

NATURE OF THE ADSORPTION REACTION OF ERYTHROCYTES
CONJUGATED WITH SOLUBLE PROTEINS

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Adsorption of erythrocytes conjugated with soluble proteins on lymphoid cells is shown to be a specific test for detecting antibody-producing cells. This reaction cannot be due to antibodies passively adsorbed on the surface of the lymphoid cells.

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According to recent reports in the literature [3], certain adsorption tests recommended for determination of antibody production by single cells are insufficiently specific, because antigen particles may be attached not only to antibody-producing cells, but also to cells passively adsorbing antibodies on their surface.

The object of the present investigation was to test the specificity and elucidate the mechanism of the reaction of adsorption of erythrocytes conjugated with protein [1].

EXPERIMENTAL METHOD

Experiments were carried out on albino rats of both sexes weighing 200-250 g. Horse serum γ -globulin (HSG), given as a single subcutaneous injection into the plantar surfaces of both hind limbs, was used as antigen for immunization of the animals and also as test antigen. One group of rats was immunized with HSG together with Freund's complete adjuvant. The dose of antigen was 10 mg per injection. The animals were sacrificed on the 5th day after immunization, and the popliteal lymph glands were removed and a suspension made from their cells. Conjugation of the erythrocytes with HSG and determination of antibody production by single cells were carried out by the method developed in our laboratory [1].

EXPERIMENTAL RESULTS

In the experiments of series I the specificity of the reaction of adsorption of protein-bound erythrocytes was studied. To do this, the reaction between conjugated and normal erythrocytes was investigated with cells obtained from unimmunized animals and from animals immunized with HSG (Table 1).

Erythrocytes conjugated with HSG became adherent in 1.3% of cases to cells isolated from rats immunized with HSG, while normal erythrocytes and erythrocytes conjugated with egg albumin became adherent in 0.1 and 0.05% of cases only. Consequently, the reaction of adsorption of erythrocytes conjugated with protein is specific. If the adsorption of erythrocytes conjugated with protein is due to interaction between antigen and antibody, preliminary contact between producer cells with soluble antigen should inhibit this process. Meanwhile, a foreign antigen should have no effect on the reaction. To discover what actually happens, a suspension of cells obtained from rats immunized with HSG was divided into three portions. The first portion was incubated for 30 min at room temperature with HSG solution (1 mg/ml), the second was treated with egg albumin (EA), and the third with saline. The cells were washed and their reaction with erythrocytes conjugated with HSG was studied (Table 2).

Hence, treatment of the cells with specific antigen caused almost complete inhibition of the adsorption reaction.

The object of the experiments of series II was to study the possibility of possible passive "induction" of the reaction. Blood serum of immunized animals and saline extracts from popliteal lymph glands cells,

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TABLE 1. Determination of Specificity of Adsorption Reaction of Erythrocytes Conjugated with Protein Antigen

Animals	Erythrocytes	Number of cells studied	Number of cells with adhesion of erythrocytes	
			ab-solute	%
Unimmunized rats	Normal	11,216	8	0.07
The same	Conjugated with HSG	9,321	8	0.085
Immunized rats	Normal	10,638	13	0.1
The same	Conjugated with HSG	5,710	78	1.3
" "	Conjugated with egg albumin	5,780	3	0.05

TABLE 2. Effect of Preliminary Treatment of Cells with Specific and Foreign Antigen on Reaction of Adsorption of Erythrocytes Conjugated with Protein

Antigen used to treat cells	Number of cells studied	Number of cells producing anti-bodies	
		ab-solute	%
HSG	6603	9	0.14
EA	6475	75	1.1
Saline	6620	84	1.2

TABLE 3. Possibility of Adsorption of Antibodies on Surface of Lymphoid Cells of Unimmunized Animals

Antigen used for immunizing donors	Preparation used for "induction"	Mode of "induction"	Number of cells investigated	Number of cells with adhesion of erythrocytes	
				ab-solute	%
HSG	Cell extract	In vitro	6833	3	0.04
The same	Serum	The same	4352	6	0.13
" "	" "	In vivo	5230	6	0.1
HSG + Freund's adjuvant	" "	In vitro	6294	8	0.12
The same	Cell extract	The same	7756	9	0.1

disintegrated by grinding, obtained from the lymph glands of rats immunized with HSG and with HSG together with Freund's complete adjuvant, were used as preparations. According to Boyden's findings [2], injection of antigen with complete adjuvant gives a much higher yield of cytophilic antibodies.

In some experiments, cells taken from unimmunized rats were incubated for 1 h at 37° with one of the preparations. The cells were then washed twice and treated with conjugated erythrocytes ("induction" in vitro).

In other experiments, the immune serum in a dose of 0.4 ml was injected into the plantar surface of both hind limbs of an unimmunized animal 24 h before the experiment ("induction" in vivo).

The results of these experiments are given in Table 3.

As Table 3 shows, lymphoid cells did not adsorb antibodies on their surface passively. Consequently, only cells actively synthesizing antibodies were detected by the erythrocyte adsorption reaction. These findings are in agreement with the results of investigations showing that cytophilic antibodies are adsorbed only on macrophages [4].

LITERATURE CITED

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