SEPARATION OF ROSETTE-FORMING CELLS WITH THE AID OF AN IMMUNOSORBENT BASED ON SEPHADEX G-25 AND G-75

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A method of separating rosette-forming cells on columns with an immunosorbent based on Sephadex G-25 and G-75 is described and has been tested. The protein antigen (bovine serum albumin – BSA) is attached by covalent bonds to the surface of the Sephadex granules oxidized by sodium periodate. Cells with receptors on their surface were tested by the rosette method. Up to 88% of the rosette-forming cells were retained on a column packed with BSA-Sephadex granules. Nonspecific retention of the cells was relatively small, about 4%.

KEY WORDS: rosette-forming cells, separation of; immunosorbents; cell receptors.

Immunosorbents based on the use of glass balls [12], sepharose [2], polyacrylamide [11], and methylmethacrylate [13] have been suggested for the separation and isolation of cells carrying immunoglobulin receptors on their surface or synthesizing antibodies. The greatest success has been attained by Henry et al. [4], who not only absorbed lymphocytes with antihapten receptors but then went on to separate them from the absorbent, i.e., essentially to isolate a clone.

Reports have recently been published [7-9] of the use of Sephadex G-25 and G-75 granules for the preparation of an immunosorbent with which serum antibodies could be isolated.

It was decided to investigate the possibility of using immunosorbents based on Sephadex granules in order to separate rosette-forming lymphocytes.

EXPERIMENTAL METHOD

The immunosorbent was prepared from Sephadex G-75 and G-25 (extra fine, fine, and coarse). The protein was bound with covalent bonds to the surface of the Sephadex granules [7, 8]. To do this, after swelling in water the Sephadex granules were treated with sodium periodate solution (0.01-0.1 M) for 1 h at room temperature. After being washed three times with distilled water the granules were placed in a solution of bovine serum albumin (BSA) (10-30 mg/ml, Koch-Light Ltd., England) for 18-20 h and at 4°C. In the course of this period the tubes were shaken several times. After rinsing four times in the cold with buffered (pH 8.0) physiological saline the immunosorbent was kept at 4°C for a few days. In some of the experiments the stroma of sheep's red blood cells was bound to the surface of the Sephadex granules. The stroma was isolated as follows. A 20% suspension of sheep's red cells was treated with 10 volumes distilled water, incubated for 10 min at 37°C, and centrifuged for 20 min at 10,000 g. The residue was washed twice in physiological saline and resuspended in the original volume.

Experiments were carried out on BALB/c mice which were immunized by a single intraperitoneal injection of alum-precipitated BSA (0.1 mg protein per mouse). The serum of the experimental mice was

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tested by the passive hemagglutination method of Boyden and Stavitsky. In some of the experiments the mice were immunized with a single intraperitoneal injection of sheep's red cells (10^8 cells).

Spleen cells were isolated on the 4th-7th day after immunization by pressing the spleen tissue through Kapron gauze into medium No. 199 and then drawing it up into a pipet several times. The trypan blue test showed that 80-85% of the cells were viable. Lymphocytes were isolated from the suspension of spleen cells by the method described in [3].

The lymphocytes were absorbed on to the immunosorbent by one of two methods.

In most of the experiments a suspension of splenic lymphocytes in medium No. 199 or in physiological saline buffered with 0.01 M phosphate buffer to pH 7.25 was passed in the cold (4°C) or at room temperature through a column (1.3 × 3.0 cm) packed with the immunosorbent and washed with buffered 0.14 M NaCl solution.

The rate of flow of the cell suspension through the column was 20-30 drops per minute. The column was then washed with buffered physiological saline (rate 150 drops per minute), and the completeness of washing verified microscopically. In some of the experiments a mixture of granules of the immunosorbent and lymphocytes was placed in a Petri dish 5.5 cm in diameter on a horizontal rotating platform (50 rpm). One hour later, to separate the cells the mixture of lymphocytes and granules of the absorbent was suspended in medium No. 199 and filtered through a layer of glass wool.

Cells with antibody molecules on their surface were tested by the rosette method [6]. BSA was bound to the surface of the sheep's red cells by means of tetraazotized benzidine [5] or tannin [1, 10].

EXPERIMENTAL RESULTS AND DISCUSSION

The investigation of the binding of protein to the Sephadex granules (the protein content in the portions of medium used to wash the immunosorbent, measured from the absorption at 280 nm, was added together) yielded results close to those of Sanderson [9]. One gram of Sephadex G-25 extra fine, G-25 coarse, and G-75 bound 10, 23, and 25-70 mg BSA, respectively.

Unlike Sephadex G-25, Sephadex G-75 was broken up by treatment with 0.1 M sodium periodate solution, but it was not damaged by a 0.01 M solution of this compound. The greater resistance of G-25 coarse, combined with the adequate size of the swollen granules (up to 300 μ), makes it advantageous to use.

Before the isolation of the rosette-forming cells (RFCs) the question of what fraction of the splenic lymphocytes was retained nonspecifically in the column under the experimental conditions used was studied. Up to 96% of the added lymphocytes were washed from the column packed with BSA–Sephadex G-25 coarse.

Nonspecific retention of the RFCs in the column was not connected with any change in the physicochemical properties of the surface of the Sephadex granules. It was absent in columns with intact or oxidized Sephadex granules. Specific retention of the RFCs varied in the experiments with BSA from 66 to 88% and in the experiments with sheep's red cells from 48 to 61%. This fact evidently depended on the concentration and avidity of the specific Ig molecules on the surface of the RFCs. Other workers give similar figures [11].

Titers of antibodies against BSA in the experimental mice in the present experiments varied greatly (from 1/128 to 1/8000). When splenic lymphocytes from mice on the 7th day of the secondary response were used, more than 80% of the RFCs were retained on the column. To test the specificity of absorption of the lymphocytes BSA (5-10 mg/ml) or ovalbumin (4.6 mg/ml) was added to their suspension before it was passed through the column. BSA prevented the specific retention of the RFCs whereas ovalbumin did not.

The results thus showed that Sephadex granules with protein antigen fixed to them by covalent bonds specifically absorb cells carrying antibody molecules (receptors) on their surface. Passage of lymphocytes through columns packed with such granules is accompanied by minimal nonspecific retention of the cells (about 4% if Sephadex G-25 coarse is used). The suggested method is labor-saving and gives highly reproducible results with the same cell population. Adsorption on a rotating platform is less convenient and gives poorer results.

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