INVESTIGATION OF SPECIFIC RETENTION OF LYMPHOCYTES PROLIFERATING UNDER THE INFLUENCE OF AN ANTIGEN IN MOUSE LYMPH GLANDS CONTAINING THE SAME ANTIGEN

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The lymph glands and spleen of mice receiving intravenous injections of spleen cells or thymocytes, labeled with thymidine-H^3, were studied radiochemically. An effect of specific fixation of the labeled cells in the lymph glands and spleen was demonstrated. It is postulated that the cells are fixed by means of antibody molecules located on the cell surface.

Small lymphocytes of the thoracic duct, if injected intravenously into a syngeneic animal, accumulate in its lymphoid organs [2, 3, 5-8]. Cells obtained from the lymph glands can also settle in the lymphoid organs of recipients. The degree of fixation of the cells increases if an antigen is present in the lymph glands and spleen. This phenomenon has been demonstrated in experiments both with intact lymphocytes [1, 9] and with lymph gland cells proliferating under the influence of antigen [4]. The latter were not retained in lymph glands stimulated by another antigen. This observation suggests the immune specificity of the phenomenon. However, stimulated lymph glands also retain nonimmune cells, i.e., there is a certain nonspecific component.

The object of the investigation described below was to reproduce the phenomenon and, by investigating spleen cells, to show how it depends on the presence of "its own" or a "foreign" antigen in the lymph gland, i.e., to examine the specificity of the phenomenon. The first steps toward the study of the mechanism of this phenomenon were also to be taken.

EXPERIMENTAL METHOD

Male BALB/C mice were immunized by intraperitoneal injection of 5×10^8 sheep's red blood cells (SRC). After 30, 42, 54, 66, and 78 h the mice received an intraperitoneal injection of 20-30 μ Ci thymidine-

TABLE 1. Scheme of Experimental Conditions

Group No.	No. of recipients	Cells injected	Time of obtaining cells after immunization of donors (in days)	Method of labeling cells	Antigen injected into recipients	Injection of anti-SRC serum into recipients	Treatment of cells with anti-mouse antiglobulin serum
1	12	Spleen	5	In vivo	SRC	—	—
1a	10	»	5	» »	»	—	—
2	10	»	3	» »	»	—	—
3	10	»	Intact donors	» »	»	—	—
4	9	»	5	» »	RRC	—	—
5	12	»	4	In vitro	SRC	—	—
6	8	»	4	» »	»	+	—
7	9	»	5	In vivo	»	—	+
8	7	Thymus	5	» »	»	—	—

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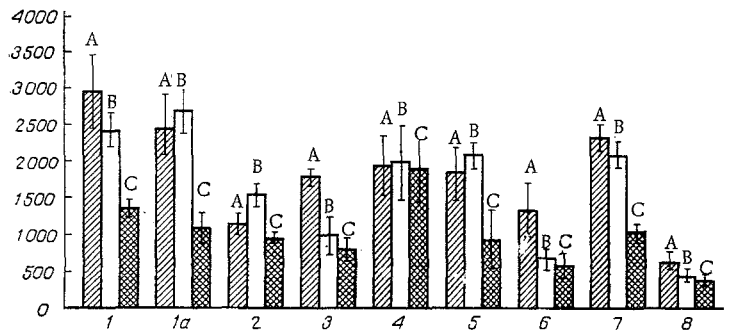

Fig. 1

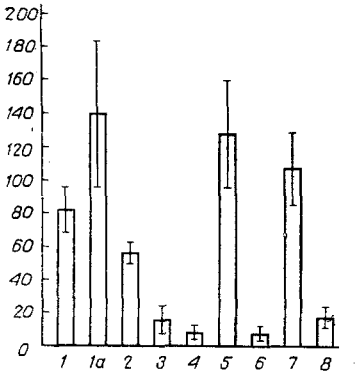

Fig. 2

Fig. 1. Radioactivity of spleen and lymph gland tissues of experimental and control groups: A) spleen; B) lymph glands on experimental side; C) lymph glands on control side of various experimental groups (Table 1). Abscissa, No. of experimental groups (for details, see Table 1); ordinate, radioactivity of splenic tissue (pulses[10 min]2 mg tissue).

Fig. 2. Increase in retention of labeled cells in experimental lymph glands compared with control. Abscissa, No. of experimental groups (details in Table 1); ordinate, percentage of radioactivity in experiment compared with control.

H^3 (specific activity 300 mCi/mmole). In one group of experiments the labeled precursor was given by the same scheme to intact animals. The animals were killed 48 and 96 h after immuniazation (at the maximum of rosette formation), and a cell suspension was prepared from the spleen (in some experiments also from the thymus) in medium No. 199 (2×10^8 cells/ml). The suspension (0.5 ml) was injected intravenously into syngeneic recipients, which had received a subcutaneous injection of 2×10^8 SRC or rat red blood cells (RRC) into the right forelimb and the right hind limb 4 h previously. In the experiments of groups 5 and 6 the mouse spleen cells were labeled with thymidine-H^3 in vitro for 1 h at 37° C (10 μ Ci/1×10^8 cells in 1 ml medium No. 199) 3 days before immunization with SRC, the cells were washed twice, and the labeled thymocytes were injected intravenously. The recipients were killed 20-24 h after receiving the injection of cells and the spleen and the axillary and popliteal lymph glands (right and left) and from some animals the liver and kidneys also were taken for investigation. The organs to be examined were weighed and hydrolyzed in concentration formic acid at 37° C for 20 h. The radioactivity of the samples (including the suspension of donors' cells) was determined with the Packard Model 3320 Tri-Carb scintillation counter.

To assess the experimental results, the radioactivity of the lymph glands of the experimental (right) and control (left) sides of the trunk was compared. The radioactivity of specimens of spleen tissue in the experimental and control observations also was compared.

In the experiments of group 6, together with donors' cells, 6 h before injection of the cells the recipients received an intravenous injection of 0.2 ml mouse anti-SRC serum (titers of hemagglutinins 1:1024). In a separate group of experiments the cells were treated for 1 h before injection with rabbit anti mouse antiglobulin serum and washed once with medium No. 199.

Altogether eight groups of experiments were carried out on 154 mice. The experimental conditions are summarized in Table 1.

EXPERIMENTAL RESULTS

In these experiments the donors' spleen cells by the beginning of the 5th day after immunization had incorporated 1.5 times more thymidine-H^3 than the spleen cells of the intact mice. This is in agreement with data in the literature showing intensive proliferation of lymphoid cells induced by antigen. In these observations the recipient thus received a population enriched with labeled cells sensitized to the particular antigen.

The distribution of the donor's cells in the recipient's body is reflected in Figs. 1 and 2. In the unstimulated lymph glands investigated only 8%, and in the spleen 44-45% of the injected labeled donor's spleen cells were retained regardless of whether they were taken from immunized or intact animals.

In the experiments of group 1 lymph glands regional with respect to the site of injection of SRC fixed 80% more labeled cells than the lymph glands on the contralateral side. In analogous experiments carried

out at another time (group 1a) 2.41 times more labeled cells accumulated in the regional lymph glands than in the glands of the opposite side. If the donor's cells were taken 48 h after immunization (group 2) the phenomenon of asymmetrical fixation of labeled cells was less marked. This could be due to the smaller number of labeled cells of the particular specificity at that time.

The results of the experiments of group 3 show that labeled cells of intact mice virtually did not accumulate selectively in the stimulated lymph glands. This does not agree with some observations in the literature [1, 9]. However, the authors cited above labeled the cells in their experiments with Cr^{51} and not with thymidine-H^3, so that practically the whole population was labeled. In the present case it was possible to study migration of cells dividing intensively in the donor's body under the influence of the antigen. In similar experiments with donors' lymph gland cells labeled with thymidine-H^3 in vitro similar results were obtained [4]. In the present experiments in which spleen cells were labeled in vitro, selective retention of the donor's cells also was observed in the antigen-containing lymph glands of the recipient (experiments of group 5).

It follows from the results of the experiments of group 4 that spleen cells, labeled with thymidine-H^3, from mice immunized with SRC accumulate almost equally in lymph glands on the side of injection of RRC and on the opposite side.

Regardless of the experimental conditions, the level of radioactivity in the recipient's liver and kidney tissue was only very slightly above the background level.

Selective retention of thymocytes in lymph glands containing the antigen was practically absent (experiments of group 8).

It can be concluded from these observations and others reported in the literature that the spleen cells in the present experiments, like the cells of the thoracic duct and lymph glands in the investigations of other workers, have the ability to be selectively concentrated in the lymphoid organs regardless of whether they are taken from immunized or intact animals. The penetration of the antigen into the lymph glands was on a much larger scale than fixation of cells proliferating under the influence of the same antigen in them. Accumulation of intact cells and cells taken from mice immunized with another antigen occurred to a much lesser degree.

Thymocytes did not exhibit taxis toward the antigen, which was characteristic of labeled immune spleen cells.

In the experiments of groups 6 and 7 the role of molecules of specific antibodies (receptors ?) on the surface of the labeled donors' cells in the fixation of these cells in the recipient's lymph glands was studied. Injection of anti-SRC serum into the recipients completely blocked the selective retention of labeled lymphocytes in the lymph glands containing antigen. It was thus shown that the antibodies effectively competed with cells proliferating under the influence of the corresponding antigen. It can be concluded that the cells are fixed by means of molecules similar to antibodies on the surface of the donor's lymphocytes. The results of experiments with uncrossed antigen indirectly confirm the role of specific receptors in the phenomenon of retention of the cells in the lymph glands containing the antigen.

The negative response obtained in the experiments with antiglobulin serum cannot be regarded as final, for during the 20-24 h that the donor's cells remained in the recipient's body the immunoglobulins on their surface could have been unblocked.

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