ELECTROPHORETIC AND IMMUNOLOGICAL CHARACTERISTICS OF Fc^+ AND Fc^- FRACTIONS OF CBA MOUSE SPLEEN CELLS

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Conditions for effective separation of Fc^+ and Fc^- cells on a monolayer of sheep's erythrocytes, sensitized with antierythrocytic antibodies, were chosen. A modification of the method, consisting of replacing the poly-L-lysine by protamine sulfate, is suggested. The electrophoretic distribution profiles of Fc^+ and Fc^- cells of the mouse spleen were established. The Fc^+ cells mainly have low electrophoretic mobility, whereas that of the Fc^- cells is higher. The Fc^+ fraction contains mainly B lymphocytes, the Fc^- fraction mainly T cells. The electrophoretic profiles of the Fc^+ and Fc^- cells overlap considerably. If spleen cells are first passed through a column with glass beads, the profiles of the Fc^+ and Fc^- cells which do not adhere to the glass overlap to a much lesser degree.

KEY WORDS: *Fc receptor*; *Fc^+ and Fc^- cells*; *electrophoretic mobility of cells*; *T and B cells*.

As a result of the successful study and discovery of the fine mechanisms of immunogenesis it is possible to make a subpopulation analysis of lymphocytes on the basis of intercellular differences in biological (differential antigens and receptor structures of the plasma membrane) and physicochemical (total electric charge, size, and density of the cells) parameters. With this approach, the comparative analysis of Fc^+ and Fc^- cells is of considerable interest, for the Fc receptor is ascribed an important role in immunological phenomena such as the incorporation of cells into the immune response [6], regulation of the immune response [5], and the phenomenon of antibody-mediated cytotoxicity [11].

The Fc receptors for antigen–antibody complexes in the peripheral lymphoid organs of mice are known to be represented on all or nearly all B cells [1, 2] and on some of the T cells [10]. Because of the predominant location of Fc receptors on the B cells of the lymph nodes and spleen of nonimmune mice, highly effective separation of T and B cells has become possible through the use of chromatographic methods based on affinity on columns containing antigen–antibody complexes [3] and adsorption on a monolayer of erythrocytes coated with antierythrocytic antibodies [9].

However, when such methods are used to separate T and B cells, allowance must be made for the quantitatively small but qualitatively important contribution of Fc^+-T lymphocytes, K cells, and nonlymphoid Fc^+ cells to the Fc fraction.

The results of an analysis of cell fractions undertaken to assess the degree of heterogeneity among Fc^+ and Fc^- cells are given below. The method of separation of the cells on a monolayer described by Kedar et al. [9] was used. In the modification of the method suggested by the present writers poly-L-lysine, binding the erythrocyte monolayer to the surface of the plastic dishes, was replaced by protamine sulfate, a protein with a high content of lysine ε-amino groups. This substitution does not change the principal properties of the monolayer (uniformity and density of distribution of the erythrocytes, firmness of binding to the petri dishes) and does not impair the efficiency of specific settling of Fc^+ cells on the monolayer, and it is accordingly very convenient to use the more readily available protamine sulfate instead of the expensive poly-L-lysine

Experiments were carried out on inbred CBA mice and rabbits. When choosing the optimal concentration of protamine sulfate, the quality of the monolayer of erythrocytes was evaluated

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The range of optimal concentrations was 20-50 mg/ml. To prepare the erythrocytic monolayer, 5.6 ml of a solution of protamine sulfate (20 mg/ml) or poly-L-lysine (50 μg/ml) in physiological saline, buffered with phosphates, pH 7.2 (PBS), in a volume of 5.6 ml, was poured into plastic Petri dishes (Falcon Plastics, 90 × 15 mm), and these were allowed to stand at room temperature for 45 min. The dishes were then rinsed several times with PBS, filled with a 1.5% suspension of sheep's erythrocytes in a volume of 5.6 ml, and allowed to stand for 45 min at room temperature. After washing with PBS, the monolayer could be kept in the refrigerator until required for use for at least 24 h. A layer of inactivated (56°C, 30 min) rabbit antiserum against sheep's erythrocytes, in a dilution of 1:50 (hemagglutinin titers 1:256-1:512), was poured over the resulting erythrocyte monolayer. The antiserum was obtained by hyperimmunization of rabbits [9]. After incubation for 45 min with antiserum at 37°C the monolayer was washed with PBS.

A suspension of spleen and thymus cells was obtained by rubbing the organs, previously cut into small pieces with scissors, through nylon gauze into Eagle's medium with inactivated (56°C, 30 min) 5% fetal bovine serum (Flow Lab). The cells were pipeted and allowed to stand in test tubes on an ice bath for 5-10 min to allow sedimentation of the cell aggregates. In some experiments the cell suspension ($60 \cdot 10^6$ cells/ml, total 5 ml) was passed through a column with glass beads (a 5-cm^3 syringe) and filled up to the 3-ml mark with beads 125-200μ in diameter (Serva) at room temperature. The yield of cells not adhering to the glass was 40-50%, and 90% of these were lymphocytes.

The cells (10^7 in 1 ml) were introduced into the dishes in a volume of 4.5 ml (altogether $4.5 \cdot 10^7$ cells) and incubated on a revolving platform (5 rpm, 10 min, 37°C), after which they were sedimented for 5 min at 100 g without cooling. The nonadherent cells (the Fc^- fraction) were washed off the monolayer with medium as the dishes were carefully revolved. The sorbent with the firmly bound cells (the Fc^+ fraction) was treated with 6.4 ml isotonic solution of NH_4Cl in 0.2 M Tris-HCl buffer, pH 7.3 to destroy the erythrocytes. The cells were carefully removed from the surface of the dishes by means of a rubber spatula.

Rabbit antiserum against mouse T cells, prepared by the writers by Golub's method [7], in the cytotoxic test of Takahashi et al. [14], in a dilution of 1:16 killed 27-34% of spleen cells, 85-100% of thymocytes, and fewer than 5% of bone marrow cells of CBA mice, whereas antiserum against total mouse γ-globulins (N. F. Gamaleya Institute of Epidemiology and Microbiology), adsorbed by the method of Forni and Pernis [6], in a dilution of 1:8 killed 46% of spleen cells and 6% of thymocytes.

The cells were separated on an apparatus for preparative cellular electrophoresis in a free flow of fluid (Elpor VaP5, from Bender, Hobein Co.). The buffer of low ionic strength for electrophoretic fractionation and the electrode buffer were prepared by von Boehmer's formulas [4]. Nonviable cells were removed from the cell suspension before electrophoretic fractionation [4].

Living cells accounted for 97-99% of the yield. The conditions for fractionation of the cells were: intensity of the electric field 80 W/cm, rate of flow of the buffer 480 ml/h, rate of feeding of cells into fractionation chamber 1.2 ml/h.

During electrophoresis on the "Elpor VaP5" instrument spleen cells from the fraction adherent to the monolayer possessed lower electrophoretic mobility than the cells of the nonadherent fraction, i.e., their distribution by charge on the cell membrane was close to that for B and T cells described in the literature [4] (Fig. 1). The greater effectiveness of electrophoretic fractionation of cells of the Fc^+ and Fc^- fractions after preliminary purification of the spleen cells on columns with glass beads will be noted (Fig. 1c); allowance must be made here for the considerable loss of cells (over 50%), with a substantial change in the quantitative proportions of cells with different electrophoretic mobilities (Fig. 2). The more effective fractionation of the Fc^+ and Fc^- cells after preliminary purification on columns with glass beads was thus accompanied by substantial changes in the relative numbers of lymphocytes at the subpopulation level.

The electrophoretic fractionation profiles of Fc^+ cells adherent and nonadherent to glass differed by a greater degree than the corresponding profiles of the Fc^- cells. The small shift of the Fc^+ fractions of splenocytes nonadherent to glass toward the cathode was probably connected with retention of the cells moving rapidly in an electric field on the column.

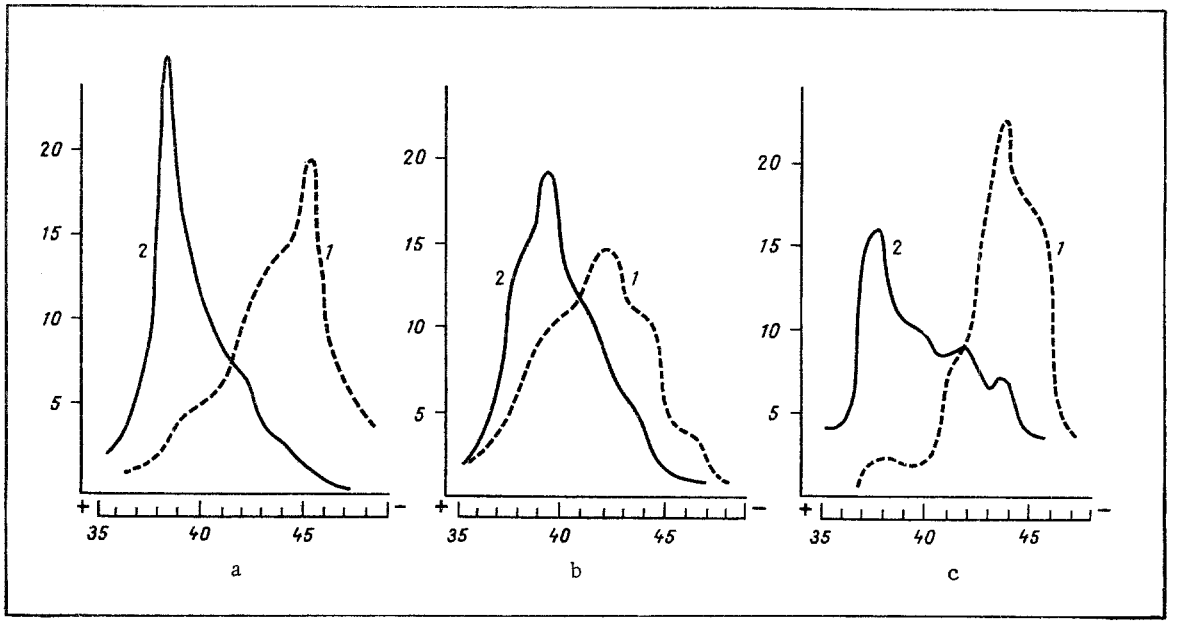

Fig. 1. Profiles of electrophoretic fractionation of splenic Fc^+ and Fc^- cells after adsorption on a monolayer by Kedar's method. a) Underlayer poly-L-lysine; b, c) underlayer protamine sulfate; b) spleen cells; c) spleen cells not adherent to glass. 1) Fc^+ cells; 2) Fc^- cells. Data on pool of spleen cells obtained from three to five mice shown in each graph. Ordinate, number of cells in individual fractions (in % of total number); abscissa, No. of fractions.

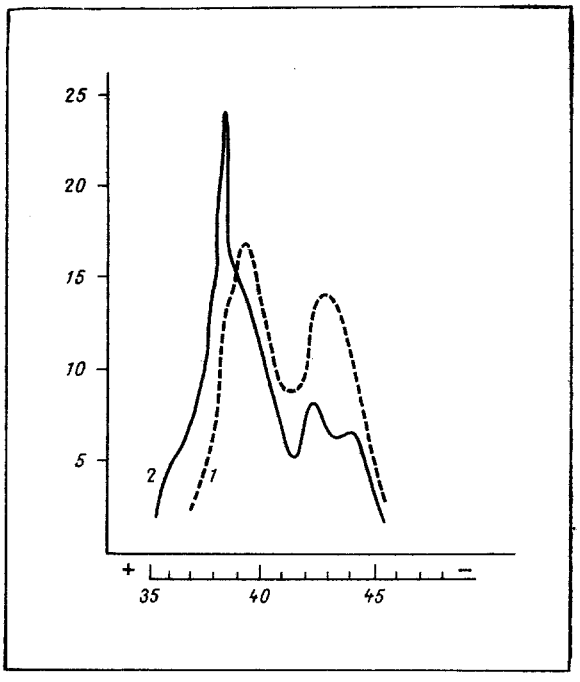

Fig. 2. Comparison of electrophoretic distribution profiles of spleen cells before and after passage of the original cell suspension through a column with glass beads (data on the pool of spleen cells obtained from three mice): 1) spleen cells before passage through column; 2) spleen cells adherent to glass. Remainder of legend as in Fig. 1

Judging from data in the literature [12], these were immature B cells and nonlymphoid cells (macrophages, polymorphonuclear leukocytes).

When poly-L-lysine and protamine sulfate were used as the underlayer for the erythrocytes highly effective fractionation of the cells was achieved (Fig. 1a, b). The distribution of cells of the Fc^+ and Fc^- fractions obtained with the Elfor VaP5 apparatus was almost the same as that expected on the basis of the results of the cytotoxicity test with anti T and antiimmunoglobulin serum. During fractionation of the cells on the monolayer (protamine sulfate underlayer) without preliminary purification on the column with glass beads, the anti-T serum had a cytotoxic action on 23% of the Fc^+ cells and on 72% of the Fc^- cells, whereas the antiimmunoglobulin serum had a cytotoxic action on 75 and 24% of cells respectively. After preliminary removal of cells adherent to glass the anti-T serum killed 6% of the Fc^+ cells and 89% of the Fc^- cells. As a result of the high purity of the fractions, it was possible to determine the ratio between subpopulations in the B fraction, i.e., the distribution of B cells at different stages of functional maturity, contaminated by only a few T cells. By a combination of the Fc monolayer method and the "Elfor VaP5" instrument, it was possible to seek and purify Fc^+-T cells which, according to Stout and Herzenberg [13], account for about 9% of all spleen cells. Work in this direction is in progress.

The results confirmed that highly purified fractions of T and B cells can be obtained by the method of Kedar et al. in the original formula [9] and they demonstrated the high effectiveness of the method as modified by the present writers. The use of protamine sulfate instead of the expensive poly-L-lysine makes the method much easier to use.

Predominance of Fc^+ cells in the splenocyte pool, migrating slowly in an electric field, was demonstrated previously by Häyry et al. [8], who studied Fc-rosette formation in cells fractionated on the "Elfor VaP4" instrument. However, the effectiveness of evaluation of Fc^+ cells by the rosette-formation method is limited by the possibility of interaction of subagglutinating doses of antibodies with erythrocytes [10]. The density of settling of antibodies on the monolayer was considerably higher, so that Fc^+ cells could be more easily determined.

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