A SIMPLE METHOD OF DETECTING Fc-RECEPTOR SITES ON CELLS

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The bacterial labeling method for detecting Fc^+-cells is based on the ability of *Staphylococcus aureus* with a high concentration of protein A (strain Cowan I) to form rosettes with cells treated with immune complexes or aggregated γ globulin. The new method differs from other methods of detection of Fc^+-cells by the simplicity of the procedure for detecting cells binding immune complexes and aggregated γ globulin.

KEY WORDS: Fc-receptor sites; Fc-positive cell (Fc^+-cell); T cell; B cell.

Receptors for the Fc region of IgG were first found with the aid of radioactively labeled antigen–antibody complexes [2] and heat-aggregated γ globulin [4] by methods of indirect immunofluorescence and autoradiography.

The writers suggest a method of identification of cells binding aggregated γ globulin and soluble immune complexes, by bacterial labeling with *Staphylococcus aureus* of a strain whose cells carry protein A, which has high affinity for the Fc region of IgG [12]. Through the Fc receptor, lymphocytes bind aggregated IgG or immune complexes, and then, acting through free Fc-sites, they bind protein A of *S. aureus*.

EXPERIMENTAL METHOD

Suspensions of lymphocytes were obtained from the thymus, spleen, and lymph nodes of CBA mice. Phagocytes were removed from the suspension of spleen cells after incubation with iron carbonyl by means of a magnet [1]. Erythrocytes were lysed with 0.83% NH_4Cl solution [3]. The method of separation of the cell population, on the "Elphor VaP 5" apparatus for preparative cellular electrophoresis, was described previously [1]. Separation of Fc^+- and Fc^--cells on a monolayer of sensitized sheep's erythrocytes was carried out by the method in [9] in the writers' own modification [1]. Populations of T and B cells were separated on columns with nylon wool [7]. The methods of obtaining rabbit antiserum against mouse T cells and of setting

TABLE 1. Binding of Aggregated Human γ Globulin by Cells of Mouse Lymphoid Organs

Source of cells	Preliminary adsorption with thymocytes	Fc-positive cells, percent; dose of aggregated γ globulin, μg					
		–	50	500	1000	2000	5000
Spleen	+	7–9 (n=4)	18–20 (n=2)	36–48 (n=3)	38–39 (n=2)	37–45 (n=3)	39–47 (n=3)
	–		33–48 (n=2)	46–60 (n=3)	47–52 (n=3)	44–68 (n=3)	79–85 (n=3)
Thymus	+	5–9 (n=4)	17–22 (n=2)	19–20 (n=3)	17–20 (n=3)	38–38 (n=2)	39–44 (n=2)
	–		17–25 (n=2)	50–57 (n=3)	53–60 (n=2)	65–70 (n=2)	88–91 (n=2)
Lymph nodes	+	7–12 (n=4)	24–25 (n=2)	24–28 (n=2)	24–27 (n=3)	26–36 (n=3)	36–53 (n=3)
	–		25–27 (n=2)	26–26 (n=2)	35–41 (n=2)	47–57 (n=2)	71–94 (n=3)

Legend. Here and in Table 2, number of experiments shown in parentheses.

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TABLE 2. Binding of Antigen—Antibody Complex by Cells of Lymphoid Organs

Source of cells	Quantity of antigen (in mg/ml) to equal standard volume of antiserum						
	K	0,5	1,0	5,0	10	50	100
Spleen	2—7 (n=3)	17—19 (n=2)	19—21 (n=2)	23—36 (n=3)	39—43 (n=3)	39—42 (n=2)	25—30 (n=2)
Thymus	7—7 (n=3)	6—12 (n=2)	10—11 (n=2)	9—13 (n=3)	5—14 (n=3)	6—9 (n=3)	3—8 (n=3)
Lymph nodes	7—12 (n=3)	22—30 (n=2)	23—29 (n=2)	23—29 (n=2)	17—38 (n=3)	36—42 (n=3)	21—27 (n=3)

TABLE 3. Ability of Separate Cell Subpopulation of Mouse Thymus and Spleen to Bind Aggregated Human γ Globulin

Source of cells	T cells (cytotoxic test), %	EA-rosette-forming cells, %	Fc-positive cells (bacterial label), %
Thymus	87—100	Under 1	17—38
Spleen	40—52	41—46	40—47
Spleen cells adherent to nylon	21—23	56—60	80—83
Spleen cells not adherent to nylon	78—81	18—20	25—29

up the cytotoxic test were described previously [1, 6, 13]. The Fc-rosette-formation test with sheep's erythrocytes coated with antierythrocytic antibodies was carried out according to the recommendations. *S. aureus* strain Cowan I was grown on Hottinger's alkaline agar. A bacterial suspension obtained 18 h after seeding was washed three times with physiological saline buffered with phosphate (PBS), treated for 3 h with 0.5% formaldehyde solution in PBS, and heated for 3 min at 80°C. The staphylococcal suspension was kept at 4°C in PBS with 0.02% sodium azide. Human γ globulin (from Sigma Chemical Co.) was first adsorbed on thymus cells (10 mg protein in 1 ml PBS to 0.2 ml of solid residue) for 30 min at 4°C. The γ globulin was aggregated for 15 min at 63°C [4]. To prepare immune complexes, human serum albumin (from Reanal, Hungary) and rabbit antiserum against this protein were used. The zone of equivalence in the precipitation test was determined by the method in [10].

Thymus, spleen, and lymph node cells (1×10^7 cells in 1 ml) were incubated with different doses (50-5000 μg) of aggregated γ globulin or with antigen—antibody complexes (0.2 ml) for 40 min at 4°C. After three washings in PBS, the cells were suspended in 0.3 ml of 5% staphylococcal suspension and incubated for 15 min at 4°C. The volume of the suspension was then made up to 10 ml. The cells were sedimented (800 rpm, 5 min). The number of rosette-forming cells was determined in a Goryaev's chamber, no fewer than 200 cells per sample being counted. Cells binding at least four staphylococci were regarded as labeled.

EXPERIMENTAL RESULTS

Binding of aggregated human IgG was detected by rosette formation with staphylococci in the mouse thymus, lymph nodes, and spleen (Table 1). In control experiments with aggregated human serum albumin, no binding of the bacterial label took place. Preliminary treatment of spleen cells with pronase, an enzyme destroying the Fc receptors, led to complete abolition of binding of aggregated γ globulin by the cells. Treatment of the cells (10^7 in 1 ml) with pronase (from Serva, 2 mg/ml) at 37°C for 30 min reduced the number of Fc^+-cells from 38 to 0%. Under these conditions pronase did not affect the viability of the cells. To detect Fc^+-cells when a high dose of γ globulin was used, preliminary adsorption of the native protein with thymocytes was essential. The optimal concentrations of protein for detection of Fc^+-cells varied within the range 0.5-5 mg/2×10^7 cells in 1 ml. Cells with Fc-receptor sites also were detected with the aid of soluble antigen—antibody complexes (Table 2). On simul-

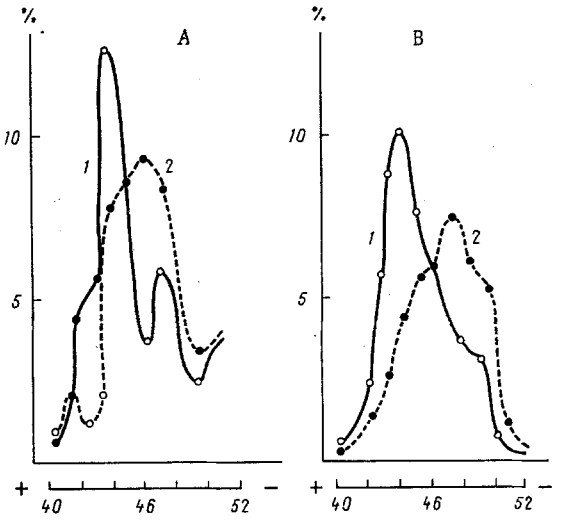

Fig. 1. Profiles of electrophoretic fractionation of Fc^+- and Fc^--cells from spleen. A) distribution of cells binding aggregated -globulin and *Staphylococcus aureus* Cowan I; B) distribution of Fc^+- and Fc^--cells after adsorption on monolayer by Kedar's method. 1) Fc^--cells; 2) Fc^+-cells. Abscissa, serial number of fractions; ordinate, number of cells in individual fractions (in percent of total number).

taneous labeling of spleen cells with aggregated γ globulin and antigen–antibody complexes the number of labeled lymphocytes (68.8%) did not exceed the number of cells binding each reagent separately (aggregated γ globulin 65.5%, antigen–antibody complexes 39%). Hence it can be concluded that both markers revealed the same cell population in the spleen. Most of the Fc^+-cells in the spleen, isolated on a monolayer of sensitized sheep's erythrocytes, were labeled by aggregated γ globulins, further evidence of the identity of the cells binding the immune complexes and aggregated γ globulin. In the thymus, however, Fc^+-cells bound aggregated γ globulin only. This may be due either to the lower density of Fc-receptor sites on thymocytes or to the existence of a receptor for aggregated γ globulin distinct from that revealed by the antigen–antibody complex.

During fractionation of the splenocytes on a column with nylon wool cells binding aggregated γ globulin were found chiefly in the B cell fraction (Table 3). A similar distribution by ability to adhere to nylon wool also was found for Fc^+-cells with the aid of antierythrocytic antibody–erythrocyte complexes. The distribution of spleen cells binding aggregated γ globulin during electrophoretic fractionation in a free flow of fluid on the "Elphor VaP 5" apparatus was similar to the profile of Fc^+-cells separated on a monolayer of sensitized erythrocytes (Fig. 1).

The suggested method of detection of cells binding aggregated γ globulin and soluble immune complexes is thus simple and effective. The identity of these cells with Fc^+-cells detected by the classical method of rosette formation with sensitized sheep's erythrocytes was demonstrated. Recently Ghetie et al. [5] suggested sheep's erythrocytes coated with protein A as a marker for IgG bound with the Fc-receptor site. The method suggested by the present writers for detection of Fc^+-cells is simpler, for it does not require isolation and purification of the protein A. This method takes less time and uses less reagents than the methods of immunofluorescence and autoradiography.

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