ANTIGENIC STRUCTURE OF SOLUBLE COMPONENTS OF GUINEA PIG LYMPH GLANDS

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Thirteen antigen components were found in a saline extract of the lymph glands. Because of their common immunologic properties with components of other organs the antigens were conventionally arranged in six groups. Some of these antigens are specific for lymph glands only, some for lymph glands, spleen, and bone marrow, some for lymph glands and liver, and so on.

The antigenic composition of organs of the lymphoid and reticulo-endothelial systems have received comparatively little study. The work of Grabar et al. [4, 7, 10] has demonstrated the complex immunochemical profile of the water-soluble fraction of the rat spleen, bone marrow, and thymus. Organ-specific antigens have been found in the spleen [7], bone marrow [4], and thymus [8, 10, 11].

Results of the immunochemical study of water-soluble antigens of guinea pig lymph glands are described in this paper.

EXPERIMENTAL METHOD

The lymph glands, spleen, bone marrow, liver, kidneys, lungs, and thymus were removed from guinea pigs weighing 450-500 g. All the organs were homogenized and the homogenates were lyophilically dried. The product represented pooled material from 40 guinea pigs. Anti-lymph gland serum (ALGS) was obtained by immunization of rabbits with homogenate adsorbed on aluminum hydroxide. The preparation was injected subcutaneously at several points, 100 mg dry weight of the substance being given at each injection. Three injections were given at intervals of 21 and 30 days. The rabbits were revaccinated three times 60 days later (intervals of 5 days). Blood was taken 14 days after the last injection. The sera from 4 rabbits were pooled and lyophilically dried. ALGS agglutinated cells of the lymph glands in a dilution of 1 : 128. Immunochemical analysis was carried out by Ouchterlony's method [1], by immunoelectrophoresis [1], by the indirect hemagglutination test (IHT) with formalinized, tanninized erythrocytes [2], and the lymphocyte-agglutination test with a suspension of lymph gland cells [6]. The gel-diffusion test was intensified by antiserum against rabbit globulins [3]. The ALGS was first exhausted with guinea pig serum: 0.5 ml of ALGS was added to 1 ml lyophilized guinea pig serum and the mixture incubated for 1 h at 37° and for 18 h at 4°. This treatment completely removed precipitins against serum antigens from the ALGS (using Ouchterlony's test and immunoelectrophoresis as the controls). In different experiments, the ALGS was additionally exhausted with lyophilized extracts of guinea pig organs: 100 mg of lyophilized tissue was added to 1 ml ALGS and the mixture was kept for 1 h at 37° and 18 h in the cold, after which the ALGS was clarified by centrifugation. Evidence of complete exhaustion was given by the negative Ouchterlony's test and IHT with the corresponding extract. For the gel diffusion test, Ouchterlony's test, and immunoelectrophoresis the ALGS was concentrated 5 times, tissue extracts were prepared in doses of 100 mg dry weight/ml 0.067 M phosphate buffer in physiological saline, pH 7.2, and the extracts were kept for 18 h at 4°, centrifuged for 1 h at 3000 g, and the residue was discarded. The concentration of the extract for treatment of the erythrocytes in the IHT was 0.5-1 mg dry weight/ml.

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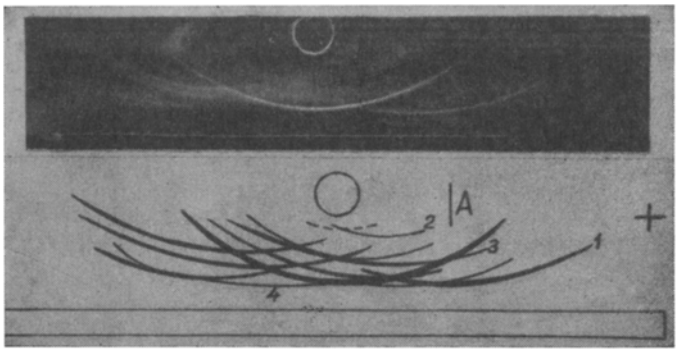

Fig. 1. Immunoelectrophoresis of water-soluble antigens of guinea pig lymph glands. Test with anti-lymph gland serum exhausted with serum of normal guinea pigs. In the diagram: A) limit of mobility of guinea pig serum albumins; 1 and 2) antigens specific for lymph glands; 3 and 4) antigens specific for lymph glands, spleen, and bone marrow. Remaining precipitation lines correspond to antigens common to all tested organs.

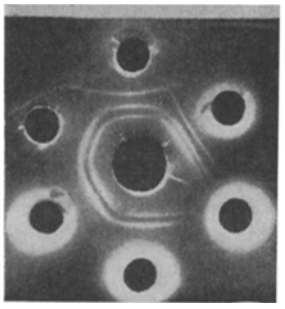

Fig. 2

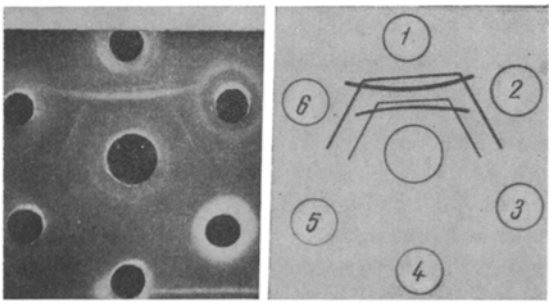

Fig. 3

Fig. 2. Immunodiffusion test by Ouchterlony's method. Central well contains anti-lymph gland serum exhausted with serum of normal guinea pigs. Peripheral wells contain saline extracts of: 1) lymph glands; 2) spleen; 3) thymus; 4) liver; 5) lungs; 6) bone marrow.

Fig. 3. Immunodiffusion test by Ouchterlony's method. Central well contains anti-lymph gland serum of guinea pigs, exhausted with serum and with liver of normal guinea pigs. Peripheral wells contain saline extracts of: 1) lymph glands; 2) spleen; 3) liver; 4) lungs; 5) kidneys; 6) bone marrow. Precipitation lines correspond to antigens specific for lymph glands and to antigens common to lymph glands, spleen, and bone marrow.

EXPERIMENTAL RESULTS

Thirteen antigens were discovered immunoelectrophoretically in the saline extract of the lymph glands (Fig. 1), two of them being organ-specific lymph glands (exhaustion of ALGS with homogenates of the spleen, bone marrow, liver, kidneys, lungs, and thymus had no effect on the precipitation lines corresponding to these antigens), two were found only in lymph glands, spleen, and bone marrow, while nine were common to all investigated organs except the thymus. Experiments in which the sera were exhausted and tests with different tissue extracts gave identical results. The immunochemical "inertia" of the thymus corresponded to its complete morphological involution (fatty degeneration). Immunodiffusion tests carried out by Ouchterlony's method, demonstrating antigen relationships between the soluble components of the lymph glands and saline extracts from various organs are shown in Figs. 2 and 3.

TABLE 1. Results of Study of Soluble Lymph Gland Antigens in IHT

Extract used to treat erythrocytes	Titer of IHT with ALGS exhausted by						
	serum	spleen	marrow	liver	kidneys	lungs	spleen and liver
Lymph glands	1/25,600	1/2560	1/5120	1/2560	1/2560	1/5120	1/1280
Spleen	1/12,800	0*	1/160	1/640	1/640	1/160	0
Bone marrow	1/3200	0	0	1/320	1/320	1/80	0
Liver	1/6400	1/1280	1/1280	0	1/1280	1/1280	0
Kidneys	1/800	0	1/40	0	0	0	0
Lungs	1/1600	0	1/40	0	0	0	0
Thymus†	0						

*Agglutination absent in ALGS dilution 1 : 10.
†Involution of organ (fatty degeneration) observed morphologically.

TABLE 2. Immunochemical Relationships between Water-Soluble Antigens of Lymph Glands and Other Tissue Systems

Group of antigens	Tested organs		Detection method
	contain	do not contain	
1st	Lymph glands	Spleen, bone marrow, liver, kidneys, lungs	Precipitation, IHT
2nd	Lymph glands, spleen, bone marrow	Liver, kidneys, lungs	" "
3rd	Lymph glands, liver	Spleen, bone marrow, liver, kidneys, lungs	IHT
4th	Lymph glands, spleen, bone marrow, liver	Kidneys, lungs	"
5th	Lymph glands, spleen, liver, kidneys, lungs	Bone marrow	"
6th	Lymph glands, spleen, bone marrow, liver, kidneys, lungs	–	Precipitation, IHT

Note. The term "group" of antigens conventionally describes soluble antigenic components of lymph glands characterized by unequal spectra of common immunochemical properties with saline extracts of various organs (from results of gel-diffusion tests and IHT).

Removal of precipitins against the soluble antigens (exhaustion of the ALGS with saline extract of lymph glands) had little effect on the ability of the ALGS to agglutinate lymphoid cells (titer of lymphocyte agglutination test reduced by half).

The results of the IHT (Table 1) indicated that three additional groups of antigen exist: 1) antigens specific for lymph glands and liver, 2) antigens common to lymph glands, spleen, bone marrow, and liver but absent from kidneys and lungs, and 3) antigens common to all organs except bone marrow. Under identical experimental conditions the titer of the IHT was lowest with erythrocytes treated with extracts of kidneys and lungs. Extract from the thymus was inactive in the IHT.

These results demonstrate the complex antigen interrelationships between soluble components of the lymph glands and other tissue systems. On the basis of these immunochemical relationships (from results of the gel-diffusion tests and IHT) with the spleen, bone marrow, liver, kidneys, and lungs six groups of water-soluble lymph gland antigens can be distinguished (Table 2).

Saline extracts of lymph glands contain almost no antigens responsible for the phenomenon of lymphocyte agglutination. For this reason observations [5] indicating that antibodies against the soluble components of

of lymphoid cells, with little effect on the cell surface, nevertheless give a marked immunodepressive effect, are of considerable interest.

Specific antigens detected by the present experiments in lymph glands supplement observations on organ-specific antigens in other parts of the lymphoid and reticulo-endothelial system [4, 7, 8, 10, 11]. Immunochemical specificity is known to be a structural manifestation of functional properties of the corresponding tissue system [9]. From this point of view immunochemical relationships between soluble components of the lymph glands, spleen, liver, and bone marrow can be regarded as a reflection of the functional pleurality of the lymph glands.

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