FORMATION OF 19S- AND 7S-ANTIBODIES IN TISSUE CULTURE AND SOME OF THEIR PROPERTIES

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UDC 612.017.1-085.23

After intratracheal immunization of rabbits with two doses of sheep's erythrocytes, lung explants synthesize predominantly 19S-antibodies whereas explants of lung, spleen, and paratracheal lymph glands of intravenously immunized rabbits form predominantly 7S-antibodies after the second injection of antigen. Some serologic and physicochemical properties of the serum 19S- and 7S- antibodies were studied.

The few investigations of the dynamics of the switch in synthesis from macroglobulin to 7S-antibodies in isolated cells from lymphoid organs which have so far been made confirm the general rules governing this process as revealed by studies of antibodies in the blood serum of immune animals [3, 5, 7, 8]. It is not yet known whether different classes of specific immunoglobulin can be produced in a culture of surviving tissue of nonlymphoid organs, or after injection of antigen by certain special methods into animals.

The present investigation was undertaken to study the dynamics of synthesis of macroglobulin and 7S-antibodies in tissue cultures of the lungs, paratracheal lymph glands, and spleen, and also to examine some of the biological and physicochemical properties of these classes of immunoglobulins.

EXPERIMENTAL METHOD

Experiments were carried out on 92 rabbits weighing 2.5-3 kg. The animals were immunized with two injections of sheep's erythrocytes in doses of 0.5 ml of a 20% suspension intravenously or into the trachea at intervals of 10 days. The rabbits were sacrificed 5 days after the first injection of antigen or 48 h after the second immunization; the antibody-forming function of the lungs, spleen, and paratracheal lymph glands was studied in a 36-h culture of surviving tissue [200 mg of tissue from the organ in 10 ml medium No. 199 in the presence of normal rabbit serum and antibiotics, and after saturation for 6 min with a gas mixture consisting of 95% O_2 and 5% CO_2; when tissues from rabbits immunized once only were cultivated, antigen (sheep's erythrocytes) was added to the medium in a dose of 0.1 ml of 50% suspension]. The following controls were used: the tissue in physiological saline at the optimal temperature (37°C) and the tissue in medium No. 199 at 0°C. Macroglobulin and 7S-antibodies in the culture fluid and blood serum were separated on a chromatographic column filled with Sephadex G-200 dextran gel (diameter 42 mm, height 940 mm, height of gel layer 620 mm, volume of material applied 0.5 ml), equilibrated with 0.01 M tris-HCl buffer, pH 8.0, adjusted to isotonicity with sodium chloride. The culture fluid was concentrated 3.5 times before chromatography by evaporation in a cellophane bag (Visking) or by mixing with Sephadex G-25. In individual experiments, to detect protein peaks and to identify the immunoglobulins in them, culture fluid mixed with an equal volume of blood serum from intact rabbits was chromatographed. Chromatographic fractions containing macroglobulin and 7S-antibodies were collected separately and concentrated with Sephadex G-25. The antibody titer was recorded by the hemagglutination test. Hemolysing and hemagglutinating properties, thermostability (heating at 70°C for 10 min), and cysteine resistance [4]

Department of Biochemistry, Kursk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 71, No. 2, pp. 61-64, February, 1971. Original article submitted July 13, 1970.

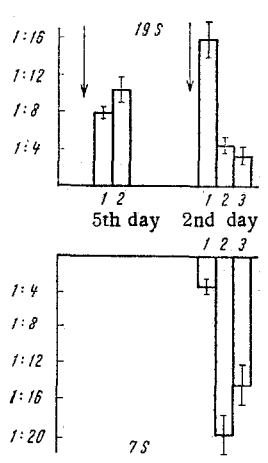

Fig. 1

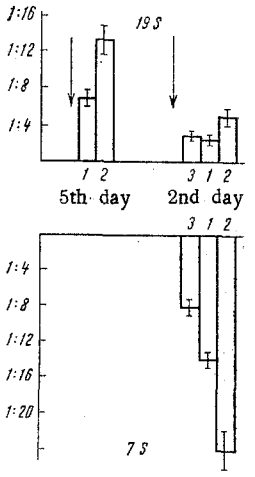

Fig. 2

Fig. 1. Synthesis of 19S- and 7S-antibodies in culture of surviving tissue of explants of lungs and some lymphoid organs of intratracheally immunized rabbits. Abscissa: 1) lung; 2) paratracheal lymph gland; 3) spleen; ordinate: titer of antibodies in hemagglutination test. Arrows denote time of immunization .

Fig. 2. Synthesis of 19S- and 7S-antibodies in culture of surviving tissue of explants of lungs and some lymphoid organs of intravenously immunized rabbits. Abscissa: 1) paratracheal lymph gland; 2) spleen; 3) lung; ordinate: titer of antibodies in hemagglutination test. Arrows denote time of immunization.

TABLE 1. Some Properties of Serum 19S- and 7S-Antibodies (Titers of Antibodies)

Material studied	19S		7S	
	hemolysins	hemagglutinins	hemolysins	hemagglutinins
Native serum	1:3,280	1:110	1:1 280	1:210
Serum treated with cysteine	0	1:10	1:640	1:160
Serum heated to 70°C	0	1:10	1:320	1:95
Incomplete antibodies of native serum	1:12,800	1:15,840	1:3,300	1:17,000
Incomplete antibodies of heated serum	1:1,350	1:17,500	1:1,280	1:15,120

were determined in the chromatographic fractions of serum antibodies of each type, and incomplete antibodies were detected by the Coombs' test.

EXPERIMENTAL RESULTS

A study of the dynamics of synthesis of macroglobulin and 7S-antibodies in explants of organs from intratracheally immunized animals showed that, after the first injection of antigen, explants of the lungs and paratracheal lymph glands synthesized 19S-antibodies only. The actual type of the antibodies formed in explants of the spleen could not be determined at these times because of the special role of this organ in immunogenesis following intratracheal immunization [1, 2]. After the second immunization, the titer of macroglobulins in the lymphoid organs fell sharply, while production of 7S-antibodies increased significantly at the same time. In the lungs, however, after the second injection of sheep's erythrocytes predominantly macroglobulins were synthesized, and the production of 7S-antibodies remained at a low level (Fig. 1).

After intravenous immunization the general principles governing synthesis of macroglobulin and γG-antibodies in the lymphoid organs and lungs were approximately the same: synthesis of 19S-antibodies was replaced by synthesis of 7S-antibodies after the second injection of antigen (Fig. 2).

The differences between the dynamics of replacement of synthesis of macroglobulin antibodies by that of γG-antibodies in the lungs compared with the spleen and paratracheal lymph glands indicate, in the writers'

opinion, that after intratracheal immunization, antibody formation in the lungs is carried out mainly by cells of nonlymphoid nature, possibly by alveolar macrophages. This hypothesis seems justified in the light of recent findings [6] demonstrating a similar function of the peritoneal macrophages. After intravenous immunization, however, these observations suggest that antibody synthesis in the lungs takes place in the lymphoid structures of this organ.

Investigation of certain biological and physiochemical properties of the serum 19S- and 7S- antibodies revealed the following patterns: macroglobulin antibodies possess marked hemolytic activity, 20-30 times greater than their hemagglutinating power. The hemolytic action of 7S-antibodies is weaker, but they are better able than 19S-antibodies to agglutinate sheep's erythrocytes. Treatment of the serum with cysteine, like heating it to 70°C, led to the almost total loss of serologic activity of the macroglobulins, whereas the hemolytic and hemagglutinating power of the 7S- antibodies still remained high (Table 1).

Besides its use for the detection of incomplete agglutinins, the antiglobulin test was also used to demonstrate properties of incomplete antibodies in the hemolysins, and the hemolytic action of incomplete macroglobulin antibodies was also found to be considerably higher than that of the 7S-antibodies. The ability of these antibodies to react serologically in the presence of antiglobulins still persisted after heating the serum.

The results indicate differences between the properties and biological role of the types of antibodies studied. In particular, with their predominantly hemolytic activity, the 19S-antibodies can evidently destroy erythrocytes rapidly and thus provide the body with antigenic information required for the subsequent synthesis of 7S-antibodies neutralizing the erythrocytes by agglutination.

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