

LOCALIZATION OF CELLS SYNTHESIZING SPECIFIC AND NONSPECIFIC GLOBULINS IN LYMPH GLANDS

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After immunization of rabbits with diphtheria toxoid proliferation of cells synthesizing specific and nonspecific immunoglobulins is found in the lymph glands. These cells are located mainly along blood vessels and sinuses. The number of cells decreases away from the lumen, the number of cells producing specific antibodies falling away more rapidly. This arrangement of globulin-producing cells corresponds to the localization of cells taking up the injected antigen.

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After injection of foreign protein into the body, cells synthesizing specific and nonspecific immunoglobulins begin to proliferate in the lymphoid organs. Some authorities consider that immunoglobulins non-specific with respect to injected antigen are "normal antibodies" against different antigens [4], while others regard them as nonspecific globulins not possessing an antibody determinant [1, 3]. The relationship between cells producing specific and nonspecific immunoglobulins to a particular antigen in the immunologic response remains unexplained [3].

A combined luminescence and serologic method was used in the present investigation to examine the relationship between localization of cells taking up antigen and cells producing specific and nonspecific immunoglobulins.

EXPERIMENTAL METHOD

Experiments were carried out on 20 rabbits weighing 2-2.5 kg, which were immunized twice with crude diphtheria toxoid in the plantar pad of the hind limb at an interval of 30 days. The dose of antigen at each injection was 160-180 Lf. The animals were sacrificed 12 and 24 h and 2, 3, and 4 days after the second injection of antigen. The regional popliteal lymph glands were fixed and embedded in paraffin wax by Sainte-Marie's method [6]. Tissue sections were stained by the direct Coons' method for antigen and by a double luminescence-serologic method for specific and nonspecific immunoglobulins using a second antibody label. In this last staining procedure, diphtheria toxoid plus diphtheria antitoxin labeled with rhodamine sulfochloride (red fluorescence) plus antibody against rabbit immunoglobulins labeled with fluorescence isothiocyanate (green fluorescence) were applied successively to the lymph gland section. Parallel control tests were carried out with sections of a nonimmune lymph gland, with heterologous antigen, and by the neutralization reaction (for details of the staining method, see [2]).

EXPERIMENTAL RESULTS

Twelve hours after injection of antigen, a large quantity of antigen was found in the popliteal lymph glands, some lying freely, some ingested by macrophages. After 24 h antigen was found mainly intracellularly. Macrophages containing antigen were located mainly in the medullary cords of the medullary zone of the lymph gland, near blood vessels and sinuses. Fewer cells containing antigen were observed in the cortex, likewise located near blood vessels. In the germ centers a small quantity of antigen was found between the cells, but only 12 h after immunization. On the 3rd and 4th days after immunization, no diphtheria toxoid could be found in the lymph glands.

The number of cells containing antigen in the lymph glands fell away from the lumen of the vessels and sinuses into the interior of the tissue, which corresponded to the distribution of free-lying antigen. This is clearly illustrated by counts of cells containing antigen in areas of the medullary cords of equal sizes but at different distances from the lumen of the vessels (Table 1).

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TABLE 1. Distribution of Cells Containing Antigen Near Vessels and Sinuses of Lymph Gland of a Rabbit Sacrificed 12 h After Second Immunization with Diphtheria Toxoid

Observation no.	Number of cells containing antigen depending on distance of area of counting away from lumen of vessel sinus							
	1-18 μ	18-36 μ	36-54 μ	54-72 μ	72-90 μ	90-108 μ	108-126 μ	126-144 μ
1	4	4	2	0	0	1	1	0
2	7	5	0	2	0	0	0	0
3	10	4	2	0	0	0	2	1
4	5	2	0	0	2	0	0	0
total	26	15	4	2	2	1	3	1

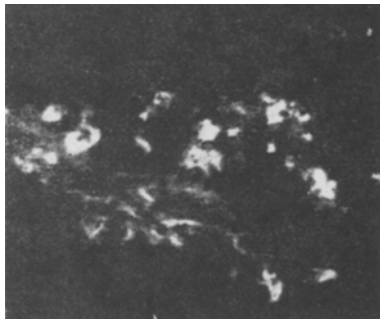


Fig. 1. Photomicrograph. Popliteal lymph gland of rabbit sacrificed 12 h after double immunization with diphtheria toxoid. Distribution of cells containing antigen along blood vessel in region of medullary cords of lymph gland. Stained for antigen by indirect luminescence-serologic method. Objective 40.

A small number of cells containing only nonspecific immunoglobulins was found in the regional lymph gland 12 and 24 h after the second immunization (Fig. 1). Cells containing specific antibodies appeared on the second day. Between the 2nd and 4th days the number of cells containing both specific and nonspecific immunoglobulins in the lymph gland increased sharply (Fig. 2). These cells belonged mainly to the plasma-cell series. Approximately 50% of fluorescent cells contained specific antibodies on the 2nd-4th days after immunization.

Most cells producing specific and nonspecific immunoglobulins were located in the medullary cords of the medullary zone, and fewer were found in the cortex of the gland. A few cells containing nonspecific globulins only were detected in the germ centers.

Globulin-producing cells in the medullary cords were arranged in groups, with the lumen of the vessel or sinus in their center. Each group consisted of cells containing specific and also nonspecific globulins. In a direction away from the lumen of the sinus or blood vessel the total number of fluorescent cells diminished. Nearer the lumen, among the fluorescent cells, more cells containing specific antibodies were observed,

while further from the lumen more cells containing nonspecific globulins were found. Figures showing the distribution of specific and nonspecific globulin-producing cells in areas at different distances from the lumen of the vessels and sinuses are given in Table 2.

The distribution of globulin-producing cells around the blood vessels and sinuses of the lymph glands as thus revealed is in good agreement with the observed distribution of plasma cells around the lumen of blood vessels [5]. Meanwhile, the problem of whether the precursors of globulin-producing cells migrate toward blood vessels and sinuses from the germ centers of secondary follicles or whether they are already present in these zones at the moment of immunization has not yet been solved [5, 7]. However, it is important to note that after immunization the location of newly formed globulin-producing cells corresponds to the location of antigen uptake, and the number of these cells bears a direct relationship to the amount of antigen ingested by macrophages in the particular zone. Zones with a considerable accumulation of antigen-ingesting cells (nearer to the lumen of the vessels and sinuses) thereby correspond to a larger number of cells containing specific antibodies compared with cells containing nonspecific globulins.

A possible explanation of this phenomenon is as follows. Injected antigen stimulates proliferation of a large number of globulin-producing cells in the lymph gland. Activation of synthesis of specific antibodies in these cells requires a relatively large quantity of specific antigenic stimulus. In areas of tissue where antigen is present but in an amount below the threshold, immunologically competent cells produce only nonspecific immunoglobulins.

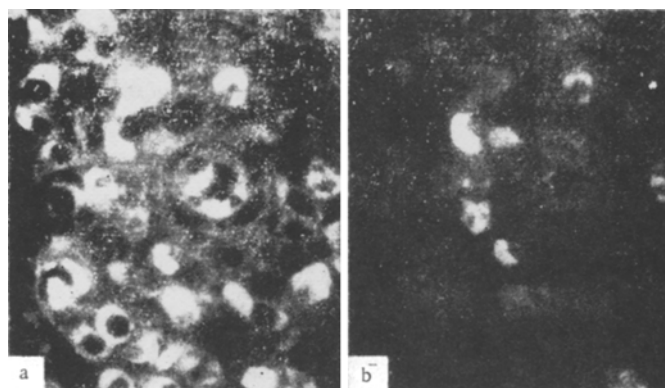


Fig. 2. Photomicrograph. Popliteal lymph gland of rabbit sacrificed 4 days after double immunization. Distribution of globulin-producing cells near lumen of vessel. a) Photograph without filter; all cells producing immune globulins (specific and nonspecific) are shown; b) photograph of the same area with red filter; only cells containing specific antibodies, staining red by this method, are seen. Simultaneous luminescence-serologic staining for specific antibodies and nonspecific immune globulins using 2 fluorochromes. Objective 90.

TABLE 2. Distribution of Cells Containing Specific Antibodies and Nonspecific Immunoglobulins Near Blood Vessels and Sinuses of Lymph Gland of a Rabbit Sacrificed 4 Days After 2nd Immunization with Diphtheria Toxoid

Observation no.	Distance of area of counting away from lumen of vessel or sinus (in μ)									
	1-18	18-36	36-54	54-72	72-90	90-108	108-126	126-144	144-162	162-180
Number of cells containing all immunoglobulins (specific and nonspecific)										
1	5	8	13	3	3	3	4	5	3	0
2	8	11	10	5	6	0	5	3	2	0
3	6	11	6	8	8	9	6	7	5	5
4	11	9	6	8	3	3	0	0	0	0
5	5	9	8	9	6	8	8	4	0	1
total	35	48	43	33	26	23	23	19	10	6
Percentage of cells containing specific antibodies relative to total number of globulin-containing cells										
1	100	88	85	100	33	37	0	0	0	0
2	100	91	90	60	67	60	67	0	0	0
3	83	100	83	87	50	45	33	14	40	20
4	91	89	83	38	0	33	0	0	0	0
5	100	89	75	23	0	12	25	0	0	0
mean	94,8	92,0	83,2	61,6	30,0	32,0	29,5	20,2	13,3	10,0

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