EFFECT OF SOMATOTROPIC HORMONE ON AUTOIMMUNE RESPONSES INDUCED IN MICE OF VARIOUS GENOTYPES BY HEATED SYNGENEIC ERYTHROCYTES

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After a single injection of $1 \cdot 10^9$ heated syngeneic erythrocytes into BALB/c and C57BL mice, antierythrocytic autoantibodies appear, the weight of the lymphoid organs is increased, and lymphoid-reticular hyperplasia is observed on the 13th day. These changes are more marked in BALB/c mice. During the development of the autoimmune response changes occurred in the number of E- and EAC-rosette-forming cells in the thymus and spleen and in the intensity of the response to immunization by sheep's red cells and *Escherichia coli* endotoxin, when distinct differences were observed between the strains. Injection of somatotropic hormone in a dose of 5 mg/kg daily for 10 days reduced the degree of development of the autoimmune response in mice of both strains, but more strongly in BALB/c mice.

KEY WORDS: *lymphoid organs; rosette- and plaque-forming cells; autoimmune reactions; somatotropic hormone.*

Research in the field of autoimmunity has developed rapidly in recent years, for the study of autoimmune processes is one way of identifying certain functional features distinguishing the immunocompetent system [1]. Much evidence has been obtained on the different causes and mechanisms of autoimmune pathology [2]. Data on T lymphocytes, acting as suppressors of immune responses, are of great importance for the understanding of the mechanisms of development of autoimmune processes [4, 7]. Spontaneous autoimmune hemolytic anemia in NZB mice has been shown to be accompanied by depression of the responses of cellular immunity, including the immunodepressive function of the thymus [3, 12]. On the basis of this fact and of the observed ability of somatotropic hormone (SH) to accelerate differentiation of T lymphocytes [6], it can be postulated that administration of SH will prevent the development of autoimmune reactions.

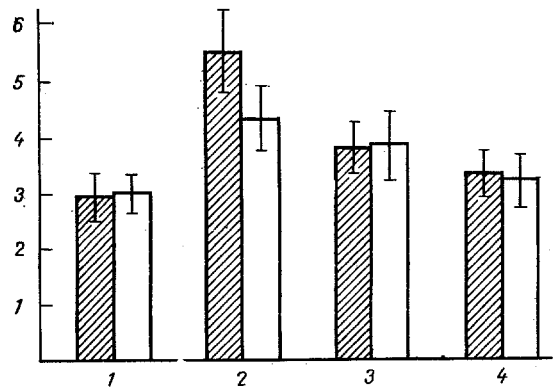

Fig. 1. Titers of antierythrocytic autoantibodies in BALB/c (shaded columns) and C57BL (unshaded columns) mice before and after injection of SH and syngeneic heated red cells. Abscissa, groups of animals: 1) intact; 2) receiving red cells; 3) receiving red cells and SH; 4) receiving SH; ordinate, $\log_2$ of autoantibody titers. Mean values of $\log_2$ and confidence limits shown.

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TABLE 1. Absolute and Relative Weights of Thymus and Spleen and Total Number of Nucleated Cells in BALB/c and C57BL Mice before and after Injection of Heated Syngeneic Red Cells and SH (M ± m)

Group of animals	Thymus					
	absolute weight, mg		relative weight, mg/g		total number of nucleated cells ($\times 10^7$)	
	BALB/c	C57BL	BALB/c	C57BL	BALB/c	C57BL
Intact	42±5,4	27±3,6	2,3±0,4	1,1±0,1	16±2,6	8±1,3
Receiving red cells	59±5,7*	39±4,0*	2,8±0,3	1,6±0,1*	16±2,4	10±1,7
Receiving red cells and SH	55±4,6	24±1,7	2,8±0,2	1,0±0,1	13±1,6	7±1,2
Receiving SH	55±5,2	29±3,6	3,2±0,3*	1,1±0,1	11±1,2*	5±0,7*

Group of animals	Spleen					
	absolute weight, mg		relative weight, mg/g		total number of nucleated cells ($\times 10^7$)	
	BALB/c	C57BL	BALB/c	C57BL	BALB/c	C57BL
Intact	122±5,1	66±5,4	6,5±0,4	2,6±0,3	19±1,3	9±0,8
Receiving red cells	143±6,4*	98±5,8*	7,8±0,3*	4,0±0,1*	22±1,5	13±2,4
Receiving red cells and SH	120±9,3	123±12,3*	6,5±0,4	4,8±0,6*	19±1,8	14±2,0*
Receiving SH	120±7,1	83±6,3	6,5±0,4	3,1±0,3	16±1,1	11±1,1

Legend to Tables 1 and 2: 1. P calculated by comparison with data for intact animals. 2. Asterisk — $P < 0.05$. 3. Results of two experiments combined in Tables 1 and 2.

EXPERIMENTAL METHOD

Female BALB/c and C57BL mice aged 3 months (obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR) were divided into four groups: 1) intact; 2) animals receiving a single intraperitoneal injection of $1 \cdot 10^9$ syngeneic erythrocytes previously heated to 49.5°C for 30 min; 3) animals receiving erythrocytes (as in group 2) and daily subcutaneous injection of SH (Biochem. Nutr. Inc., Cleveland, Ohio, USA), obtained from Dr. Sorkin (Switzerland), in a dose of 5 mg/kg daily for 10 days (starting from the day of injection of the erythrocytes); 4) mice receiving SH as in group 3. The control for the formation of antierythrocytic autoantibodies consisted of the direct Coombs' test [11]. On the 13th day after injection of the erythrocytes the mice were killed, their lymphoid organs were weighed, and the total number of nucleated cells in them, the number of cells forming spontaneous rosettes with sheep's erythrocytes (E-rosette-forming cells, E-RFC) [14] in the thymus and spleen, the number of cells forming rosettes with sheep's erythrocytes previously treated with complement and hemolytic serum (EAC-rosette-forming cells, EAC-RFC) [5] in the spleen, and the number of T lymphocytes in the spleen (by the microcytotoxic test with anti-T serum [10]) were counted. Squash preparations of lymphoid organs were strained by the method of Stockinger and Kellner [13]. On the 13th day after the injection of red cells some of the animals were immunized by intraperitoneal injection of $2 \cdot 10^8$ sheep's red cells or 15 μg of *Escherichia coli* endotoxin, and the number of plaque-forming cells (PFC) in the spleen was counted on the 5th day after immunization by the method of local hemolysis in a liquid monolayer [8]. The results were analyzed by Student's method.

EXPERIMENTAL RESULTS

As Fig. 1 shows, short-term antigenic loading led to the appearance of antierythrocytic autoantibodies with a peak on the 13th day in 40-50% of BALB/c and C57BL mice. In the BALB/c mice with a positive Coombs' test the titers of autoantibodies were higher than in the C57BL mice. If SH was injected together with the red cells it not only reduced the number of mice with a positive Coombs' test but also lowered their antierythrocytic autoantibody titers. The action of SH was stronger in BALB/c mice.

In the mice of both strains in which autoantibodies were formed the absolute and relative weights of the thymus and spleen were increased although the number of nucleated cells in them was unchanged (Table 1). The increase in weight of the lymphoid organs took place on account of lymphoid-reticular hyperplasia: The number of large macronucleolar basophilic lymphocytes (blast cells) and reticular cells was increased by three to four times. Injec-

TABLE 2. Number of RFC in Spleen and Thymus and of PFC in Spleen of BALB/c and C57BL Mice before and after Injection of SH and of Heated Syngeneic Red Cells (M ± m)

Group of animals	Number of E rosettes per 10^3 thymus cells		Number of E rosettes per 10^3 spleen cells		Number of EAC rosettes per 10^3 spleen cells		Number of PFC per 10^6 spleen cells					
							immunization with sheep's red cells				immunization with E. coli endotoxin	
	BALB/c	C57BL	BALB/c	C57BL	BALB/c	C57BL	BALB/c	C57BL	BALB/c	C57BL	BALB/c	C57BL
Intact	0,72±0,14	0,12±0,1	0,93±0,2	1,3±0,3	45,5±4,2	62±10,0	105±10,3	53±9,7	2,8±0,4	33±6,4
Receiving red cells	0,22±0,09*	0,5±0,2*	2,14±0,2	1,4±0,5	30,5±3,1*	104±12,3*	197±20,4*	13±5,2*	5,2±0,55*	15±3,1*
Receiving red cells and SH	0,5±0,06	0,12±0,1	1,31±0,1	2,4±0,8	27,0±2,4*	113±18,6*	117±12,7	40±6,3	2,9±0,32	89±12,5*
Receiving SH	0,63±0,11	0,16±0,1	2,73±0,7*	0,2±0,1*	45,8±3,6	67±11,2	197±23,5*	89±9,5*	4,1±0,5*	43±5,7

tion of SH after injection of red cells prevented the development of splenomegaly and of lymphoid-reticular hyperplasia in BALB/c mice only. After injection of the hormone alone there was virtually no change in the weight of the lymphoid organs except the weight of the thymus in the BALB/c mice, despite a decrease in the total number of nucleated cells in them. The results of the psychological analysis revealed a twofold increase in the relative percentage of large and medium macronucleolar hyperbasophilic lymphocytes (activated cells) under the influence of SH. A very small increase in the number of lymphocytes of this type also was observed in animals into which red cells and SH were injected simultaneously. The number of T lymphocytes in the spleen also was reduced in BALB/c mice with a positive Coombs' test. SH increased the number of T lymphocytes in the animals of both group 3 and group 4. In the C57BL mice the number of T lymphocytes was unchanged.

Despite differences between strains in the numbers of E-RFC and EAC-RFC and of PFC after immunization with sheep's red cells and *E. coli* endotoxin in the BALB/c and C57BL mice and the changes in these indices after injection of syngeneic red cells (Table 2), injection of SH prevented these changes in the mice of both strains, with the exception of the number of EAC-RFC in the spleen. Injection of the hormone alone altered only the amplitude of the immune response in all the animals; it increased the number of PFC in the spleen after immunization with both sheep's red cells and *E. coli* endotoxin.

The results indicate the development of autoimmune reactions after injection of syngeneic heated red cells in mice of different genotypes. The cytological picture of the lymphoid organs, the number of T lymphocytes in the spleen, the number of E-RFC and EAC-RFC in the thymus and spleen, and the character of the immune response to thymus-dependent and thymus-independent antigens suggest that the mechanism of development of these autoimmune reactions differs in BALB/c and C57BL mice. In BALB/c mice it is possibly due to a reduction in the immunodepressive function of the thymus after injection of syngeneic red cells modified by a physical factor. Meanwhile in C57BL mice, autoantibody formation, indicating potentiation of the reactions of humoral immunity, was independent of the thymus. It is accordingly probable that SH has different effects on the development of the autoimmune process in mice of different genotypes.

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SECRETION OF SUPPRESSOR FACTORS BY MOUSE LYMPHOCYTES ON CONTACT WITH SYNGENEIC AND XENOGENEIC RED CELLS

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During incubation of spleen cells of immune mice *in vitro* with syngeneic and xenogeneic red cells a factor (or factors) with immunosuppressive activity is secreted into the medium. Secretion of the suppressor factor by spleen cells of nonimmune mice takes place only on contact with xenogeneic red cells.

KEY WORDS: *immune response; immunosuppression.*

Evidence has now been obtained that the immune response is under the control of T cells and, in particular, through the secretion of humoral factors [1, 8]. It has been shown, for instance, that on incubation of human T lymphocytes (strain MOLT) with sheep's red cells secretion of a factor (or factors) reducing the number of antibody-forming cells (AFC) in a culture of spleen cells of mice immunized with sheep's red cells *in vitro* [6] occurs. Activated mouse lymphocytes, when incubated with antigen, secrete a dialyzable factor which enhances the immune response [5, 7].

In this investigation the ability of mouse lymphocytes to secrete an immunosuppressive factor on contact with syngeneic and xenogeneic red cells was studied.

EXPERIMENTAL METHOD

(CBA × C57BL)F_1 mice were immunized intravenously with $2 \cdot 10^8$-$3 \cdot 10^8$ sheep's red cells. Three days later some of them were killed to obtain the test factor. Experimental immunized

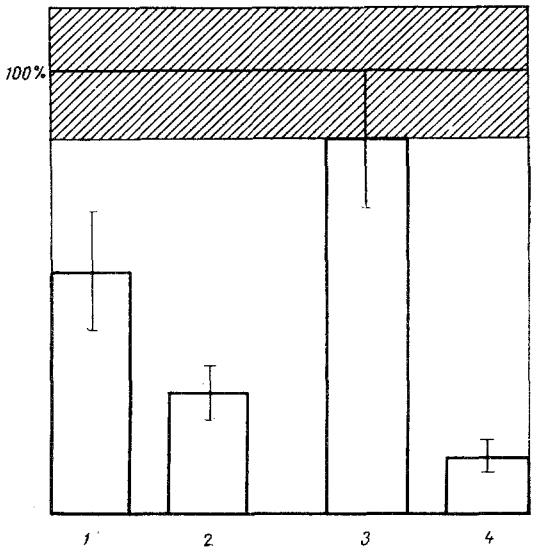

Fig. 1. Effect of supernatants obtained on contact between spleen cells and red cells on immune response: 1) nonimmune mouse lymphocytes + sheep's red cells; 2) immune mouse lymphocytes + sheep's red cells; 3) nonimmune mouse lymphocytes + syngeneic red cells; 4) immune mouse lymphocytes + syngeneic red cells. Continuous line in shaded zone indicates PFC level in control (in mice immunized with sheep's red cells), taken as 100%. Shaded zone indicates 95% confidence limits.

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