changed) modification of Jerne's method is given by reproduction of Andersen's phenomenon of immune blocking [5] and the recording of translucency and lysis of erythrocytes in the peripheral zone of the plaque (Fig. 2).

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ANTIGEN-DEPENDENT INDUCTION OF A NONSPECIFIC HUMORAL FACTOR BLOCKING ROSETTE-FORMING CELLS in vitro AND in vivo

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Isologous serum of mice immunized with rabbit globulin (IARS) was shown to contain a factor inactivating rosette-forming cells (RFC) in vitro from CBA mice immunized with sheep's erythrocytes. If the mice were immunized with sheep's erythrocytes after preliminary injection of IARS, the number of RFC at the peak of the immune response was about 30% of their number in mice receiving normal isologous serum together with sheep's erythrocytes. The decrease in the number of RFC took place on account of cells not containing θ antigen. Passive immunization with IARS did not affect proliferation of antibody-forming cells or synthesis of antibodies against sheep's erythrocytes.

KEY WORDS: *rosette-forming cells; antibody-forming cells; θ antigen; isologous antirabbit serum.*

The possibility of uncoupling the processes of antibody production and accumulation of rosette-forming cells (RFC) during immunization of mice with sheep's erythrocytes (SE) was demonstrated previously: This result was obtained by preliminary injection of foreign protein into the same animals [2].

It was decided to investigated the mechanism of the above phenomenon since it provides an approach to the explanation of processes regulating the kinetics of RFC in the course of the immune response.

EXPERIMENTAL METHOD

SE and rabbit immunoglobulins, obtained by precipitation with ammonium sulfate at 40% saturation, were used as the antigens. The immunoglobulin preparations were purified from large aggregates by ultracentrifugation of 105,000g for 2 h at 20°C. The immunoglobulin preparations did not contain hemagglutinins against SE.

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TABLE 1. Inactivation of RFC in Spleen of Mice Immunized with Sheeps' Erythrocytes by Isologous Antirabbit Serum in vitro

Serum for treatment of lymphocytes	Number of RFC per 10^3 splenic lymphocytes (M ± m)		
	dilution of serum		
	1:40	1:20	1:10
Normal mouse	15,0±0,1 (14,6—15,4)	15,3±0,2 (14,3—16,3)	14,0±0,3 (12,8—15,2)
Isologous antirabbit	10,8±0,1 (9,8—11,8)	9,0±0,4 (7,9—10,1)	9,0±0,2 (8,3—9,7)
No treatment (control)		17,5±0,2 (16,9—18,1)	

Legend. Mean number of RFC in parallel tests from pool of spleen cells from 10 mice shown in the table. Confidence interval calculated for arithmetic mean values with a level of significance of 99%.

TABLE 2. Effect of Isologous Antirabbit Serum on Immune Response of CBA Mice to Sheep's Erythrocytes (M ± m)

Serum for passive immunization	Number of RFC per 10^3 lymphocytes	Number of AFC per spleen	Titer of hemagglutinins	
			total	7S
Normal mouse	15,0±0,4 (13,7—18,8)	45242,0±723,2 (47534,6—52119,8)	1/683±1/103 (1/245—1/1121)	1/3,3±1/0,5 (1/1,6—1/5,0)
Isologous antirabbit	5,4±0,2 (4,7—6,2)	51830,0±3456,5 (39733,4—63944,1)	1/320±1/55 (1/101—1/539)	1/5,2±1/1,3 (1/1,8—1/8,6)
Control (injection of physiological saline)	17,3±0,3 (16,4—18,8)	40531,0±245,9 (39793,4—41268,6)	1/224±1/13 (1/115—1/333)	1/2,5±1/0,4 (1/0,8—1/4,2)

Legend. Each group consisted of 10 CBA mice. Confidence interval calculated for arithmetic mean values with level of significance of 99%.

Mice of strain CBA were immunized by the scheme suggested previously [2]. Initially four intravenous injections of rabbit immunoglobulins were given at intervals of 24 h. Three days later $5 \cdot 10^8$ SE were injected intravenously. The number of antibody-forming cells (AFC) was determined by Jerne's direct method [5]. RFC were determined by a modified Biozzi's method [4]. The number of RFC carrying θ antigen was determined with the aid of anti-θ serum and complement, as described previously [1]. Hemagglutinins were titrated with a microtitrator of the Takatsi system. Antibodies against rabbit globulin were determined by the passive hemagglutination method. SE were sensitized with rabbit globulins with the aid of glutaraldehyde (Sigma) by Avrameas' method [3].

EXPERIMENTAL RESULTS

Injection of rabbit immunoglobulins into the mice before the injection of SE did not affect proliferation of AFC or the production of antibodies against SE. Under these conditions, however, a decrease of 70% was found in the number of RFC formed in response to immunization with SE. These results were in full agreement with those obtained by the writers previously [2].

We postulated that as a result of immunization of the mice with the first antigen a factor capable of inactivating RFC arising in response to injection of the second antigen appeared in their serum. This hypothesis was tested by experiments both in vitro and in vivo, using isologous serum of CBA mice immunized with rabbit globulins. As in the other experiments, protein in a dose of 1 mg/ml was injected four times at intervals of 24 h, and serum was obtained 7 days after the last injection of the protein antigen. The serum was inactivated at 56°C, exhausted by absorption with SE, and sterilized by passage through Millipore filters with a pore diameter of 0.22 μ. Antibodies against rabbit globulins were found in low titer (1:8) in this serum by the passive hemagglutination test. On that basis the serum was described as isologous antirabbit serum (IARS). In the experiments in vitro, spleen cells of CBA mice, obtained on the fifth day after immunization with $5 \cdot 10^8$ SE, were treated with IARS. Spleen cells (10^7) were incubated with different dilutions of serum in 1 ml medium No. 199 for 45 min at 4°C. The cells were then sedimented by centrifugation and the super-

natant was removed, after which the cells were used for the rosette-formation test without further washing. As Table 1 shows, incubation of spleen cells of an immune mouse with IARS leads to a decrease in the number of detectable RFC; the inhibitory activity of the serum, moreover, rises with an increase in its concentration. Since the IARS was nontoxic for spleen cells and did not contain antibodies against SE, its action could be ascribed to blocking of the antigen-binding receptors of the splenocytes.

In other experiments it was shown that injection of IARS prevents the accumulation of RFC in mice immunized with SE, although it does not affect proliferation of AFC and hemagglutinin production. IARS obtained by the method described above was injected intraperitoneally over a period of 5 days in a total volume of 0.5 ml. Simultaneously with the first injection of serum, $5 \cdot 10^7$ SE were injected intravenously and the mice were killed four days later. Instead of IARS, animals of the control group received isologous normal serum treated in the same way as the IARS. The results in Table 2 show that passive immunization of mice with IARS leads to a decrease of about 70% in the number of detectable RFC formed in response to immunization with SE. Injection of normal serum had no effect on the accumulation of RFC. As a result of injection of IARS the relative number of RFC carrying θ antigen was changed. Whereas mice of the control groups receiving SE had about 28% of θ-positive RFC, in mice receiving injections of IARS as well as SE, all the detectable RFC carried θ antigen and could be eliminated by treatment with anti-θ serum and complement. On this basis it can be concluded that IARS prevents the accumulation of θ-negative RFC or of rosette-forming B cells.

The writers demonstrated earlier that a decrease in the number of cells forming rosettes with SE as a result of the preceding injection of a protein antigen takes place on account of rosette-forming B cells [2]. It can be concluded from a comparison of these results with those described above that the effect of injection of isologous serum into mice receiving protein antigen is equivalent to the effective result of immunization with this antigen. This provides additional grounds for the conclusion that RFC accumulation is prevented by a factor contained in the serum of animals repeatedly immunized with an unrelated antigen. It also follows from the results of this investigation that this factor can block antigen-binding receptors of RFC.

Work is now in progress to study the nature of the serum factor blocking the receptors of RFC and preventing their accumulation in vivo in the course of immunization. The fact that this factor can be extracted from IARS by preformed immune complexes formed by mouse antibodies suggests that this factor may be an antibody against aggregated mouse immunoglobulins.

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