INACTIVATION OF ROSETTE-FORMING CELLS OF THE SPLEEN OF IMMUNIZED MICE BY ANTIBODIES AGAINST AGGREGATED MOUSE IMMUNOGLOBULINS

A. Ya. Kul'berg, V. V. Khorobrykh, I. A. Tarkhanova, M. N. Svirezheva, T. A. Golovanova, and D. R. Kaulen

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The nature of the antigen-binding receptors of the spleen cells of immunized mice was investigated by means of a rabbit antiserum specifically identifying aggregated mouse immunoglobulins. This antiserum was found to inactivate about 70% of rosette-forming cells (RFCs) in vitro and its inhibitory activity was greater than that of monoreceptor anti-μ serum. All RFCs inactivated by anti-μ were also sensitive to treatment with antiserum against aggregated immunoglobulins. RFCs sensitive to the action of antiserum against aggregated immunoglobulins belong to the class of lymphocytes not containing θ-antigen. On the basis of these findings and the results of immunochemical analysis of antibodies against aggregated immunoglobulins it can be concluded that these antibodies block the antigen-binding receptors of immune lymphocytes of bone-marrow origin (B cells); these receptors can be regarded as immune complexes fixed on the surface of the cells.

It was shown recently that lymphocytes of bone-marrow origin (B cells) can bind immune complexes [1, 3, 5] or nonspecifically aggregated γG globulin [4] in vitro. It is therefore logical to suggest that the B cells in the immunized individual carry antigen–antibody complexes on their surface and that the antibodies fixed in this way constitute a large proportion of the total pool of antigen-binding immunoglobulin receptors belonging to the lymphocytes of this type [9].

As a result of aggregation with antigen, antibodies are known to acquire new antigenic specificity; the determinant groups that arise are similar in structure with antigenic determinants characteristic of heat-aggregated immunoglobulin [7, 8]. If the B cells in the immune animal do in fact carry antibodies on their surface in the form of a complex with antigen, their antigen-binding ability can be suppressed with the aid of antibodies against aggregated immunoglobulin.

To test this hypothesis the effect of antiserum against aggregated mouse immunoglobulins on the rosette-forming cells (RFCs) of the spleen was studied in mice immunized with sheep's red cells.

EXPERIMENTAL METHOD

The immunoglobulin fraction of mouse serum (precipitated by ammonium sulfate at 50% saturation) was heated for 20 min at 63°C; the aggregated proteins were separated by centrifuging at 105,000 g for 2 h, emulsified in Freund's complete adjuvant, and injected into the popliteal lymph glands of rabbits. Reimmunization was carried out 3 months later. The antiserum was absorbed with a twofold excess of aggregate-free mouse immunoglobulins (the upper third of the supernatant obtained by centrifuging the heated immuno-

N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR O. V. Baroyan.)
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TABLE 1. Inactivation of Rosette-Forming Spleen Cells of Mice Immunized with Sheep's Red Cells by Means of Antiserum against Homologous Aggregated Immunoglobulins (M ± m and 99% confidence limits)

Expt. No.	Serum	No. of RFCs per 10^3 lymphocytes	Inactivation of RFCs (in %)
1	— —	24,1±0,5 (23,1÷25,1)	—
	AAS —	8,0±0,7 (6,0÷10,0)	66,8
	Anti-μ —	15,1±0,5 (13,5÷16,7)	37,3
	Anti-γ —	1,1±0,4 (−0,2÷2,5)	95,4
	Anti-μ → AAS	6,2±0,7 (3,9÷10,1)	74,3
	Anti-μ → anti-γ	0 —	100
2	Complement	16,5±0,4 (15,3÷17,7)	—
	Complement → anti-μ	12,6±0,5 (12,1÷14,1)	23,6
	Complement → AAS	8,3±0,2 (7,6÷9,0)	50,3
	Anti-θ + complement	11,0±0,6 (9,1÷12,9)	33,3
	Anti-θ + complement → anti-μ	4,2±0,4 (3,0÷5,4)	74,5
	Anti-θ + complement → AAS	1,3±0,4 (0,0÷2,6)	92,1

globulins) and sheep's red cells. The absorbed antiserum (AAS) did not react in the double diffusion test in agar with aggregate-free immunoglobulins or with purified mouse IgM, IgG (γG_1 and γG_2) or IgA, but it gave a very distinct precipitation line with heat-aggregated mouse immunoglobulins.

Mice of line CBA were immunized with sheep's red cells in a dose of $5 \cdot 10^8$ cells and the number of RFCs in the spleen was determined on the fifth day by the method of Biozzi et al. [2]. To evaluate the action of AAS and of polyvalent rabbit serum against mouse immunoglobulins or of specific rabbit antiserum against the μ chains of mouse IgM on the RFCs, $20 \cdot 10^6$ spleen cells (washed 3 times with medium No. 199) were suspended in 2 ml of the same medium and incubated with the corresponding antiserum for 40 min at 4°C. The excess of serum was removed and the cells were used without washing to determine the number of RFCs. In the control tests the cells were incubated with normal rabbit serum taken in the same dilutions as the antiserum. To eliminate cells containing θ-antigen, $5 \cdot 10^6$ spleen cells in a volume of 0.5 ml were incubated for 45 min at 37°C with AKR anti-θ C3H serum (final dilution 1:600) and with rabbit complement (final dilution 1:5). In the dose used and in the presence of complement the anti-θ serum caused the death of 99% of the thymocytes but did not affect the viability of the bone-marrow cells or of the antibody-producing cells detectable by Jerne's method [6] in the spleen of the immunized mice.

EXPERIMENTAL RESULTS

Incubation of the spleen cells with AAS was accompanied by a decrease in the number of RFCs detected by 66.8% (Table 1). In its ability to inactivate RFCs this antiserum was much more effective than anti-μ serum but less effective than the polyvalent antiglobulin serum, which inactivated nearly all the RFCs in these experiments. All cells sensitive to treatment with anti-μ serum were inactivated by AAS. If the cells were first incubated with anti-μ serum and later with AAS the number of inactivated RFCs remained the same as when the cells were treated with AAS only. Neither AAS nor the other antisera, in the dilutions used, had any effect on the viability of the spleen cells.

RFCs sensitive to the action of AAS belong to the class of lymphocytes not containing θ-antigen. The θ-positive RFCs were eliminated by incubating the spleen cells with anti-θ serum and complement. As a result of this treatment the number of RFCs detected fell by 33% below the control level (cells treated with complement and normal mouse serum; Table 1, experiment No. 2). Nearly all the θ-negative RFCs could be inactivated by AAS. Most of the RFCs in this population also were sensitive to the action of anti-μ serum.

The RFCs inactivated by AAS thus did not contain θ-antigen but possessed immunoglobulin receptors of the μ type. Both these properties are characteristic of lymphocytes of bone-marrow origin – B cells [9, 10]. Since AAS is specific for aggregated mouse immunoglobulins it can be concluded that the rosette-forming cells of bone-marrow origin appearing in the mouse spleen contained aggregated immunoglobulins on their surface. It will be noted that the RFCs detected in the spleen of normal mice were not inactivated by AAS or by anti-μ serum. This may mean that they were either thymus-dependent lymphocytes or B-lymphocytes not containing aggregated immunoglobulins on their surface.

The nature of the aggregated immunoglobulins found on the RFCs and the mechanism of their fixation on the cell can be judged on the basis of the results of serologic analysis of the AAS. Tests showed that antibodies against heat-aggregated immunoglobulins can be removed from the AAS by adsorption with agglutinates of sheep's red cells and mouse IgM hemagglutinins. The source of the latter was a mouse antiserum obtained on the third day after immunization with $5 \cdot 10^8$ red cells containing no detectable quantities of hemagglutinins resistant to the action of mercaptoethanol. When red cell agglutinates with IgG hemagglutinins from hyperimmune mice were used as the immunosolvent, the effectiveness of adsorption of the antibodies against aggregated immunoglobulins was significantly less than in the case of agglutinates with IgM antibodies. These differences were confirmed by the use of AAS in the Coombs' test. Although AAS, unlike anti-μ serum, did not lead to an increase in the titer of IgM hemagglutinins, it definitely increased the degree of hemagglutination induced by IgM antibodies. This effect was not observed when IgG hemagglutinins were used. The data described above can thus be regarded as proof that the AAS was specific for aggregated IgM antibodies, although the possibility cannot be ruled out that it gives a cross reaction also with IgG antibodies in the immune complex.

As the results of the experiments in vitro show, B-lymphocytes bound the immune complexes either through receptors for the Fc fragment of IgG [3] or through receptors for the third component of complement [5]. Whereas in the first case IgG antibodies could be fixed in the immune complex but not IgM [3], by means of the third component of complement in principle antibodies of all classes having the property of binding this component of complement in the presence of antigen could be fixed on the B-lymphocyte. Considering these findings and also the fact that the AAS interacted effectively with IgM antibodies in the immune complex it can be postulated that fixation of immune complexes in vivo on RFCs of bone-marrow origin takes place with the participation of complement. It is impossible at present to give an unequivocal answer to the question whether the immune complexes fixed on the RFCs are antigen-binding receptors, but this suggestion seems very likely in the light of the results showing the ability of AAS to inactivate the RFCs. In that case the accumulation of rosette-forming cells in the course of immunization can be directly correlated with the circulation of antigen–antibody complexes in the immune organism.

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