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INHIBITION OF FORMATION OF CELLS SECRETING ANTIBODIES AND ANTIGEN-DEPENDENT NONSPECIFIC IMMUNOGLOBULINS IN MICE TREATED WITH ISOLOGOUS ANTIERYTHROCYTIC IMMUNOGLOBULINS

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It was shown previously that treatment of animals with syngeneic immunoglobulins (Ig), conjugated with cellulose (cel) and containing antibodies against sheep's red blood cells (SRBC), leads to inhibition of specific reactivity: On immunization of such animals with SRBC the number of antibody-forming cells (AFC) in their spleens was found to be an order of magnitude less than in animals receiving the antigen alone [2]. Anti-idiotypic antibodies inhibiting antibody production against SRBC were found in the serum of animals areactive to SRBC [3].

Experiments to study induction of tolerance with the aid of antigen showed that not only are AFC not formed in tolerant animals in response to injection of homologous antigen, but there is no increase likewise in the number of cells producing antigen-dependent nonspecific Ig (NIGFC) [4, 5].

The aim of this investigation was to study how reactivity to SRBC, induced by injection of idiotype-positive syngeneic Ig into mice (Ig-anti-SRBC) affects the formation of NIGFC.

EXPERIMENTAL METHOD

(CBA × C57BL/6)F_1 mice were obtained from the Stolbovaya Nursery, Academy of Medical Sciences of the USSR. Normal serum Ig (norm Ig) and Ig-anti-SRBC were conjugated with oxidized cellulose (norm Ig-cel and Ig-anti-SRBC-cel respectively) [2]. The authors are grateful to Professor A. E. Gurvich for providing the oxidized cellulose.

Areactivity to SRBC was induced in the mice by subcutaneous injection of Ig-anti-SRBC-cel at two points, followed 1 month later by injection of Ig-anti-SRBC in a dose of 0.2 mg protein per mouse. Normal mice, and also mice receiving norm Ig-cel, followed by norm Ig at two points, subcutaneously at the same time (0.2 mg per mouse in each case), served as the control. The animals were given an injection of 5×10^8 SRBC, 2×10^8 hens' red blood cells (HRBC), or Eagle's medium, 7 days later. On the 4th day the total number of cells forming Ig (IgFC) [10] and the number of 19S AFC [9] was determined in the spleens of individual animals. The number of NIGFC was calculated as the difference between the number of IgFC and the number of AFC per 10^6 living cells. The results are presented as arithmetic means with standard error.

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TABLE 1. Inhibition of AFC and NIGFC Formation in Mice Immunized with SRBC after Preliminary Treatment of the Animals with Idiotype-Positive Ig

Preparation injected			No. of AFC against SRBC/10^6 cells	No. of NIGFC/10^6 cells
norm. Ig-cel, norm. Ig	Ig-anti-SRBC-cel, Ig-anti-SRBC	SRBC		
—	—	—	0,9±0,9	2190±502
—	—	+	1849±243	10260±806
+	—	+	1432±244	443±1125
—	+	+	150±28	3785±741

Legend. In each group 10-12 mice were used.

TABLE 2. Specificity of Inhibition of AFC and NIGFC

Preparation injected		No. of AFC/10^6 cells		No. of NIGFC/10^6 cells
Ig preparations	antigen	against SRBC	against HRBC	
—	—	0	0	2 842±675
	SRBC	2354±195	3±2	14 670±1 299
	HRBC	—	263±50	14 392±2 606
Norm. Ig-cel + norm. Ig	—	4±2	7±4	3 916±643
	SRBC	1781±244	1±1	12 550±559
	HRBC	—	263±57	12 989±2 597
Ig-anti-SRBC-cel + Ig-anti-SRBC	—	8±3	9±5	4 207±813
	SRBC	164±43	4±3	5 779±883
	HRBC	—	320±47	16 137±2 667

Legend. In each group seven mice were used.

EXPERIMENTAL RESULTS

The first step was to determine how injection of idiotype-positive Ig affects the number of IgFC in the spleens of animals not subsequently immunized with SRBC. For this purpose a group of mice was immunized with Ig-anti-SRBC-cel, reimmunized 1 month later with solutions of the preparation of Ig-anti-SRBC, and 7 days later they were given an injection of Eagle's medium. The number of IgFC was determined on the 4th day. Injection of idiotype-positive Ig itself was found not to cause any significant changes in the number of IgFC in the animals. In intact mice, for instance, the number of IgFC was 2842 ± 675, compared with 4207 ± 813 per 10^6 living cells in the experimental mice.

Injection of SRBC into intact mice caused a sharp increase in the number of AFC and NIGFC. The number of NIGFC was more than five times greater than the number of AFC (Table 1). Preliminary (before SRBC) injection of norm Ig-cel and norm Ig into the mice had virtually no effect on the increase in number of AFC and NIGFC during subsequent immunization of the animals with SRBC (Table 1), and inhibition of the increase in the number of AFC and NIGFC in this group did not exceed 23 and 10% respectively.

By contrast with this, preliminary treatment of the mice with Ig-anti-SRBC-cel and Ig-anti-SRBC induced both marked areactivity to SRBC and a sharp decrease in NIGFC formation in them. On immunization of these animals with SRBC the formation of only 150 ± 28 AFC against SRBC and of 3785 ± 741 NIGFC was observed compared with 1849 ± 243 AFC against SRBC and 10,260 ± 806 NIGFC in mice receiving antigen only (Table 1). Injection of idiotype-positive Ig thus caused inhibition of the antigen-dependent increase in the number of AFC and NIGFC by 92% and 80% respectively.

It was shown previously that areactivity induced by injection of Ig-anti-SRBC-cel and Ig-anti-SRBC is antigen-specific in character [2]. It was interesting to test whether inhibition of NIGFC formation under these conditions is antigen-specific also. For this pur-

pose animals treated with Ig-anti-SRBC-cel and Ig-anti-SRBC were immunized with HRBC, which do not give cross reactions with SRBC [8]. Mice with induced areactivity to SRBC, injected with SRBC or Eagle's medium, and also normal mice and animals receiving norm Ig-cel and norm Ig and immunized with SRBC, HRBC, or medium, served as the control.

It will be clear from Table 2 that immunization of intact mice with SRBC or HRBC caused the formation both of AFC of corresponding specificity and of NIGFC. Preliminary treatment of the animals with norm Ig-cel and norm Ig did not prevent the increase in the number of AFC and NIGFC on subsequent injection into these animals of SRBC or HRBC. Conversely, in mice treated with Ig-anti-SRBC-cel and Ig-anti-SRBC a good response was observed only to injection of HRBC, but not of SRBC. Injection of SRBC into areactive animals, just as in the previous series of experiments, caused inhibition of formation of AFC to SRBC and of NIGFC (by 93 and 86% respectively), but injection of HRBC induced the formation of AFC to HRBC and of corresponding NIGFC, in virtually the same numbers as in the control.

The results are evidence that injection of idiotype-positive Ig-anti-SRBC into animals induces antigen-specific areactivity not only at the level of AFC formation, but also at the level of formation of antigen-dependent NIGFC.

Differences in behavior of NIGFC during stimulation of mice areactive to SRBC by SRBC and HRBC also show that different lymphocyte subpopulations take part in NIGFC formation under the influence of these two antigens. Stimulation by each antigen of its "own" NIGFC population was demonstrated previously on other experimental models [1, 7].

We know that inhibition of the immune response to SRBC induced by preliminary injection of Ig-anti-SRBC-cel and Ig-anti-SRBC is connected with stimulation of clones of anti-idiotypic cells, and not with peripheral neutralization of antigen by antibodies injected from outside [3]. The decrease in NIGFC formation under these conditions also is connected with inhibition of cells carrying idiotypic determinants similar to those of AFC against SRBC. Cells of this kind may be both antigen-specific inducers of NIGFC and precursors of NIGFC. Data on the presence of common idiotypic determinants on antibodies and antigen-dependent nonspecific Ig [6, 11], are evidence in support of this hypothesis.

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