and this may influence the results of immunologic tests, although the characteristic arrangement of specific thymocyte antigens on the surface of the cells, demonstrated in the present experiments, does not confirm an important role of nucleic acids in determining antigenic differences between lymphoid cells.

The presence of isolated cells in the spleen and lymph glands preserving their fluorescence even after careful absorption of the antithymocyte serum indicates the possible existence of a special type of lymphocytes entering the general circulation and lymphoid organs from the thymus. As yet, however, there is no direct proof that lymphocytes can leave the thymus. The immunofluorescence method, with its ability to differentiate thymocytes from lymphocytes of extrathymic origin, may prove useful for the solution of this problem.

LITERATURE CITED

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