8. V. A. Chernov, Cytostatic Substances in the Chemotherapy of Malignant Neoplasms [in Russian], Moscow (1964).
9. G. Epstein et al., Anal. Chem., 27, 1435 (1955).
10. D. L. Hill, A Review of Cyclophosphamide, Springfield (1975).
11. N. K. Nerne and A. A. Nordin, Science, 140, 450 (1963).
12. B. J. Phillips and P. J. Cox, Chemotherapy, 7, 335 (1976).

MECHANISMS OF HYPOREACTIVITY IN MICE AFTER INJECTION OF LYSED ERYTHROCYTE ANTIGEN

T. V. Anfalova and L. A. Pevnitskii

UDC 612.017.1-06

A state of specific hyporeactivity to sheep's red cells (SRBC) was induced in mice by injection of hemolyzed SRBC. Blocking serum factor in these mice was shown not to be identical in the character of its action with antired-cell antibodies, but to be probably an antigen–antibody complex. After combined injection of hemolyzed SRBC and cyclophosphamide (CP) into mice production of blocking serum factor was suppressed but the reactivity of the mice to SRBC was considerably reduced. It is suggested that in this case inactivation of the immunocompetent cells took place through the combined action of CP and SRBC antigen in a nonimmunogenic form.

KEY WORDS: antigen of hemolyzed red cells; serum blocking factor; cyclophosphamide; suppression of immune response.

Hemolyzed sheep's red cells (SRBC) obtained by treating SRBC with distilled water, followed by ultracentrifugation, if injected into mice, induce a state of hyporeactivity to SRBC [1, 2]. In our own experiments [1], unlike those of Auerbach et al. [3, 4], the blood serum of mice treated with hemolysate possessed blocking activity, which disappeared after absorption with native SRBC.

The object of this investigation was to study the factors determining the state of reduced reactivity arising as a result of injection of hemolyzed SRBC into mice.

EXPERIMENTAL METHOD

The method of obtaining the hemolyzed SRBC was described previously [1]. Male CBA, (CBA × C57BL/6)F_1, and (DBA/2 × C57BL/6)F_1 mice weighing 20-26 g were obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR. SRBC hemolysate was injected either in a dose of 0.5 ml daily on 5 successive days or as a single dose 2.5 ml intraperitoneally (both methods were equally effective).

The reactivity of the animals was determined from the number of 19S-antibody-forming cells (AFC) in the spleen on the 5th day after intraperitoneal injection of 2×10^8 SRBC (the method of local hemolysis in agar [7]).

Antired-cell sera were prepared from the blood of mice immunized singly or repeatedly with SRBC. The sera were inactivated at 56°C for 30 min and were kept at −20°C before use.

The results were subjected to statistical analysis by Student's t-test.

EXPERIMENTAL RESULTS

The SRBC hemolysate had very low immunogenicity and, consequently, it induced only weak production of 19S-AFC (in these experiments on average 200 AFC per spleen, i.e., only 2 or 3 times more than the

Laboratory of Immunogenetics, Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bochkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 3, pp. 253-256, March, 1979. Original article submitted June 16, 1978.

0007-4888/79/8703-0245$07.50

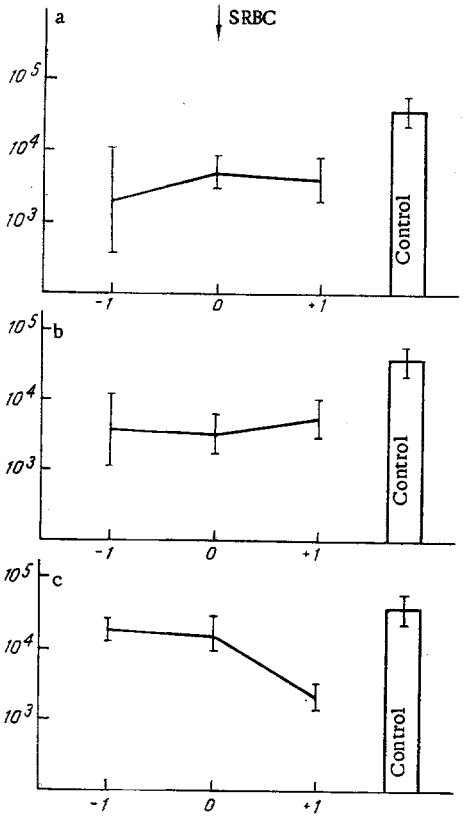

Fig. 1. Effect of blocking and antired-cell serum on immune response of intact mice: a) serum of mice receiving one injection of SRBC; b) serum of mice receiving five injections of SRBC; c) blocking serum (obtained from mice on 12th day after injection of hemolysate). Columns show response of intact mice to test injection of SRBC. Abscissa, day of injection of sera in relation to time of injection of SRBC; ordinate, number of AFC in spleen.

background level in nonimmunized mice). Nevertheless, in conformity with data in the literature [5], it could rightly be assumed that the formation of even a small quantity of antibodies (possibly of a different class from IgM) could inhibit the immune response of the animals to subsequent injection of SRBC.

The writers showed previously [1] that blood serum obtained from mice treated with hemolysate (conventionally described as blocking serum) reduces AFC production when injected into intact mice, and that the maximum of inhibition is observed if the serum is injected 24 h after the SRBC. With these results in mind, in the experiments of series I the inhibitory action of blocking serum was compared with the effect of antired-cell sera obtained after 1 or 5 injections of native SRBC (the titer of the sera in the hemagglutination test was 1 : 160, 1 : 4096, and 1 : 4096 respectively).

The sera were injected intraperitoneally, at different times relative to the injection of SRBC, in a dose of 0.4 ml in a dilution of 1 : 2.

As Fig. 1 shows, depression of the immune response by antired-cell sera was similar regardless of the method by which they were obtained. Considerable depression of the immune response was observed regardless of the time of injection of the serum. Maximal depression of AFC production by the blocking serum was observed when it was injected 24 h after the SRBC. Differences in the character of action of the sera compared thus suggest that the blocking factor in the serum of the hyporeactive mice was not identical with ordinary antired-cell antibodies.

Some workers associate the suppressive action of hemolysate with the formation of an antigen–antibody complex [3, 4]. Efforts were made to study this by incubating the antired-cell serum with the hemolysate (ratio 1 : 2) in vitro for 30 min at 37°C before injecting it into the animals.

TABLE 1. Effect of Combination of SRBC Hemolysate + Antiserum of Immune Response of CBA Mice

Material injected	Day of injection of reagents relative to test index of SRBC					
	experiment 1				experiment 2	
	−1	0	+1	control	+1	control
Hemolysate	—	24 040 (15 260—37 870) $n=5$	28 220 (14 160—56 270) $n=5$	25 380 (14 810—43 500) $n=6$	—	11 060 (7 952—15 380) $n=5$
Antired-cell serum	539 (324—896) $n=5$	1 573 (788—3 139) $n=5$	3 514 (1 967—6 276) $n=5$		1758 (962—3207) $n=6$	
Hemolysate + antiserum	650 (443—954) $n=6$	1 808 (845—3 808) $n=5$	1 533 (818—2 936) $n=6$		2186 (1246—3843) $n=6$	

Legend. Here and in Tables 2 and 3 geometric mean values and (in parentheses) confidence intervals ($P \leq 0.05$) for number of AFC in spleen are given.

TABLE 2. Blocking Properties of Mouse Serum after Combined Injection of SRBC Hemolysate and CP

Expt.	Immune response of mice receiving serum		
	control (serum not injected)	antiserum of mice receiving hemolysate	antiserum of mice receiving hemolysate + CP
1	17 320 (21 740—14 150) $n=6$	2551 (667—9671) $n=6$	11 070 (7 709—15 630) $n=6$
2	6 653 (3 200—13 830) $n=4$	589 (216—1604) $n=6$	3 504 (938—12 650) $n=5$

Legend. CP injected 24 h after hemolysate; sera obtained on 10th day after injection of SRBC hemolysate.

TABLE 3. Effect of Combined Injection of Hemolysate and CP on Immunologic Reactivity of Mice

Line of mice	Immune response to test injection of SRBC					
	control mice	mice receiving CP	mice receiving hemolysate	mice receiving CP followed 1 day later by hemolysate	mice receiving CP and hemolysate simultaneously	mice receiving hemolysate followed 1 day later by CP
CBA	25 120	24 250	7439	6604	2595	2403
	(19 200—32 820)	(17 456—33 700)	(6407—8639)	(5423—8042)	(1987—2876)	(1864—3097)
	n=6	n=6	n=6	n=6	n=6	n=6
BDF_1	31 340	32 265	3720	3791	1607	1482
	(22 240—44 220)	(24 360—40 770)	(2703—5117)	(2596—4862)	(569—4535)	(1387—1558)
	n=6	n=6	n=6	n=8	n=5	n=7

Legend. Test injection of SRBC given 14 days after injection of CP; CBA mice received 150 mg/kg CP, BDF_1 mice 100 mg/kg CP.

It follows from Table 1 that the hemolysate alone did not affect the immune response, whereas the antiserum depressed it. In one experiment injection of a mixture of SRBC hemolysate+antiserum led to more marked depression of the immune response 1 day after immunization than after injection of the antiserum alone. This effect was not observed in another experiment. This indicates that it is indeed possible, in principle, to inhibit AFC formation by a combination of hemolysate+antibodies, but in vitro it is evidently not always possible to create the optimal relative proportions of the ingredients of the complex, so that its inhibitory action is not exhibited.

In view of these results indicating the probable role of antibodies or the antigen–antibody complex in this state of hyporeactivity, in the next series of experiments injection of hemolysate was combined with injection of cyclophosphamide (CP). CP was given in doses causing virtually complete suppression of antibody formation, but not inducing prolonged depression of immunoreactivity of the animals (ability to respond to SRBC was restored after 7-10 days). It was expected that inhibition of production of blocking antibodies under the influence of CP would render the hemolysate unable to induce a state of hyporeactivity.

As the results in Table 2 show, CP in fact inhibited the production of blocking factor in the animals receiving the hemolysate: The sera of these mice lost their ability to inhibit the immune response of intact animals to SRBC. However, at the same time, CP did not change the state of hyporeactivity arising in the mice after injection of hemolysate (Table 3).

It will be clear from Table 3 that 2 weeks after injection of CP alone the reactivity of the animals was completely restored. The hemolysate caused significant inhibition of ability to respond to SRBC. The effect of combined injection of the hemolysate and CP depended on the time of injection of the immunodepressant: If it was injected 24 h before the hemolysate the immune response of the animals to the test injection of SRBC was the same as after injection of the hemolysate alone; if CP was given at the same time as or 24 h after the hemolysate, even stronger depression of the immune response was observed.

The main factor concerned in the formation of hyporeactivity after injection of the SRBC hemolysate was thus the serum blocking factor – antibodies or (more probably) an antigen–antibody complex. Meanwhile the hemolysate, under certain conditions, behaved as a true tolerogen: After injection together with CP it caused specific inhibition of reactivity in the absence of serum blocking factors. It can tentatively be suggested that by the mechanisms of its development this state differs from the "classical" type of drug-induced tolerance to SRBC (clonal elimination), an essential condition for which is intensive antigenic stimulation before injection of CP. As was shown above, the hemolysate possessed extremely weak immunogenic properties. Accordingly it is reasonable to suggest that in this case what happens is inactivation of the immunocompetent cells under the influence of the immunodepressant and antigen in a nonimmunogenic form, as has been demonstrated for nonimmunogenic preparations of levan [6].

LITERATURE CITED

1. T. V. Anfalova and L. A. Pevnitskii, Byull. Éksp. Biol. Med., No. 3, 310 (1978).
2. T. N. Anderson et al., J. Exp. Med., 136, 1666 (1972).
3. R. Auerbach, Am. Zool., 15, 209 (1975).
4. R. Auerbach and J. Roethle, Science, 183, 332 (1974).
5. G. Haughton and D. Nash, Transplant Proc., 1, 616 (1969).
6. J. G. Howard, in: Immunological Tolerance: Mechanisms and Potential Therapeutic Applications, New York (1974), p. 163.
7. N. K. Jerne and A. A. Nordin, Science, 140, 405 (1963).