ISOLATION OF 7S-IMMUNOGLOBULINS (IgG) DETECTABLE ON SPLEEN CELLS AND IN MOUSE SERA IN THE EARLY STAGE OF RAUSCHER'S LEUKEMIA

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In the early stage of Rauscher's leukemia, IgG capable of specific and selective fixation on the surface of the leukemic cells, as shown by the results of an indirect cytotoxic test and the indirect immunofluorescence test, can be isolated at low pH values from the spleen cells of C57BL/6 and BALB/c mice inoculated with Rauscher's virus together with Freund's complete adjuvant. The use of leukemic cells fixed with 1% glutaraldehyde as the immunosorbent makes it possible to isolate IgG, also possessing the properties of antibodies against antigens of the leukemic cell membrane, from the 7S-fraction of the serum.

KEY WORDS: Rauscher's leukemia; isolation of antibodies in leukemia; cytotoxicity of antitumor 7S-antibodies.

Experiments on mice infected with Rauscher's virus (RV) together with Freund's complete adjuvant (FCA) have shown that in the initial stage of leukemia the 19S-fraction of the serum exhibits specific cytotoxic properties whereas the 7S-fraction of the same sera can block the cytotoxicity of the macroimmunoglobulins [2, 4]. Facts have also been obtained on the basis of which the two types of antibodies can be linked with the group-specific surface antigen of mouse leukemic cells [3].

The possibility of isolating immunoglobulins of the IgG class, with the properties of antibodies, from the spleen cells of mice with developing leukemia by the method of elution at low pH values, and also from the serum (7S-fraction) of animals inoculated with RV-FCA, was investigated with the aid of an immunosorbent prepared from fixed or living leukemic cells.

EXPERIMENTAL METHOD

FCA (Difco, USA) was injected intraperitoneally in a dose of 0.1 ml into C57BL/6 mice aged 1.5-2 months and, 6 days later, plasma from leukemic BALB/c mice in a dilution of 1:10 was injected intravenously in a dose of 0.2 ml. The titer of the RV preparation was 10 p. f. u./0.2 ml. The BALB/c mice were infected either with RV alone (leukemic plasma in a dilution of 10^{-2}-10^{-3}) or with the FCA-RV combination. Blood for serologic tests was taken from the retro-orbital sinus at various times after injection, the sera from the mice of each group being pooled. The 19S- and 7S-fractions were obtained by gel-filtration on Sephadex G-200, equilibrated with 0.1 M NaCl in Tris-HCl buffer 0.05 M, pH 7.3; in the process of elution the concentration of immunoglobulins was reduced by 15-20 times.

Preparation of Eluates from Spleen Cells (ESC). The residue of nucleated spleen cells from mice of a given group, washed 6-8 times with Hanks's solution, pH 7.2, was suspended in isotonic glycine-HCl

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TABLE 1. Cytotoxic Test of Monospecific Antibodies against Mouse IgG with Target Cells Treated with ESC of Mice Inoculated with FCA-VR and Developing Rauscher's Leukemia

Materials fixed in vitro on target cells	Rabbit antibodies against mouse IgG	Target cells			
		BALB/c leukemic cells		spleen cells of intact BALB/c mice	
		percentage of dead cells	index of cytotoxicity	percentage of dead cells	index of cytotoxicity
ESC of BALB/c mice:					
6-8 days after injection of RV	1:8	57±5,4	0,48±0,08	39±2,0	0,1±0,01
Intact	1:8	26±5,6	0,10±0,01	34±4,0	0,03±0,02
ESC of C57BL/6 mice:					
10-12 days after injection of RV	1:8	68±6,0	0,62±0,07	40±2,0	0,13±0,02
Intact	1:8	29±3,0	0,13±0,08	35±3,5	0,05±0,02
Control elution, pH 7.2, from C57BL/6 spleen cells (10-12 days after injection of RV)	1:8	18±2,0	—	32±4,6	—
Control elution, pH 7.2, from C57BL/6 spleen cells (10-12 days after injection of RV)	Exhausted with mouse IgG preparation	14±2,0	—	11±2,0	—

Note. Here and in Table 2 mean results of experiments of series III-IV are given.

TABLE 2. Cytotoxic Test of Monospecific Antibodies against Mouse IgG with Target Cells Treated with ESA and 7S-Fractions of Sera of Mice Developing Rauscher's Leukemia

Materials fixed in vitro on target cells	Rabbit antibodies against mouse IgG	Target cells			
		BALB/c leukemic cells		spleen cells of intact BALB/c mice	
		percentage of dead cells	index of cytotoxicity	percentage of dead cells	index of cytotoxicity
ESA of C57BL/6 mice (7-9 days after injection of RV):					
With BALB/c leukemic cells	1:8	83±7,5	0,78±0,12	38±5,2	0,11±0,02
With spleen cells of intact mice (control)	1:8	22±3,0	—	31±3,0	—
7S-Fraction of C56BL/6 sera 7-9 days after injection of RV	1:8	72±3,5	0,63±0,03	38±1,5	0,12±0,02
Intact mice (control)	1:8	21±3,0	—	31±2,0	—

buffer, pH 2.8, or citrate–phosphate buffer, pH 2.6, at the rate of 0.2 ml to 10^8 cells. After incubation for 40 min at room temperature, the supernatant was clarified by centrifuging at 8000 rpm and the reaction was adjusted to neutral by the addition of $NaHCO_3$. The ESC and serum fractions were concentrated 8-10 times with Bio-Gel P-6 (USA) and Liphogel (England) and dialyzed at 4°C against physiological saline in phosphate buffer or Hanks's solution, pH 7.2. As the control elution from the spleen cells phosphate buffer, pH 7.2, was incubated with the cells, concentrated, and dialyzed under the same conditions as the ESC.

Preparation of Eluates of Serum Antibodies (ESA). The serum immunoglobulins were fixed on spleen cells of leukemic and intact (control) BALB/c mice in two variants, using washed living cells or cells previously fixed with 1% glutaraldehyde as the sorbent. Whole sera or concentrated 7S-fractions were incubated with the sorbent cells (0.4-0.6 ml to 10^8 cells) for 30 min at 37°C, and then for 2 h at 4°C; the cells were washed 6-8 times with Hanks's solution or phosphate buffer, pH 7.2, and treated with acid buffer, pH 2.8, the procedure of elution of the immunoglobulins being repeated as described above. The presence of IgG in the ESC and ESA was verified by the precipitation test in agar, using a test system consisting of monospecific rabbit antibodies against mouse IgG and 7S-fractions of the sera of intact mice. The ability of the eluted immunoglobulins to undergo specific fixation on the surface of the leukemic cells was tested in the indirect cytotoxicity (ICT) and indirect immunofluorescence (IF) tests with monospecific rabbit antibodies against mouse IgG. Antibodies were isolated on immunosorbent from a preparation of mouse IgG obtained by ion-exchange chromatography on DEAE-cellulose by Adinolifi's method [5] and treated with

glutaraldehyde. The ICT test was set up by incubating target cells with the test material (0.1 ml to 5×10^5 cells) at 37°C for 20 min, and then at 4°C for 1 h; the thrice washed cells were treated with rabbit antibodies at 37°C for 20 min, washed, and incubated with fresh complement at 37°C for 45 min. The percentage of dead cells and the index of cytotoxicity (IC) were determined by the standard method [1]. The specificity of the reaction was verified by the use of: a) control elution from the spleen cells; b) a preparation of rabbit antibodies, previously exhausted on immunosorbent from mouse IgG. The indirect immunofluorescence test was carried out on preparations of an artificial monolayer of living cells attached to a slide by Dorfman's method [6] by treating them successively with the test or control materials and then with rabbit antibodies against mouse IgG labeled with fluorescein isothiocyanate. All the sera, ESC, and ESA used in the experiments in vitro were previously tested for toxicity (with normal syngeneic cells and with leukemic cells, without complement) and for absence of anticomplementarity.

EXPERIMENTAL RESULTS

1. Detection of Specific Immunoglobulins (Antibodies) in ESC. The precipitation test in agar revealed IgG in the ESC of mice with developing leukemia, but also in eluates from the cells of intact animals. No immunoglobulins were found in the ESC of BALB/c mice with advanced leukemia. The indirect cytotoxic test (Table 1) showed that IgG eluted from mouse cells in the early stage of Rauscher's disease could be selectively fixed on the surface of the leukemic cells and gave a high cytotoxic-effect of antibodies against mouse IgG (IC 0.4-0.7). Meanwhile the ESC of intact mice increased the percentage of cells containing immunoglobulins on their surface only very slightly compared with the effect of the control elution (IC 0.07-0.14). Treatment with neither eluate altered the cytotoxicity of the rabbit antibodies significantly against the spleen cells of intact BALB/c mice. The difference between the results obtained by treatment of the target cells with unabsorbed rabbit antibodies and with rabbit antibodies absorbed previously by the mouse IgG preparation indicates that this method reveals the "background" presence of IgG on the surface of more than 20% of spleen cells from intact mice but only 2-4% of cells from leukemic spleens.

The indirect immunofluorescence test also demonstrated the presence of antibodies in eluates from the experimental mice; treating monolayers of leukemic BALB/c spleen cells with ESC of mice inoculated with FCA-RV led to specific fluorescence of the great majority of the cells in the test with rabbit antibodies against mouse IgG, but not IgM, labeled with fluorescein isothiocyanate.

2. Detection of Specific IgG (7S-Antibodies) Eluted after Adsorption of the Sera on Leukemic Cells. As the results in Table 2 show, 7S-globulins from the sera of mice with the initial stage of leukemia can be fixed specifically on leukemic cells (IC = 0.6-0.7), but not on the spleen cells of uninfected BALB/c mice (IC = 0.09-0.13). The eluates obtained at low pH values from the immunosorbent consisting of leukemic BALB/c cells previously washed after fixation of the sera (or 7S-fractions) of mice with a developing leukemia on them in vitro, contained specific IgG. These immunoglobulins completely preserved their antibody activity in the indirect cytotoxic test and the indirect immunofluorescence test, in which they reacted selectively with Rauscher's leukemia cells (IC and IF index 0.7-0.8). No antibodies could be detected in eluates obtained after adsorption of the same sera on the spleen cells of intact BALB/c mice. Similar results were obtained in the immunofluorescence test with a monolayer of leukemic cells.

The results described above show that eluates containing IgG and obtained from spleen cells of mice with a developing Rauscher's leukemia at low pH values, like 7S-immunoglobulins absorbed from the serum of the same mice on Rauscher's leukemia cells and then eluted from the washed sorbent, equally exhibit activity attributable to the presence of specific antibodies.

The technique described in this paper – the use of leukemic cells fixed with glutaraldehyde as the immunosorbent – can be used to isolate serum antibodies against the antigens of leukemic cell membranes.

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