STUDY OF LYMPHOCYTES DAMAGED BY SPECIFIC ANTIGENS IN VITRO IN SUSPENSIONS AND TISSUE SLICES

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On the 6th to 14th day after immunization of mice with simple proteins (egg albumin, bovine serum albumin) or alloplastic skin grafting specific antigens in high concentrations had a cytotoxic action in vitro on suspensions of lymph glands, spleen, and thymus of these mice, causing death of not more than 30% of the cells. A study of native preparations of tissue slices of the lymphoid organs of such mice showed that the cells damaged by the specific antigens were localized principally in the thymus-dependent zones of the lymph glands and spleen. It is postulated that the injury to the cells was caused either by humoral factors (antibodies, lymphotoxin) or by direct interaction between the antigen and sensitized cells which are precursors of the effector immune cells.

There is experimental evidence to show that specific antigens may have a cytotoxic action on the polymorphs [1, 4, 9, 16] and lymphocytes [5-7, 11, 12, 14, 19] of sensitized animals.

However, whereas damage to polymorphs under such conditions is evidently caused by humoral factors [3, 16], the nature of the lesion of the lymphocytes and the mechanism of their injury by specific antigens remain unexplained. This investigation was devoted to the study of these problems.

EXPERIMENTAL METHOD

Mice (26) of line B10D2 were skingrafted in the dorsal region with alloplastic skin (1 cm^2) from C57BL/10 Sn mice, four mice received autografts, and 22 mice were immunized by intradermal injection of 0.05 mg (0.025 ml) of commercial preparations of egg albumin or bovine serum albumin (BSA), with or without Freund's complete adjuvant, in the inguinal region.

The localization of the antigen-sensitized cells in the lymphoid organs of the sensitized mice was determined from their response to the cytotoxic action of specific antigens. Slices of tissue about 1 mm thick and up to 0.25 cm^2 in area were cut with sharp-pointed ophthalmic scissors or with a razor blade from the spleen, thymus, and regional lymph glands of the experimental and intact mice. The surface of the tissue slice was carefully washed with Hanks's solution to remove damaged and adjacent cells. The prepared slices were placed on a slide with wells in medium No. 199 or Hanks's solution containing antigens (control without antigen) so that the solution covered the surface of the slice. The antigen was the supernatant obtained after centrifuging (1500 rpm, 10 min) the lymph gland cells, frozen and thawed three times (5,000,000 cells/ml), of the mice acting as donors of the skin grafts. The lymph glands of animals immunized with the proteins were treated with 1% solutions of BSA or egg albumin. The slices were incubated in a moist chamber at 37°C for 30 min. The liquid was carefully drawn off and 0.05% trypan blue solution with eosin was added; after 15 sec the surface of the slice was covered by means of a coverslip. The specimens were examined under the microscope with oblique illumination (80-100×). Some speci-

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TABLE 1. Injury to Cells of Lymphoid Organs of Sensitized Mice in Vitro under the Influence of Specific Agents

Cells	Material used for sensitization	Antigen tested*	Days after sensitization†		
			6	8	14
Lymph gland cells	Egg albumin	Egg albumin	0,29±0,06	0,25±0,04	0,30±0,07
	Allograft	Lymph gland cells of donor mice	0,26±0,09	0,21±0,05	0,25±0,02
Spleen cells	Egg albumin	Egg albumin	0,17±0,02	0,16±0,03	0,22±0,02
	Allograft	Lymph gland cells of donor mice	0,20±0,03	—	0,24±0,08
Thymo-cytes	Egg albumin	Egg albumin	0,08±0,03	0,05±0,04	0,06±0,04
	Egg albumin with complete adjuv.	The same	—	0,12±0,04	0,14±0,03
	Allograft	Lymph gland cells of donor mice	0,16±0,01	0,19±0,02	0,18±0,03

* The antigens tested had no cytotoxic action (index < 0.08; P > 0.05) on cells of intact animals or of mice immunized with a different protein or receiving autografts.

† Mean cytotoxic indices of series of experiments with confidence limits corresponding to P < 0.05 (group of 4 to 8 mice) are indicated. Cytotoxic indices calculated relative to control (the same cells incubated with nonspecific antigen or without antigen).

mens were not stained and were examined under the ML-2 luminescence microscope. The stained cells in the first series of experiments or the diffusely luminescent cells in the second series were dead (antigen-sensitized). In preparations of this type, especially the experimental series, in which some of the cells were stained, it was relatively easy to distinguish the cortex of the lymph glands, the follicles, the adjacent paracortical zone, and the medulla. The experimental specimens were compared with the controls. Besides the specific antigens, crossed tests were carried out with other (nonspecific) antigens and also with antigens prepared from syngeneic lymph glands.

Meanwhile the lympholysis test [6] was carried out in vitro with a cell suspension prepared from slices of the same lymphoid organs. Samples of whole and diluted antigens were treated with an equal volume (0.05 ml) of a cell suspension (2,000,000/ml) from the regional lymph glands, spleen, or thymus of the mice receiving the skin grafts, mice immunized with the proteins, or intact animals. Other control tests were set up (see above). The samples were incubated at 37°C for 1 h. Dead (stained) and living cells were counted after the addition of trypan blue with eosin and the cytotoxic index was calculated [2].

EXPERIMENTAL RESULTS

Cells injured by the specific antigens (antigen-sensitized cells) were found in cell suspensions prepared from the lymphoid organs of the mice sensitized by allografts or proteins. Morphologically (after staining with trypan blue) these cells were lymphocytes although some blast cells also were found among them. The same antigens had no cytotoxic action on the cells of intact animals (Table 1). The lymphoid cells of animals sensitized by allografts were not destroyed in the presence of simple proteins (egg albumin, BSA) or of antigens of syngeneic lymph glands. Antigens of allogeneic lymph glands of mice belonging to other lines (A, CBA, C3H/He, DBA/1) could exert a cytotoxic action if they contained at least one alloantigen of the H-2 locus, which occurs in mice of the donor (C57BL/10 Sn) line. The specificity of the cytotoxic effect of the antigens was confirmed in mice sensitized with simple proteins (egg albumin, BSA) also. The lymphocytes of mice sensitized with egg albumin were destroyed by this antigen but not by BSA or the antigens of allogeneic lymph glands.

After immunization with proteins but without adjuvants, no cells sensitized to them were found in the thymus of the mice, but after allografting cells sensitized to antigens of the mice acting as donors of the graft were found in the thymus [7]. If, however, the mice were immunized with proteins together with Freund's complete adjuvant, the number of dead cells in suspensions of thymocytes after incubation with specific antigen was significantly greater than in the controls, although the cytotoxic index was usually smaller than 0.15 (Table 1). Consequently, the differences in the numbers of cells in the thymus injured by

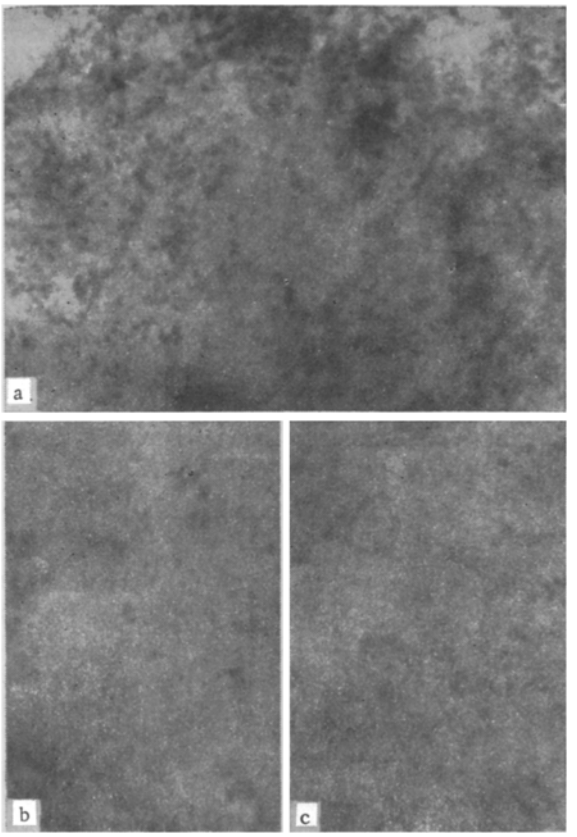

Fig. 1. Localization of antigen-sensitized cells in lymph glands: a) section through regional lymph gland of mouse on 8th day after allografting, incubated with donors' antigens. Dark, dead cells can be seen in the interfollicular zone of the cortex; b) section through the same lymph gland without donors' antigen; c) section through lymph gland of intact mouse incubated with donors' antigens. Here and in Fig. 2, native preparations after addition of trypan blue with eosin, 100×.

the antigen after immunization with proteins and after allografting were quantitative rather than qualitative. The protein antigens were cytotoxic in concentrations not below 0.1-0.05%, and antigens of the lymph glands were cytotoxic in dilutions not exceeding 1 : 80-1 : 160.

Active guinea pig complement (batch No. 137, Belorussian Institute of Epidemiology, Microbiology, and Hygiene), added to the cell suspension after the specific antigen, not only did not strengthen the cytotoxic action of the antigen, but weakened it somewhat. The cytotoxic action of the specific antigen on the sensitized lymphocytes was thus evidently not dependent upon complement.

The localization of the antigen-sensitized cells in the lymphoid organs was studied during the period when the results of the cytotoxicity test with their suspensions showed the greatest number of these cells, i.e., on the 6th-14th day after allografting or immunization (Table 1). Cells stained with trypan blue and eosin after the addition of specific antigen were found in all preparations of these lymph glands from mice receiving allografts or mice immunized with simple proteins. They were arranged as discrete foci or diffusely in the cortex between and around the follicles (Fig. 1) and in the paracortical zone. Usually no such cells could be seen in the lymph gland follicles. Some of them were evidently in the medulla, for after the addition of specific antigen dead cells were found there in rather larger numbers than in the control specimens. Examination of intravital specimens of lymph glands of the experimental mice in the lumi-

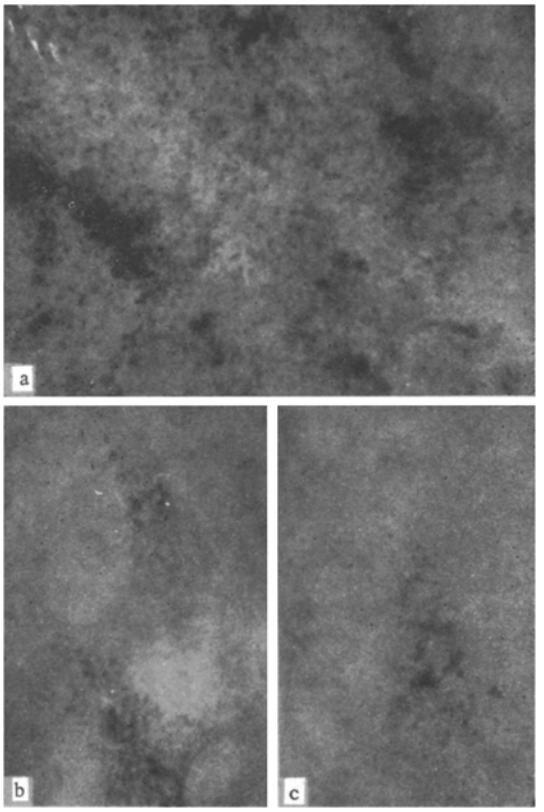

Fig. 2. Cells injured by antigen (dark, dead) in spleen of mouse on the 6th day after immunization with egg albumin (a); section through the same spleen, incubated with BSA (b), and section through spleen of intact mouse incubated with egg albumin (c).

nescence microscope after the addition of specific antigen revealed groups of diffusely luminescent cells (injured by antigen) in their cortex, but significantly fewer were found in the control specimens.

Large concentrations of antigen-sensitized cells were seen near the arterioles in the spleen of the mice after allografting or immunization with proteins (Fig. 2), and groups of these cells were sometimes in direct contact with their walls. In the control preparations (Fig. 2) only single stained cells or small groups of them were visible (Fig. 2). Just as in the experiments with suspensions of lymph gland cells, guinea pig complement did not increase the damage to the sensitized cells arising under the influence of the specific antigen. The localization of the antigen-sensitized cells in the lymph glands and spleen described above correspond to the thymus-dependent zones of these organs [17, 18].

A moderate number of stained cells, which in the control preparations were the only cells, could be seen in the cortex or, less frequently, in the medulla of the thymus of mice receiving allografts, after the addition of specific antigens.

Cells destroyed by large doses of specific antigens were thus found in cell suspensions and native slices prepared from the lymphoid organs of mice receiving skin allografts or mice immunized with proteins, by the use of the cytotoxic test. Exogenous complement did not increase the degree of injury and the accumulation of endogenous complement was improbable during such a short period (1 h) of incubation of the cell suspensions washed with Hanks's solution. The participation of humoral factors (antibodies or the antigen–antibody complex) in the phenomenon described likewise cannot be completely ruled out. Furthermore, mediators of hypersensitivity of delayed type, such as lymphotoxin [10, 20] or "transfer factor" [15], liberated under the influence of antigen by the sensitized lymphocytes and exerting a nonspecific action on other cells of lymphoid organs, could also participate in this process. However, first, lymphocytes are

relatively resistant to lymphotoxin [13, 20], and its liberation has been observed under conditions very different from those obtained in the present experiments, namely during incubation of sensitized cells, even with small doses of antigen, for a long time (from 4 h to 3 days) [13, 20]. Second, in the present experiments the insufficient quantity of fluid of the lymph gland cells obtained from the sensitized mice and incubated with specific antigens (1 h), when added to lymph gland cells of intact mice, had no cytotoxic action on them and evidently did not contain the soluble cytotoxic factor. It therefore seems more likely that injury to the cells was produced by interaction between the antigen and specific receptors located on the surface of the sensitized cells and injuring their membranes. However, since the cells injured by specific antigens in vitro were found not only in the lymph glands and spleen, where they accounted for less than 30% of the whole cell population, but also in the thymus, it can be postulated that they belong to the cell population of thymus origin [10]. They are located predominantly in the thymus-dependent zones of the lymph glands and spleen and under ordinary conditions of antigenic stimulation they could be structurally or physiologically isolated from the excess of antigen and could serve as precursors of the effector immune cells [8].

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