SYNTHESIS OF ANTIBODIES AGAINST SOLUBLE PROTEINS BY SINGLE CELLS

Ya. S. Shvartsman

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As a result of the recent increase in interest in the study of antibody synthesis by single cells, methods have been developed for carrying out such investigations [1, 4, 5, 6, 8]. However, none of these methods is intended for detecting the synthesis of antibodies against soluble proteins by single cells. Because of inadequate specificity, the reaction of adsorption of polystyrene latex particles loaded with protein has proved unsuitable for this purpose [4]. These facts have made it necessary to develop new methods to study the synthesis of antibodies against soluble protein by single cells.

This paper describes the results of investigations conducted in this direction.

EXPERIMENTAL METHOD AND RESULTS

Experiments were carried out on albino rats of both sexes weighing 200-250 g. The animals were immunized with horse serum γ-globulin (HGG) with an adjuvant of the Freund type, and in control experiments with HGG, egg albumin, Aberdeen complex salmonella antigen (AC) or with adjuvant alone. A single dose of antigen was injected subcutaneously into the plantar surface of the hind limbs.

The dose of proteins per injection was 10 mg, the dose of complex antigen 0.3 mg, and the dose of adjuvant 0.1 ml. The animals were sacrificed on the 5th-6th day after immunization, the popliteal lymph glands were extracted, and a suspension of single cells prepared from them [2]. The residue of washed cells was diluted with balanced salt solution in a ratio of 1:8 and to it was added an equal volume of suspension of particles or erythrocytes loaded with the test antigen. After incubation, a small volume of this mixture was applied to a glass slide covered with mineral oil, a cover slip was placed over it, and the preparation was studied under the microscope with an immersion phase-contrast optical system. As insoluble substrate for the test antigen, more than 30 batches of polystyrene latex and of the copolymer of styrene with α-methylstyrene, four samples of different glands of zeolites, two samples of activated charcoal particles*, and sheep's erythrocytes were used. In addition, an aggregated HGG obtained from A. M. Olovnikov at the N. F. Gamaleya Institute was also tested. The protein was adsorbed on the surface of the polystyrene, zeolite and charcoal particles in buffered mixtures of different pH values. To conjugate the protein with the erythrocytes, bis-diazotized benzidine (BDB) was used [3]. To prepare erythrocytes conjugated with protein and agglutinated by a serum specific to the conjugated protein up to high dilutions, optimal proportions of protein, erythrocytes, and BDB had to be ensured during conjugation. The best results were obtained in the following experimental conditions. Into a test tube measuring 40 × 120 mm, 10 mg protein dissolved in 10 ml 0.15 M phosphate mixture (pH 7.3) and 1 ml of a 50% suspension of erythrocytes were poured. To this mixture was added 2 ml of a solution prepared from 1 ml BDB and 14 ml of 0.15 M phosphate mixture (pH 7.3). The mixture was heated in a water bath at 30° for 15 min with vigorous mixing. The tube was then rapidly cooled and centrifuged at 0° for 5 min at 700 rpm. The residue was washed twice with 10 ml of solvent and a 5% suspension of erythrocytes made from it, kept at 4°, and used in experiments for 5-6 days. The quality of the suspension thus prepared was checked by the agglutination reaction on slides.

The results of preliminary experiments with all carriers of the test antigen except erythrocytes proved unsatisfactory. Latex, zeolite, and charcoal particles, loaded with protein, adhered to the surface of the cells not only of immune, but also of normal animals. Experiments with the aggregated HGG required the presence of a borate buffer in the medium, and this caused serious injury to the cells.

*The polystyrene latex was obtained from the Leningrad Research Institute of Polymerization Plastics and the Leningrad Technological Institute, the zeolites from the Institute of Silicate Chemistry, Academy of Sciences of the USSR, and the activated charcoal from the S. M. Kirov Military Medical Academy.

Leningrad Research Institute of Vaccines and Sera (Presented by Active Member of the Academy of Sciences of the USSR P. F. Zdrodovskii). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 62, No. 12, pp. 75-77, December, 1966. Original article submitted May 11, 1965.

TABLE 1. Effect of Temperature and Duration of Incubation on Reaction between Conjugated Erythrocytes and Cells of Immune and Normal Animals

Temperature (in degrees)	Exposure (min)	Immunized animals			Unimmunized animals		
		number of cells studied					
		total	with adhesion of erythrocytes	%	total	with adhesion of erythrocytes	%
37	15	4 389	207	4,71	5 500	8	0,15
37	30	2 988	57	1,91	5 810	23	0,45
20	30	5 104	80	1,56	5 222	9	0,17
20	60	5 461	128	2,34	6 161	8	0,13

TABLE 2. Analysis of the Specificity of the Reaction of Adsorption of Erythrocytes Conjugated with Protein Antigen by Means of BDB

Antigen for immunization of animals	Test antigen	Number of cells studied		
		total	with adhesion of erythrocytes	%
HGG + adjuvant	Erythrocytes conjugated with HGG	5,150	168	3.25
The same	Erythrocytes not conjugated	5,086	11	0.21
HGG	Erythrocytes conjugated with HGG	3,155	23	0.73
The same	Erythrocytes not conjugated	3,114	7	0.22
Adjuvant	Erythrocytes conjugated with HGG	3,071	9	0.29
The same	Erythrocytes not conjugated	3,041	7	0.23
Egg Albumin + adjuvant	Erythrocytes conjugated with HGG	5,138	12	0.23
Aberdeen complex salmonella antigen	The same	5,320	5	0.09

The object of series I of the main experiment was to determine the optimal conditions for reaction between the producer cells and the conjugated erythrocytes (Table 1).

The maximal number of cells with adhesion of erythrocytes (4.71%) was found in the experiments in which a mixture of cells with erythrocytes was incubated at 37° for 15 min. After an exposure of 30 min, the number of such cells was much smaller (1.91%). The reason for this was that with the longer exposure in the water bath, many of the cells formed large conglomerates with the erythrocytes, which could not be studied under the microscope.

In the experiments of series II the specificity of the reaction of adsorption of the erythrocytes was studied.

The mixture of cells with erythrocytes was incubated for 15 min at 37° (Table 2).

The results of these experiments showed that the reaction of adsorption of erythrocytes conjugated with protein by means of BDB is a sufficiently reliable test for studying synthesis of antibodies by single cells.

At the same time, when a single injection of small doses of soluble protein is given, only a small part of the total cell population is concerned in immunogenesis. In such a situation, therefore, the experiments should be carried out in two stages; initially the reaction between the cells and unsensitized erythrocytes should be studied, in order to obtain details of the nonspecific "background," and only then should experiments be carried out with conjugated erythrocytes to study antibody synthesis by the cells.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of the first issue of this year.
