MECHANISM OF BLOCKADE OF EA-ROSETTE FORMATION BY ANTISERA AGAINST CELL SURFACE ANTIGENS

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UDC 612.112.94.017.1-06

KEY WORDS: EA-rosette formation; Fc receptor; antiserum; T and B lymphocytes.

Receptors for the Fc fragment of the IgG molecule are present on the majority of B lymphocytes, a subpopulation of T lymphocytes, mast cells, monocytes, macrophages, and polymorphs of man and mice [5]. Their biological role has not been adequately explained [9, 11]. Assessment of the function of the Fc receptor requires data on its representation on different cell subpopulations and on its spatial and functional connection with known receptor structures and with antigens of the outer cell membrane [11]. To assess the connection of the Fc receptor with cell surface antigens the method of blockade of EA-rosette formation by antibodies against antigens represented on the plasma membrane of the cell is widely used [12, 13]. However, some workers question the reliability of this method for evidence has been obtained that blockade of the Fc receptor is nonspecific in character [4].

The object of this investigation was to study the mechanism of blockade of EA-rosette formation by antisera against cell surface antigens.

Inbred BALB/c mice, (CBA × C57BL/6)F_1 hybrids, and rats of the August A strain were used. Antiserum against mouse cells was obtained by repeated weekly immunization of rabbits with brain homogenate of (CBA × C57BL/6)F_1 mice in Freund's complete adjuvant, subcutaneously [6]. The animals were exsanguinated 1 week after the last immunization and the sera were inactivated (30 min at 56°C) and absorbed with erythrocytes and liver powder from mice of the same strains as were to be used for the subsequent investigation. The γ-globulin fraction was isolated from the serum by salting out with Na_2SO_4 at 40% saturation. The protein content in the γ-globulin fraction of this serum was 14 mg/ml. Antiserum against mouse immunoglobulins was obtained by subcutaneous immunization of rabbits at an interval of 2 weeks with mouse γ-globulin (sessional dose 10 mg) in Freund's complete adjuvant [7]. Serum was obtained 10 days after repeated immunization, inactivated (30 min at 56°C), and absorbed twice with erythrocytes and twice with thymocytes from mice of the strains to be tested. Before use the antisera were ultracentrifuged for 40 min at 145,000g [3]. Hemolytic serum was obtained by intravenous hyperimmunization of rabbits with sheep's erythrocytes [8].

The sheep's erythrocytes for EA-rosette formation were sensitized by subagglutinating doses of antierythrocytic serum [8]. Erythrocytes (0.3 ml of a 5% suspension), washed three times and sensitized by antibodies, were mixed with an equal volume of suspension of the test cells ($3 \cdot 10^6$ cells/ml) and centrifuged at 100g for 5 min at 20°C. Before the rosettes were counted the cells were allowed to stand on an ice bath for 2 h or overnight. Cells were considered to be rosette forming if they bound at least four erythrocytes. To inhibit EA-rosette formation the cells ($3 \cdot 10^6$-$5 \cdot 10^6$ in 0.2 ml) were incubated with equal volumes of antisera in different dilutions for 30 min at 4°C. After two washings with Eagle's medium, the EA-rosette formation test was carried out [12]. Inhibition of EA-rosette formation was calculated as a percentage by the equation:

$$x = \left(1 - \frac{a}{b}\right) \times 100\%,$$

where x is the percentage of inhibition of EA-rosette formation; a the percentage of EA-rosettes in the experiment; b the percentage of EA-rosettes in the control (normal rabbit serum).

Laboratory of Cytochemistry and Molecular Biology of Immunogenesis, Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 90, No. 10, pp. 453-456, October, 1980. Original article submitted August 17, 1979.

TABLE 1. Inhibition of EA-Rosette Formation by Anti-immunoglobulin Serum in Spleen of (CBA × C57BL/6)F_1 Mice*

Expt. No.	Dilution of antiserum	Inhibition of EA-rosette formation, %
1	1:16	96,3
	1:32	85,3
	1:64	86,4
	1:128	52,9
	1:256	35,6
	1:512	13,1
2	1:16	81,1
	1:32	70,5
	1:64	87,7
	1:128	71,7
	1:256	62,3
	1:512	31,6

*For each dilution of antiserum splenocytes were taken from five mice.

TABLE 2. Inhibition of EA-Rosette Formation by Anti-Baθ-γ-globulin in Mouse Spleen*

Strain of mice	Dilution of anti-Baθ-γ-globulin	Inhibition of EA-rosette formation, %
(CBA× ×C57BL/6) F_1†	1:16	97,7±0,3
	1:32	95,7±5,1
	1:64	97,1±0,9
	1:128	89,0±14,3
	1:256	85,5±9,0
	1:512	46,5±1,0
BAL B/c	1:16	100
	1:32	96,3
	1:64	100
	1:128	87,6
	1:256	53,2
	1:512	0

*At each point spleen cells were taken from five mice.
†Combined results of four experiments.

The indirect immunofluorescence test was carried out by the method described in [10], using donkey serum against rabbit globulins, labeled with fluorescein isothiocyanate (from the N. F. Gamaleya Institute of Epidemiology and Microbiology) as the second reagent. This antiserum was usually absorbed by thymocytes and splenocytes of mice or rats of the test strain.

EXPERIMENTAL RESULTS

Antiserum against mouse immunoglobulins effectively inhibited EA-rosette formation in the spleen cell population of the (CBA × C57BL/6)F_1 mice (Table 1) whereas normal rabbit serum had only slight inhibitory action (18% in a dilution of 1:16). This result is in agreement with those obtained by other workers [13] for inhibition of EA-rosette formation by anti-immunoglobulin serum: antisera reacting with the surface of a B-lymphocyte such as for example, anti-Ia, anti-Ly 4.2, anti-MBLA, and anti-immunoglobulin sera, were shown to induce indirect blockade of the Fc receptor located on these cells. On the basis of these observations it might be supposed that the anti-immunoglobulin serum ought to block EA-rosette formation only partially in a population of bone marrow cells, for according to the results of the immunofluorescence test, 9% of bone marrow cells carry surface immunoglobulin, whereas 45% of karyocytes carry the Fc receptor. However, in this case also almost complete blockade of EA-rosette formation was obtained (85% in a dilution of 1:16).

Anti-T-immunoglobulin also induced strict blockade of EA-rosette formation over a wide range of concentrations in splenocyte populations from (CBA × C57BL/6)F_1 and BALB/c mice (Table 2). The total inhibition cannot be explained by inadequate absorption of the anti-T-

TABLE 3. Nonspecific Inhibition of EA-Rosette Formation in Rat Spleen by Anti-Baθ-γ-globulin in Rat Spleen

Anti-Baθ-γ-globulin 1:32	Thymocytes		Rat splenocytes	Inhibition of EA-rosette formation, %
	of (CBA × C57BL/6)F_1 mice	of August A rats		
+	2×10^6	—	2×10^5	98
+	2×10^5	—	2×10^6	67,6
+	2×10^4	—	2×10^6	24,9
+	2×10^3	—	2×10^6	24,9
—	2×10^6	—	2×10^6	0
+	—	2×10^6	2×10^6	93,2
+	—	2×10^5	2×10^6	0
+	—	2×10^4	2×10^6	0
+	—	2×10^3	2×10^6	6,7
—	—	2×10^6	2×10^6	0

immunoglobulin, for in the indirect immunofluorescence test this reagent labeled 29.9% of (CBA × C57BL/6)F_1 mouse spleen cells. Since the cells with Fc receptors in the spleen are predominantly B lymphocytes [1, 2, 9, 11], complete inhibition of EA-rosette formation by anti-T-immunoglobulin cannot be attributed to selective blockade of the Fc-receptor on cells carrying antigen recognizable by the antibodies, and it must be more universal in character.

In the next series of experiments the possibility of blockade of the Fc receptor on some cells by immune complexes formed through interaction between antibodies and antigens on the plasma membrane of other cells, not expressing the Fc receptor, was analyzed. Rabbit immunoglobulin against mouse T cells cross-reacted with rat spleen and thymus cells. In the indirect immunofluorescence test in titers of 1:8 and 1:16 it labeled 14.6 and 6% of splenocytes and 100% of thymocytes, respectively, of August A rats. Of the total number of rat spleen cells 46% carry the Fc receptor. In the rat splenocyte population anti-T-cell immunoglobulin inhibited EA-rosette formation by only 27.2%. The addition of autologous thymocytes or of mouse thymocytes to the test system led to potentiation of the suppressor action of anti-T-immunoglobulin on EA-rosette formation in the splenocyte population. Complete blockage was observed when equal numbers of splenocytes and thymocytes were mixed (Table 3). An increase in the relative content of cells expressing the corresponding antigen in the test system thus led to potentiation of the inhibitory action of the anti-T-immunoglobulin. EA-rosette formation may be suppressed by Fc receptor blockade on some cells by antigen–antibody complexes formed during interaction between antibodies and antigens of the outer cell membrane of other cells, not necessarily carrying Fc receptors.

Consequently, the method of EA-rosette formation blockade by antisera cannot be used to determine the percentage of cells in a population carrying a test antigen and Fc receptor simultaneously.

LITERATURE CITED

1. V. V. Malaitsev and I. M. Bogdanova, Byull. Eksp. Biol. Med., No. 10, 451 (1977).
2. V. V. Malaitsev and I. M. Bogdanova, Byull. Eksp. Biol. Med., No. 5, 446 (1979).
3. E. L. Alexander and P. A. Henkart, Clin. Exp. Immunol., 33, 332 (1978).
4. A. Basten et al., J. Exp. Med., 141, 547 (1975).
5. H. B. Dickler, Adv. Immunol., 24, 167 (1974).
6. E. Golub, Cell. Immunol., 2, 353 (1971).
7. R. M. Gorczynski, Immunology, 32, 709 (1977).
8. E. Kedar et al., J. Immunol., 112, 26 (1974).
9. M. Miyama et al., Cell. Immunol., 35, 253 (1978).
10. G. Möller, J. Exp. Med., 114, 415 (1961).
11. C. R. Parish, Transplant. Rev., 25, 98 (1975).
12. G. Sarmay et al., Immunology, 36, 339 (1979).
13. V. Schirrmacher et al., J. Exp. Med., 141, 1201 (1975).