EFFECT OF ANTILUNG AUTOANTIBODIES ON SYNTHESIS OF HETEROHEMAGGLUTININS

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Synthesis of heterohemagglutinins by explants of lungs, paratracheal lymph glands, and spleen after intratracheal immunization of rabbits with sheep's red cells was investigated. Besides the antigen, protein extracts of homologous lung tissue either containing autoantibodies in a titer of 1:16 or not containing them also were injected intratracheally. The use of the tissue culture method showed that antilung autoantibodies activate synthesis of heterohemagglutinins in the lung without influencing their formation in the paratracheal lymph glands and spleen. A factor activating the antibody-synthetic function of the spleen, which was not an autoantibody, was found to appear in the lungs of the intratracheally immunized animals.

The development of the pathological process in acute and chronic pneumonias of nonspecific character is accompanied by the synthesis of antilung antibodies [2, 6]. The possibility of the production of autoantibodies in the lungs after immunization of animals with noninfectious antigen has also been demonstrated by the writers' investigations [4, 5]. However, the pathophysiological role of antilung antibodies has so far received little study.

In the investigation described below an attempt was made to determine the role of antilung autoantibodies in the synthesis of heterohemagglutinins following intratracheal immunization of animals with corpuscular antigen.

EXPERIMENTAL METHOD

Experiments were carried out on 50 rabbits weighing 2.5-3 kg. All the animals were immunized by intratracheal injection of sheep's red cells in a dose of 0.25 ml of a 50% suspension per kg body weight, given either once or twice at an interval of 10 days. The rabbits were also injected intratracheally with protein extracts of homologous lung tissue obtained by Kaplanskii's method [3] from the organs of preliminarily immunized or unimmunized animals (the protein content was determined by the biuret method and was 1.8 ± 0.3 and 2.4 ± 0.2 g%, respectively).

The experimental animals were divided into four groups: the rabbits of group 1 (15 animals) were immunized by two intratracheal injections of sheep's red cells accompanied by intratracheal injection of 1 ml protein extract of lung containing autoantibodies in a titer of 1:16; the rabbits of group 2 (15 animals) were immunized by intratracheal injection of sheep's red cells only (control); the rabbits of group 3 (15 animals) received intratracheal injections of sheep's red cells and 1 ml protein extract from the lung of an unimmunized rabbit (the extract contained no autoantibodies); the rabbits of group 4 (5 animals) received an intratracheal injection of sheep's red cells and 1 ml protein extract from the lung of an immunized rabbit, but after preliminary exhaustion of the autoantibodies in the passive hemagglutination test.

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TABLE 1. Effect of Lung Autoantibodies on Synthesis of Heterohemagglutinins in Lung Explants (titer in passive hemagglutination test)

Group of animals	Conditions of immunization	Times of investigation		
		after 1st immunization		after 2nd inmunization
		48 h	5 days	48 h
1	Sheep's red cells and lung extract containing autoantibodies in titer of 1:16	1:23 ± 1.167	1:35 ± 1.14	1:151 ± 1.233
	Control tissue culture	1:5 ± 1.146	1:6 ± 1.239	1:8 ± 1.00
	P	<0,005	<0,005	<0,001
2	Sheep's red cells only	1:10 ± 1.117	1:15 ± 1.156	1:43 ± 1.213
	Control tissue culture	1:2 ± 1.146	1:3 ± 1.183	1:5 ± 1.107
3	Sheep's red cells and extract of lungs of unimmunized rabbits	1:10 ± 1.107	1:16 ± 1.119	1:44 ± 1.18
	Control tissue culture	1:2 ± 1.148	1:3 ± 1.183	1:5 ± 1.107
	P	>0,5	>0,5	>0,5
4	Sheep's red cells and extract of lung with exhausted autoantibodies	—	—	1:36 ± 1.14
	Control tissue culture	—	—	1:4 ± 225
	P	—	—	>0,5

Note: All results significant with respect to tissue culture control ($P < 0.005$). Tissue culture control carried out with maximal titer of hemagglutinins. P denotes significance of differences compared with group 2 (control).

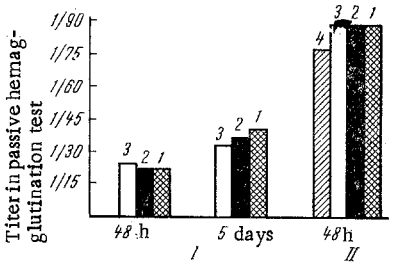

Fig. 1

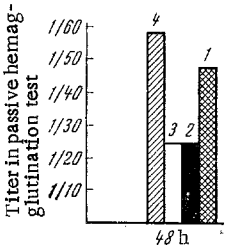

Fig. 2

Fig. 1. Effect of antilung autoantibodies on synthesis of heterohemagglutinins by explants of paratracheal lumph glands: I) first immunization; II) second immunization. Here and in Fig. 2: 1) double immunization of animals with sheep's red cells and parallel intratracheal injection of 1 ml protein extract of lungs containing autoantibodies in titer of 1:16; 2) immunization with sheep's red cells only; 3) intratracheal injection of sheep's red cells and 1 ml extract of lungs of unimmunized animal; 4) intratracheal injection of sheep's red cells and 1 ml extract of lungs with exhausted autoantibodies.

Fig. 2. Effect of intratracheal injection of lung extracts on synthesis of heterohemagglutinins by spleen explants (after second immunization).

The rabbits were sacrificed 48 h and 5 days after the first injection of antigen and 48 h after the second immunization.

The antibody-synthetic activity of explants of the lungs, paratracheal lymph glands, and spleen was studied in 36-h cultures of the surviving tissue. For this purpose, 200 mg of the tissue of the organ to be tested was placed in 10 ml medium No. 199 in the presence of normal rabbit serum (5%) and antibiotics and saturated for 6 min with a mixture of 95% O_2 plus 5% CO_2. In the case of cultivation of tissue from animals immunized once only, antigen (sheep's red cells) was added to the medium as a 50% suspension in a dose of 0.1 ml. Tissue kept in physiological saline at 37°C and tissue in medium No. 199 at 0°C acted as the control. Antibodies against sheep's erythrocytes in the culture fluid were determined by the hemagglutination test. Autoantibodies were studied by Boyden's passive hemagglutination test. Protein extracts of the lungs of immunized and intact animals, whose heterohemagglutinins had first been exhausted by sheep's red cells, acted as the antigen for the passive hemagglutination test.

EXPERIMENTAL RESULTS

The results of these experiments showed that intratracheal injection of protein extracts containing autoantibodies into rabbits previously immunized intratracheally with sheep's red cells was accompanied by a clearly defined increase in the synthesis of heterohemagglutinins in the lung. This pattern could be detected throughout the experiment after both the first and the second injection of antigen. The activating effect of the extracts was evidently due to their content of autoantibodies, for no such activation of antibody synthesis against heterologous antigen could be found (Table 1) in the animals of the control groups receiving, besides sheep's red cells, lung extracts not containing autoantibodies (extracts of the lungs of unimmunized animals; extracts with exhausted autoantibodies).

As a result of treatment with antilung autoantibodies, there was no activation of the antibody-forming function of the paratracheal lumph glands (Fig. 1).

Some interesting features were discovered when antibody-formation by splenic explants was studied (Fig. 2). After the second immunization, for instance, there was some increase in the titers of hemagglutinins in the culture fluid of the spleen from animals into which extracts of the lungs of immunized rabbits had been injected intratracheally along with the sheep's red cells. In that case, the activation of immunogenesis could not be ascribed to the antilung autoantibodies, for an almost identical increase in the titers was observed after injection both of extract containing autoantibodies ($1:47 \pm 1.114$) and extracts with exhausted autoantibodies ($1:57 \pm 1.076$). The antibody titers in the animals receiving immunization only and in the animals immunized and receiving parallel injections of extracts from unimmunized rabbits were $1:22 \pm 1.14$ and $1:22 \pm 1.164$ respectively (the difference is statistically significant, $P < 0.001$; see Fig. 2).

It can accordingly be postulated that during intratracheal immunization of animals with sheep's red cells, besides autoantibodies some other factor stimulating antibody synthesis in the spleen is produced in the lungs. By way of an hypothesis, the action of this factor can be regarded not only from the standpoint of its activation of the immunocompetence of the corresponding spleen cells, but also of its action on the lung-blood barrier, as a result of which the antigen enters the general circulation in larger quantities, and the intensity of the response of the spleen, manifested as hemagglutinin synthesis, is correspondingly increased.

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