CHARACTERISTICS OF CYTOTOXIC T LYMPHOCYTES ELUTED FROM ALLOGENEIC TARGET CELLS

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The increase in the specific cytotoxic effect of immune lymphocytes after their adsorption on the corresponding target cells (TC) and subsequent elution with pronase is due, not to increased cytotoxic activity of individual cells, but to a quantitative increase in the proportion of effector T cells in the population. The eluted and original immune lymphocytes are indistinguishable in the kinetics of their adsorption on TC. The fraction of eluted lymphocytes contains twice as many T cells and four to five times as many cells synthesizing DNA, on account of an increase in the percentage of medium-sized and large lymphocytes.

KEY WORDS: T lymphocytes; cytotoxic effect; allogeneic target cells.

Recent investigations have shown that the heterogeneity of the T-lymphocyte population is due to the diversity and complexity of their function. Effector T cells of transplantation immunity as a rule account for 1-2% of the lymphocyte population of mice immunized in vivo with allogeneic cells [7, 9, 12]. By the use of natural immunosorbents, the fraction of lymphocytes immune to particular H-2 antigens could be selectively removed from the population, concentrated on a monolayer of the corresponding target cells (TC) [1, 2], and subsequently eluted with EDTA or in an acid pH [11], by trypsin [8], and by pronase [3]. In the last case a combination of the methods of elution and removal of B cells led to an elevenfold increase in the cytotoxic activity of the effector lymphocytes, which was observed only in a specific allogeneic system [3].

The object of this investigation was to characterize the fraction of eluted cytotoxic T lymphocytes.

EXPERIMENTAL METHOD

The strain of inbred mice and the methods of immunization with the allogeneic tumor, absorption and elution by pronase, determination of the cytotoxic effect (CE) of the lymphocytes, and separation of the T and B lymphocytes [10] were described previously [2, 3]. For autoradiographic investigation 10^7 lymphocytes were incubated in 1 ml medium No. 199 containing 1 μCi/ml of thymidine-^{3}H (specific activity 1 Ci/mmole) for 1 h at 37°C with periodic shaking. Films were fixed with methanol for 20 min, coated with fine-grain type M emulsion, exposed for 10 days, and stained with Giemsa stain. In each film 1000 cells were counted and the diameter of the nucleus measured. Small (under 7 μ), medium-sized (7-10 μ), and large (over 10 μ) lymphocytes were distinguished.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1a that the number of eluted lymphocytes needed to destroy the same number of TC was only about one eighth of their initial number. This increase in activity could be due either to an increase in the concentration of effector T lymphocytes on account of removal of cells not fixed to the particular monolayer, or to an increase in cytotoxic activity per cell as a result of contact with TC for several hours [6].

Plotting the number of TC undergoing lysis as a function of the number of lymphocytes on a logarithmic scale showed that the corresponding curves were in fact parallel straight lines with the same gradient for the intact and eluted immune lymphocytes (Fig. 1a). Since the angle of slope of both straight lines was close to

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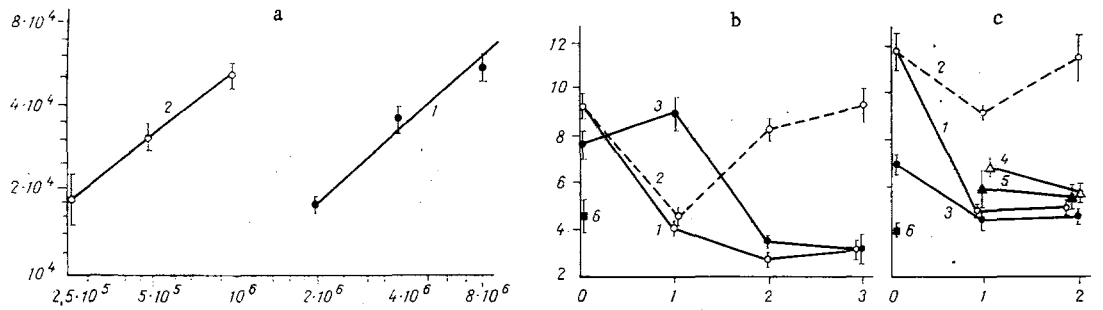

Fig. 1. Kinetics of direct CE (a) and adsorption (b and c) of intact and eluted immune lymphocytes. a: 1) Intact immune lymphocytes; 2) eluted immune lymphocytes. Abscissa, number of lymphocytes; ordinate, number of TC undergoing lysis. b and c: 1) Immune lymphocytes not fixed to TC of recipient A; 2) not fixed to TC of recipient B10; 3) immune lymphocytes eluted from A TC, tested immediately after removal of pronase (b) or after incubation for 1 h at 37°C (c); 4) original immune lymphocytes treated with pronase and tested immediately after its removal; 5) the same, tested after incubation for 1 h at 37°C; 6) normal B10 lymphocytes. Abscissa, time of adsorption (in h); ordinate, liberation of ^{51}Cr (in counts/min·10^3) after incubation of A TC with immune B10 anti-A lymphocytes.

45°, it can be concluded that in both cases CE was the result of "single hit inactivation," i.e., single interactions between the effector cell and TC; this is evidence that cytotoxic activity, expressed per cell, was the same for both populations studied. Assuming that one effector lymphocyte kills only one target cell, it can be calculated from the data shown in Fig. 1a that there is one such lymphocyte in every 120 cells (0.8%) in the initial suspension of immune lymphocytes and in every 14 cells (7%) in the eluted fraction. This means that the adsorption–elution method increases the number of active lymphocytes in the population by eight to nine times, in agreement with the increase in CE [3].

Evidence of the absence of qualitative differences between the initial and eluted effector lymphocytes is also given by the data on the kinetics of their adsorption on TC. It will be clear from Fig. 1b that adsorption for 1 h led to nonspecific fixation of immune lymphocytes to TC of both donor and recipient. Conversely, after incubation for 2 and 3 h with TC, the CE of lymphocytes not fixed to the recipients' TC was restored, but that of lymphocytes not fixed to the donors' TC disappeared. Lymphocytes eluted from the donors' TC were not adsorbed on the same TC in the course of 1 h, but were completely adsorbed during incubation for 2 h. This delay in adsorption of the eluted immune lymphocytes compared with the initial cells may have been due to the time occupied in regeneration of the membrane of the lymphocytes, damaged by pronase during their elution from TC. To test this hypothesis, in the next experiments eluted lymphocytes were incubated for 1 h at 37°C before adsorption on TC. It will be clear from Fig. 1c that in this case the kinetics of adsorption of the eluted and intact immune lymphocytes agreed completely.

If the increase in CE after elution was connected with an increase in the concentration of the effector lymphocytes, an increase in the number of T cells in the eluted fraction would be expected. In fact, the percentage of T cells in the fraction eluted from the donors' TC was twice as great as their percentage in the original immune population (Fig. 2). Removal of B cells led to a further increase in the number of T cells, and during a combination of the adsorption–elution method and the method of fractionation of T and B cells the number of T cells came close to 100%. Conversely, in the fraction of immune lymphocytes eluted from the recipient's TC the percentage of T cells decreased (Fig. 2).*

The morphological composition and proportion of DNA-synthesizing cells of four populations of immune lymphocytes were studied: intact, their "T fraction," eluted, and their "T fraction." As Fig. 3 shows, the first two populations were almost indistinguishable in their morphological composition and in the number of DNA-containing cells. They comprised 70-80% of small, 11-20% of medium-sized, and 4-7% of large lymphocytes. Conversely, the eluted lymphocytes and their "T fraction," which were themselves similar, differed from the first two populations by the considerable decrease in the percentage of small lymphocytes to 38-42 and the increase in the percentage of medium-sized lymphocytes to 39 and of large lymphocytes to 19-22. The

*The values shown in Fig. 2 for the content of T cells are too low because of the necessity of using complement in a dilution of 1:8; in higher concentrations the complement was found to be toxic for lymphocytes treated with pronase.

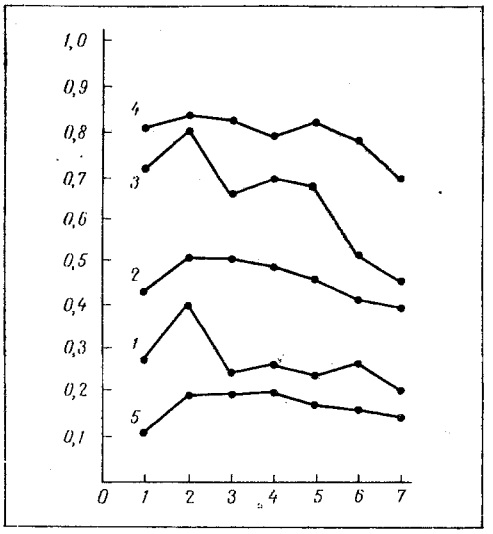

Fig. 2. Cytotoxic action of anti-θ serum on B10 anti-A lymphocytes: 1) intact immune lymphocytes; 2) their "T fraction"; 3) immune lymphocytes eluted from A TC; 4) "T fraction" of immune lymphocytes eluted from A TC; 5) immune lymphocytes eluted from B10 TC. Abscissa, dilutions of serum (in $\log_2$); ordinate, cytotoxic index.

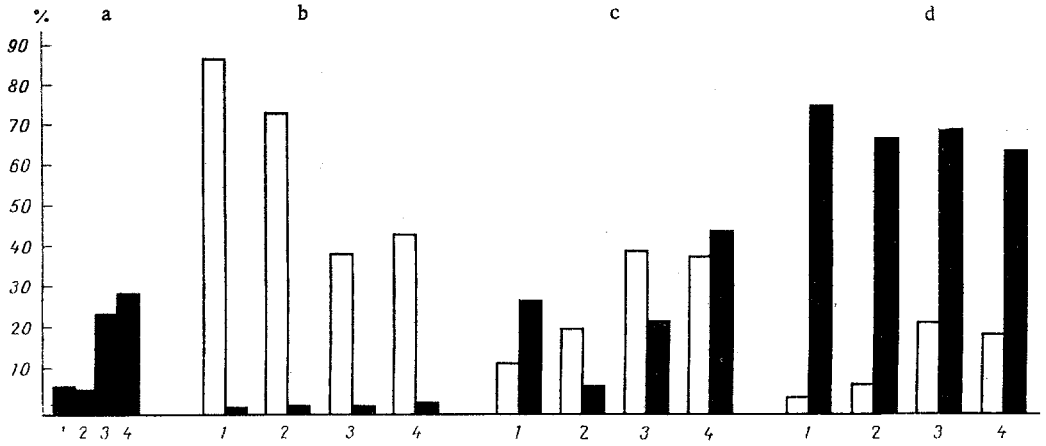

Fig. 3. Morphological characteristics and DNA synthesis of four populations of immune lymphocytes: a) total number of labeled lymphocytes; b) small; c) medium-sized; d) large lymphocytes. 1) Intact immune lymphocytes; 2) their "T fraction"; 3) eluted immune lymphocytes; 4) "T fraction" of eluted immune lymphocytes. Unshaded columns give total number of lymphocytes (in %); black columns show lymphocytes labeled with thymidine-^{3}H (in %).

percentage of DNA-synthesizing cells also was increased to 27-29 on account of an increase in the number of medium-sized and large lymphocytes. At the same time, within this morphological group the relative proportion of labeled cells either remained substantially unchanged (among the large lymphocytes) or rose slightly in the "T fractions" of the eluted small and medium-sized lymphocytes. The fraction of eluted lymphocytes thus differed from the original in containing four to five times more cells synthesizing DNA, in connection with the increase in the percentage of medium-sized and large lymphocytes in the population. These quantitative changes agree with the data for specific fixation of the DNA-synthesizing fraction of immune lymphocytes to TC [5]. The problem of whether receptors of eluted lymphocytes react selectively with "special" H-2 specificities, like the receptors of the original immune lymphocytes [4], requires further study.

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PREPARATION OF LIPOSOMES WITH IMMUNOLOGICAL SPECIFICITY

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Artificial lipid vesicles (liposomes) containing immunoglobulins were obtained. The immunoglobulins contained in the liposomes were shown to preserve their immunological activity: Liposomes containing rabbit anti-mouse immunoglobulin agglutinate under the influence of donkey anti-rabbit immunoglobulin or mouse serum. Liposomes containing inulin-^{3}H, carrying immunoglobulins against antigenic determinants of the cell surface were selectively bound by target cells but not by control cells. Specific binding with cell surface antigenic determinants was also demonstrated for liposomes carrying nonimmune globulins as well as immunoglobulins. It was shown by the direct immunofluorescence method that nonimmune globulins, in the form of complexes with immune liposomes, are selectively bound by target cells. With the aid of such liposomes it is possible to supply substances selectively to certain types of cells and also to "fit" new antigens into the cell membrane.

KEY WORDS: liposomes; immunoglobulins; antigens.

Reports have recently been published on the introduction of substances incorporated in to artificial lipid vesicles (liposomes) into cells in vivo and in vitro [3, 7, 10-12]. However, opportunities for using liposomes as carriers of substances in vivo are limited by the specificity of their distribution among the tissues [7, 9]. For the directional introduction of substances with the aid of liposomes it would be worthwhile to be able to bind liposomes selectively with cells of a particular type.

This paper describes a method of obtaining liposomes with immunological specificity and their selective binding to cells.

EXPERIMENTAL METHOD

Inulin-^{3}H (specific activity 690 mCi/mmole) was obtained from the Radiochemical Centre, Amersham, England. Concanavalin A was kindly provided by V. I. Gel'fand. Rabbit and rat immunoglobulins were obtained

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