INVESTIGATION OF THE ACTION OF ANTILYMPHOCYTIC PREPARATIONS ON THE FORMATION AND OPERATION OF IMMUNOLOGICAL MEMORY

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Experiments on mice showed that antilymphocytic preparations almost completely prevent the formation of an immunological memory to sheep's erythrocytes and have only a slight effect on its operation in response to repeated injections of the antigen. If, however, spleen cells were treated with antilymphocytic globulin in vitro, they equally lost their ability to induce a primary and a secondary immune response.

The study of the immunodepressive properties of heterologous antilymphocytic serum (ALS) has been stimulated by the demands of medical practice. ALS and immunoglobulin isolated from it are known to effectively depress the formation of humoral antibodies in animals immunized by soluble and corpuscular antigens [1, 4, 7], and also to retard the development of transplantation immunity [1, 11, 14]. Accordingly, antilymphocytic preparations have been used in conjunction with other immunodepressants in clinical practice during homografting of organs [5, 13]. Besides its great practical importance, the study of the mechanism of action of ALS is also of great theoretical interest because this agent can be used as an instrument to study different forms of immune response.

The objective of the present investigation was to study the action of heterologous ALS and antilymphocytic globulin (ALG) on the formation and operation of the immunological memory of mice immunized with two small doses of sheep's erythrocytes and, also, to compare the action of ALG on the primary and secondary reactions of lymphocytes to this same antigen in the intact animal and after treatment of spleen cells with ALG in vitro.

EXPERIMENTAL METHOD

Adult noninbred albino mice and mice of line A/He were used in the experiments. ALS was obtained by the method of Levey and Medawar [10] by intravenous immunization of rabbits with cells from the thymus and lymph glands of line A/He mice ($1 \cdot 10^9$ cells per course). The titer of ALS in the lymphocyte agglutination test was 1:256. ALG was obtained from the laboratory of immunology of the Moscow Research Institute of Epidemiology and Microbiology, Ministry of Health of the RSFSR,* by salt fractionation using ammonium sulfate from the serum of a horse immunized with small lymphocytes taken from the thymus and spleen of noninbred mice. The preparation was used as a solution containing 4.7% protein. The ALG titer in the lymphocyte agglutination test was 1:1280. Both preparations were absorbed by mouse erythrocytes until complete elimination of the hemagglutinins had been obtained.

ALS and ALG were injected intraperitoneally into mice in doses of 0.25 and 0.2 ml respectively. The mice were immunized intravenously with sheep's erythrocytes either in a single dose of five hundred million cells or in two doses, each of one million cells, with an interval of 3 weeks.

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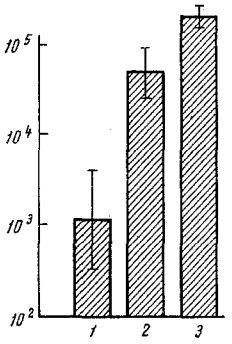

Fig. 1

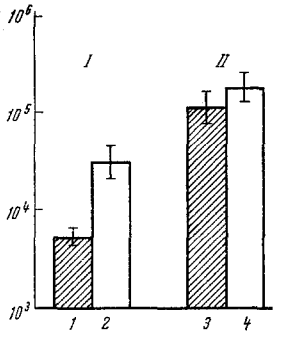

Fig. 2

Fig. 1. Effect of ALS on formation and operation of the immunological memory: 1) injection of ALS before first injection of antigen; 2) injection of ALS before revaccination; 3) control. Ordinate, number of antibody-forming cells in spleen.

Fig. 2. Effect of ALG on primary (I) and secondary (II) immune response to immunization of mice with sheep's erythrocytes: 1, 3) experiment; 2, 4) control. Ordinate, number of antibody-forming cells in spleen.

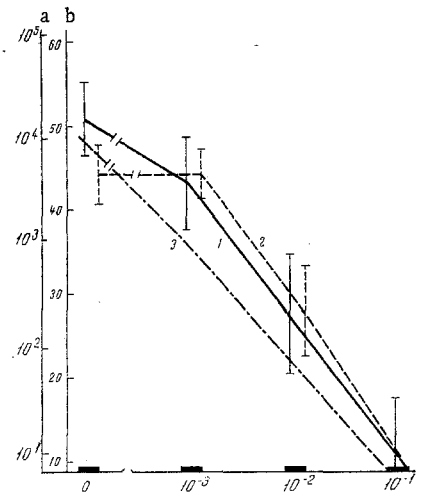

Fig. 3. Number of antibody-forming cells and total number of nucleated cells in spleen of irradiated recipients receiving spleen cells treated with ALG in vitro: 1) transfer of sensitized cells $+1 \cdot 10^6$ sheep's erythrocytes; 2) transfer of intact cells $+5 \cdot 10^8$ sheep's erythrocytes; 3) total number (in millions) of nucleated cells (combined data). Abscissa, ALG concentration (relative to original preparation); ordinate: a) number of antibody-forming cells, b) number of nucleated cells.

In the experiments to study the action of ALG on cells in vitro, various dilutions of the preparation (1:10, 1:100, and 1:1000) were used. Suspensions of spleen cells of intact A/He mice, or of mice of the same line sensitized several weeks earlier by one million sheep's erythrocytes, made up in medium No. 199 were incubated at 37°C for 30 min with different dilutions of ALG in the presence of guinea pig complement, diluted 1:10 with physiological saline. The cells were washed twice with medium No. 199 and injected in doses of a hundred million into noninbred mice which had been irradiated in a dose of 700-750 R from a cobalt source. The recipients received injections of sheep's erythrocytes: either one million cells mixed with cells of sensitized donors, or five hundred million cells after injection of the cells of intact donors. The number of antibody-forming cells in the spleen was determined on the 4th or 5th (in the experiments with transfer of cells) day after immunization by the local hemolysis in gel test [9]. The geometric mean number of antibody-forming cells in the spleen and its confidence limits were calculated.

EXPERIMENTAL RESULTS

In the experiments of series I, conducted on 60 mice, the effect of ALS on the formation of the immunological memory to sheep's erythrocytes and on its operation in response to repeated injection of antigen was investigated. Injection of ALS was combined with either the first or second injection of antigen (2 days before and on the same day as immunization). Analysis of the results (Fig. 1) shows that the combination of ALS with the sensitizing injection of antigen almost completely prevented the formation of the immunological memory, whereas injection of ALS together with the reacting injection of antigen did not prevent the revaccination reaction, although it slightly weakened it. The results indicate that the process of memory formation is particularly sensitive to the action of ALS, whereas injection of the preparation against the background of an already formed memory was less effective. Similar results were obtained by Kraskina et al. [2] in experiments with the O-antigen of Salmonella typhi.

The object of the next series of experiments, conducted on 40 mice, was to study the effect of ALG on the primary and secondary immune response to a single injection of five hundred million, or two injections, each of one million sheep's erythrocytes. ALG was injected in two doses into the mice 2 days before and on the same day as primary immunization, while to study the secondary response, it was injected 2 days before and on the same day as the reacting injection of antigen. The results of these experiments (Fig. 2) showed that ALG, although inhibiting the primary immune response, as did ALS, was ineffective in depressing the revaccination reaction.

The next step was to ascertain whether this same pattern holds good in the case of direct contact between ALG and cells of the sensitized and intact animal in vitro. Cells treated with ALG were cultivated in vivo in the irradiated recipients, and their immunological reactivity was investigated. These experiments were carried out on 90 mice. It is clear from Fig. 3 that treatment of spleen cells with ALG in vitro led to a sharp decrease in their immunological reactivity, regardless of whether the donors of the cells had been previously immunized with a small dose of antigen or not.

The impression was obtained that ability to give a secondary response was lost under these conditions perhaps to a rather greater degree than the ability to give a primary response.

In fact, the weakest of the ALG concentrations used (1 : 1000) reduced the immunological reactivity of the cells of the previously sensitized donors but had no effect on the cells of intact donors. When the ALG concentration was increased, the immunological reactivity of both populations fell equally. It is clear from Fig. 3 that within the range of ALG concentrations from 1 : 1000 to 1 : 10 the logarithm of the number of antibody-forming cells was inversely proportional to the logarithm of ALG concentration. It is interesting to note that a similar mathematical relationship was observed by Barth [3] under different conditions, when ALG was given to the mice before immunization.

It is clear from Fig. 3 that treatment of spleen cells with ALG in vitro prevented their repopulation in the recipient's spleen. This fact has also been observed by other workers [7, 8]. Conversely, when ALG was injected in vivo, hypoplasia of the lymphoid and hematopoietic tissue usually did not arise [1, 6]. This may be evidence of the existence of tissue barriers protecting the cambial cells of the lymphoid tissue against the action of antilymphocytic preparations.

The differences between the action of ALG on lymphoid cells sensitized with antigen in vivo and in vitro, observed by the present writers and others [7, 8], can be interpreted similarly. Apparently the "memory cells" (the y-cells, in the terminology of Sercarz and Coons [12]) are just as sensitive to ALG as ordinary immunocompetent lymphocytes (x-cells), but in situ, in the intact spleen, they are in areas of lymphoid tissue which are further from the capillaries and are thus "screened" from the action of ALG by other lymphoid cells.

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