SELECTIVE COMPETITIVE INHIBITION OF ACCUMULATION OF ROSETTE-FORMING CELLS DURING THE IMMUNE RESPONSE

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Repeated injection of rabbit immunoglobulins into mice before immunization with sheep's red cells inhibits the process of accumulation of rosette-forming cells but does not affect proliferation of antibody-forming cells or hemagglutinin production. Under these conditions the number of rosette-forming cells is reduced on account of the B cells, whose antigen-binding receptors may be blocked by means of antibodies against aggregated mouse immunoglobulins and polyA:polyU complex. Repeated injection of the competing antigen was shown to stimulate the formation of immunological memory for the second antigen. The question of the origin of the immune rosette-forming B cells and their effect on the formation of immunological memory is discussed.

KEY WORDS: rosette-forming cells; aggregated immunoglobulin; immunological memory.

Competition between unrelated antigens is a question of great theoretical and practical interest. This phenomenon can be expressed as inhibition of proliferation of antibody-forming cells and inhibition of antibody biosynthesis as a result of the preceding injection of an unrelated antigen [9]. However this leaves unanswered the question of the effect of competition between antigens on the accumulation of lymphocytes carrying antigen-binding receptors (rosette-forming cells — RFCs). It is also important to establish whether during competition between antigens the inhibition of RFC accumulation in response to the second antigen can take place without any significant effect on antibody production. The solution of these problems would contribute to the understanding of the regulatory role of RFCs in the primary and secondary immune response. An investigation was accordingly carried out to discover whether antibody production and RFC accumulation during immunization of mice with sheep's red cells can be dissociated by a previous injection of foreign protein. The process of formation of immunological memory for the second antigen also was studied under these conditions.

EXPERIMENTAL METHOD

Sheep's red cells and immunoglobulins obtained from pooled sera from at least five normal rabbits by precipitation with ammonium sulfate at 40% saturation were used as the antigens. To remove large aggregates from the protein preparations they were ultracentrifuged at 105,000g for 2 h at 20°C. When these protein preparations were used in a dose of 1 mg/ml, no normal hemagglutinins against sheep's red cells could be discovered in them.

Mice of strain CBA were first immunized intravenously with rabbit immunoglobulins. Protein in a dose of 4 mg was injected in four fractions at daily intervals. Three days after the last injection of protein the mice were immunized intravenously with $5 \cdot 10^8$ sheep's red cells. In other experiments, 3 days after completion of the cycle of injection of the protein antigen the mice were immunized with 10^6 sheep's red cells and then reimmunized 40 days later with $5 \cdot 10^8$ sheep's red cells.

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TABLE 1. Selective Competitive Inhibition of RFC Accumulation during Primary Immune Response (M ± m)

Group of animals	Index	Time of investigation of animals after injection of second antigen (days)			
		1	3	5	7
Experimental	Number of RFCs in spleen ($\times 10^3$)	104,5± 5,9 (5)	392,8± 64,5 (8)	704,0± 104,8 (16)	661,0± 153,9 (4)
	Number of AFCs in spleen ($\times 10^3$)	1,7± 0,3 (8)	19,2± 1,7 (8)	14,0± ,29 (16)	2,8± 0,6 (4)
	Titers of 19S antibodies in blood serum	1/6—1/12 (8)	1/376± 1/129 (10)	1/1680± 1/94 (16)	1/1440± 1/539 (5)
	Titers of 7S antibodies in blood serum	0 (8)	0—1/40 (10)	1/555± 1/49 (16)	1/560± 1/90 (5)
Control	Number of RFCs in spleen ($\times 10^3$)	135,2± 14,8 (5)	509,1± 60,3 (7)	2456,8± 148,2 (20)	1231,6± 156,4 (5)
	Number of AFCs in spleen ($\times 10^3$)	1,3± 0,2 (8)	8,0± 1,3 (7)	14,4± 2,7 (20)	2,7± 0,3 (5)
	Titers of 19S antibodies in blood serum	0	1/80± 1/13 (10)	1/2112± 1/79 (20)	1/2304± 1/275 (5)
	Titers of 7S antibodies in blood serum	0 (8)	0 (10)	1/600± 1/40 (20)	1/604± 1/10 (5)

Legend. Number of animals tested shown in parentheses.

TABLE 2. Changes in Number of RFCs Inactivated by AAS and polyA:poly U Five Days after Immunization with Sheep's Red Cells in Animals Previously Receiving Four Injections of Rabbit Immunoglobulins

Group of animals	Reagent	Number of RFCs	
		per 10^3 nucleated spleen cells	per spleen (× 1000)
Experimental	—	9,2± 0,3 (8,2÷ 10,2)	1259,8± 63,3 (1059,1÷ 1460,5)
	AAS	8,6± 0,3 (7,6÷ 9,6)	1182± 66,1 (972,7÷ 1391,3)
	PolyA : polyU	7,8± 0,4 (6,0÷ 9,8)	1069,6± 55,2 (894,6÷ 1244,6)
Control	—	25,0± 0,4 (23,6÷ 26,4)	3432,2± 150,2 (2956,0÷ 3908,4)
	AAS	11,2± 0,4 (9,8÷ 12,6)	1545,0± 102,4 (1220,4÷ 1868,6)
	PolyA : polyU	13,6± 0,3 (12,6÷ 14,6)	1865,8± 73,6 (1632,5÷ 2099,1)

Legend. Ten mice tested in each group of experiments. Confidence limits of arithmetic mean, with a 99% level of significance, given in parentheses.

The immune response against sheep's red cells was assessed from the number of antibody-forming cells (AFCs), determined by the direct and indirect methods of Jerne and Nordin [7], the number of RFCs, determined by a modified method of Biozzi et al. [4], and the titer of 19S and 7S hemagglutinins. To study the characteristics of the immunoglobulin receptors of RFCs the spleens of the immunized mice were treated with antiserum against mouse immunoglobulins (AAS) and with a polyA:polyU complex. To eliminate lymphocytes carrying θ antigen, the spleen cells were incubated with anti-θ serum (C3H anti-AKR) in the presence of rabbit complement. The method of obtaining the AAS and the method of treatment of the cells with AAS, polyA:polyU, and anti-θ serum were described previously [2, 3].

EXPERIMENTAL RESULTS

Repeated injection of rabbit immunoglobulins into mice, before immunization with sheep's red cells, led to inhibition of the accumulation of RFCs in the spleen but did not affect hemagglutinin production or proliferation of AFCs (Table 1). The greatest difference between

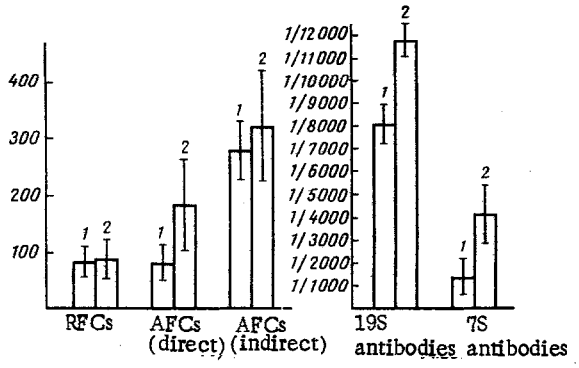

Fig. 1. Number of RFCs and AFCs and titers of 19S and 7S antibodies in animals previously immunized repeatedly with rabbit's immunoglobulins, during secondary immune response to sheep's red cells. Ordinate, left: number of RFCs and AFCs, right: antibody titers; abscissa, index studied. 1) Control; 2) experiment.

the number of RFCs in the control and experimental groups was observed on the fifth day after injection of sheep's red cells.

If the first antigen was injected all at once, but in the same dose as when given in divided doses, its effect on the immune response to sheep's red cells depended on the interval between injections of the first and second antigens. When this interval was 11 days, the immune response as reflected in all the indices studied was indistinguishable in the animals of the experimental group from that in the animals receiving sheep's red cells only. When the interval between the single injection of immunoglobulins and of sheep's red cells was 3 days, in the animals of the experimental group the number of AFCs was twice as high in animals of the experimental group by the fifth day of immunization with the second antigen and the hemagglutinin titers were higher, whereas the number of RFCs in these animals was significantly lower.

It can be concluded from the results of these experiments that only after repeated injection of the first antigen was it possible for the processes of antibody production and RFC accumulation in response to the second antigen to be completely dissociated. This scheme of immunization was therefore used to investigate the effect of this competing antigen on the formation of immunological memory for the second antigen.

The secondary response to sheep's red cells in the animals of the experimental group was considerably higher (Fig. 1) than in the controls. Since during the first immunization with sheep's red cells only 10^6 cells were injected, it was impossible directly to assess the effect of the competing antigen on RFC accumulation under the conditions of the primary immune response. However, it can be assumed that with this experimental scheme the competing antigen caused a disturbance of RFC accumulation in response to sheep's red cells, just as in the experiments described above. In that case it can be postulated that in the course of the primary immune response feedback exists between the accumulation of cells of immunological memory and of RFCs.

The RFCs detected in the spleen of immune animals are known to belong to two populations of lymphocytes: thymus-dependent and of bone-marrow origin [1, 8]. The characteristic feature of rosette-forming B cells discovered in the spleen of mice immunized with sheep's red cells is that they can be inactivated by antibodies against aggregated mouse immunoglobulin and polyA:polyU [1]. By the use of this test and also of the method of detecting T cells by means of anti-θ serum [8], an attempt was made to discover which population of RFCs it was whose accumulation was prevented by repeated injection of the competing antigen.

The population of RFCs from the spleen of mice on the fifth day after immunization with $5 \cdot 10^8$ sheep's red cells was investigated. As Table 2 shows, RFCs in the spleen of mice previously receiving four injections of rabbit's immunoglobulin were not inactivated by antiserum against aggregated mouse immunoglobulin or polyA:polyU. These cells contained θ antigen on their surface, for they could be eliminated by treatment with anti-θ serum in the presence of

complement. About 60% of cells in the spleen of the control group of mice, which received sheep's red cells only, were inactivated by AAS and polyA:polyU; these cells did not contain θ antibodies on their surface. Hence it follows that the total number of RFCs was reduced under the influence of repeated injection of the first antigen on account of rosette-forming B cells.

The possibility of inactivation of rosette-forming B cells by antibodies against aggregated mouse immunoglobulin is evidently attributable to the fact that antigen—antibody complexes adsorbed by the cells act as antigen-binding receptors of the B cells [2]. Evidence in support of this view is given by the fact that antibodies against aggregated mouse immunoglobulin cross-react with immune complexes [5, 6]; the latter can be fixed to the Fc fragment on the receptors and to the third component of complement, found on the cytoplasmic membrane of the B cells [1, 8].

It can accordingly be postulated that the competing antigen (injected repeatedly) prevents the appearance of B cells carrying immune complexes in response to a second, unrelated antigen. In that case the phenomenon described can be explained in the light of the hypothesis that, through competition for lymphocyte receptors, immune complexes formed by antibodies against the first antigen competitively inhibit the fixation of immune complexes with antibodies against the second antigen on B lymphocytes.

As was demonstrated above, the competing antigen does not affect hemagglutinin production. Consequently, the possibility of formation and persistence of immune complexes formed by sheep's red cells under these conditions ought not to differ from that in animals immunized with sheep's red cells only.

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