ANTAGONISM BETWEEN THE ACTION OF ANTILYMPHOCYTIC SERUM AND CYCLOPHOSPHAMIDE DURING INDUCTION OF IMMUNOLOGICAL TOLERANCE TO SHEEP'S ERYTHROCYTES

L. A. Pevnitskii and R. K. Andreson

UDC 612.017.1.014.46:615.365.018.53+615.277.3

Experiments on mice showed that antilymphocytic serum from rabbits and horses and also horse antilymphocytic globulin suppress the primary immune response to sheep's erythrocytes, but immunological tolerance to this antigen does not arise. If administered together with cyclophosphamide, which in combination with sheep's erythrocytes induces a state of immunological tolerance to them, these preparations not only do not facilitate the formation of tolerance, but on the contrary, they prevent its development. The possible mechanisms of this phenomenon are discussed.

One way by which immunodepressive therapy can be made more effective is by using a combination of different immunodepressants. For example, it has been shown that the combined administration of one of the most widely used immunodepressants, antilymphocytic serum (ALS), with certain chemical compounds and, in particular, with cyclophosphamide (CP) leads to a more marked immunodepressive effect, and this combination has been used to produce immunological tolerance to transplantation antigens [14, 15].

Previous investigations have shown that CP can be used to produce tolerance to sheep's erythrocytes in adult animals [5, 7, 11].

EXPERIMENTAL METHOD

Experiments were carried out on adult noninbred albino mice and mice of line A/He. Three preparations of ALS and antilymphocytic globulin (ALG) were used: rabbit ALS, horse ALS, and horse ALG,* which were obtained from animals immunized with lymph gland and thymus cells from mice. All preparations were absorbed by mouse erythrocytes and blood plasma, and the titer of hemagglutinins in the reaction with mouse erythrocytes after absorption was below 1:10. The titer of rabbit ALS in the lymph-agglutination test was from 1:256 to 1:512, and the titer of horse ALG was 1:1280. The total dose of ALS (ALG) received by the mice after two intraperitoneal injections was 0.4-0.6 ml per animal.

The immunodepressive activity of ALS and ALG was determined by the degree of suppression of the primary immune response after intraperitoneal immunization of mice with 6×10^9 sheep's erythrocytes (the preparations were injected 2 days before and on the same day as immunization, and the number of antibody-forming cells in the spleen was investigated by the method of Jerne and Nordin [12] on the 5th day after immunization).

*The authors are grateful to N. A. Kraskina for providing the preparations of horse ALS and ALG.

Laboratory of Immunological Tolerance, N. G. Gamaleya Institute of Epidemiology and Microbiology, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. V. Vygodchikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 73, No. 2, pp. 64-67, February, 1972. Original article submitted June 25, 1971.

Tolerance to sheep's erythrocytes was obtained by the scheme described previously [5]: Intraperitoneal injection of 6×10^9 sheep's erythrocytes, followed 42-46 h later by intraperitoneal injection of 200 mg/kg CP. The state of tolerance was tested 2 weeks later by intravenous injection of 5×10^8 sheep's erythrocytes, and on the 4th day after the test injection the number of antibody-forming cells in the mouse spleen was counted.

In the experiments to study the effect of ALS and ALG on induction of tolerance, the animals were injected with antilymphocytic preparations in the above doses 2 days before and on the same day as injection of 6×10^9 sheep's erythrocytes and subsequent injection of CP, and tests were carried out 2 weeks later. Animals of the control groups received either ALG (ALS) or CP or ALS (ALG) + CP without any corresponding injection of the antigen. Just as in the experimental groups, the test injection was given 2 weeks after administration of the immunodepressants.

The numerical results were analyzed by statistical methods (calculation of the geometric mean, confidence intervals, and P).

EXPERIMENTAL RESULTS

The experiments of series I showed that all the ALS and ALG preparations investigated suppressed to some extent the primary immune response of mice to sheep's erythrocytes: the mean number of antibody-forming cells in the experimental animals was 8770 and in the controls 47,970 ($P = 0.009$; aggregated results of three experiments). However, a state of immunological tolerance did not arise under these circumstances: all the experimental animals reacted to subsequent injection of sheep's erythrocytes by a normal immune response, the intensity of which was actually higher than in the control animals not receiving ALS (ALG) (Fig. 1).

The results indicate that under these conditions ALS (ALG), in combination with antigen, cannot induce tolerance to sheep's erythrocytes in mice.

The results of the experiments of series I, in which the effect of ALS (ALG) on induction of tolerance to sheep's erythrocytes was investigated with the aid of CP, are given in Fig. 2. They show that animals receiving 6×10^9 sheep's erythrocytes and CP developed a state of immunological tolerance: their reaction to a test injection of sheep's erythrocytes was significantly less in intensity ($P < 0.001$) than that of control animals of all groups. Injection of either ALS or CP caused suppression of the reaction to sheep's erythrocytes by 50-67% compared with normal immunized mice. Combined administration of ALS and CP gave a summation effect: the number of antibody-forming cells was reduced by 6-7 times compared with the control. However, administration of ALS (ALG) before the treatment designed to induce tolerance (6×10^9 sheep's erythrocytes + CP) led not to a decrease, but to an increase ($P < 0.001$) in the animals' response to the test injection of antigen compared with tolerant mice treated with sheep's erythrocytes and CP but without ALS.

The antilymphocytic preparations (ALS and ALG) thus not only did not help to create immunological tolerance to sheep's erythrocytes by means of CP, but they had the opposite action.

The fact that by itself ALS cannot induce immunological tolerance when given with sheep's erythrocytes can be explained on the basis of the writers' previous findings [6], which showed that only those agents which possess a maximum of depressive action when injected in the initial phase of the immune response (the first 2 days after injection of antigen) can be used to produce immunological tolerance to sheep's erythrocytes. ALS is not one of these agents, for its immunodepressive action is exhibited particularly strongly on administration before antigenic stimulation [1, 2, 9, 12]. In this respect, the action of ALS is similar to that of immunodepressants such as irradiation, phenylalanine mustard, and tetraethyleneimidopiperazine-N,N'-diphosphoric acid, which are unable to induce tolerance to sheep's erythrocytes if given before immunization [6].

It is more difficult to explain the paradoxical effect of ALS in relation to the tolerance-inducing action of CP. The following suggestion seems most probable. As has already been said, ALS acts mainly on the "resting" cell population. CP, on the other hand, is most effective against the population of lymphoid cells which is "activated" by the antigen. It has also been shown that the targets of the immunodepressive action of ALS are antireactive cells (ARC) of thymus origin [8, 18], which play an important role in the reaction of mice to sheep's erythrocytes [10, 19]. It is suggested that during induction of immunological tolerance in mice to sheep's erythrocytes by means of CP, the targets for the action of this agent are also ARC of

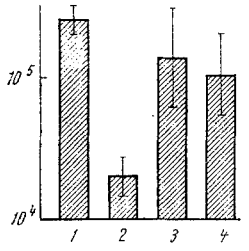

Fig. 1

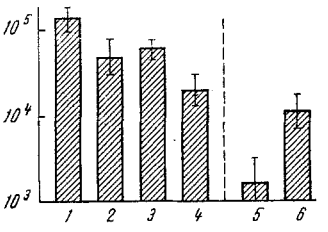

Fig. 2

Fig. 1. Administration of ALS in order to induce immunological tolerance to sheep's erythrocytes. Abscissa, preliminary treatment before test injection of antigen: 1) ALS+ 6×10^9 sheep's erythrocytes; 2) ALS; 3) 6×10^9 sheep's erythrocytes; 4) no treatment given before test injection; ordinate, here and in Fig. 2, number of antibody-forming cells in spleen after test injection of 5×10^8 sheep's erythrocytes.

Fig. 2. Combined administration of ALS and CP to induce immunological tolerance to sheep's erythrocytes. Abscissa, preliminary treatment before test injection of 5×10^8 sheep's erythrocytes: 1) no preliminary treatment given; 2) CP; 3) ALS; 4) ALS+ CP; 5) 6×10^9 sheep's erythrocytes+ CP; 6) ALS+ 6×10^9 sheep's erythrocytes+ CP.

thymus origin [17, 20]. Injection of a large dose of sheep's erythrocytes activates these cells, and CP injected in this phase of the immune response selectively eliminates the ARC activated by the antigen, and this is one of the principal factors in the establishment of the state of specific immunological tolerance [4, 16].

Taking all these findings into consideration, the following picture can be proposed for the processes taking place after injection of ALS, sheep's erythrocytes, and CP. The ALS which is injected first inactivates part of the ARC population, as a result of which these cells do not react to the subsequent injection of erythrocytes. That part of the ARC population which is in an "unactivated" state is less exposed to the action of subsequently injected CP, just as it is when this agent acts on the "resting" population of lymphoid cells [3]. As a result, complete elimination of all ARC does not take place by the action of CP, and that part of the ARC population which is inactivated by ALS remains in the body. In the course of time the reactivity of these cells (or of their progeny) is restored, and these animals then respond to the test injection of sheep's erythrocytes by the formation of a larger number of antibody-forming cells than tolerant mice which have not received ALS.

Irrespective of the interpretation proposed above, the results show that combined administration of different immunodepressants does not always lead to an increase in their effect. This effect may differ in character when attempts are made to obtain ordinary immunodepression or immunological tolerance, and it evidently depends on the properties of the immunodepressants used and on the character of the antigen.

LITERATURE CITED

1. R. K. Andreson, Byull. Éksperim. Biol. i Med., No. 3, 84 (1970).
2. N. A. Kraskina et al., Zh. Mikrobiol., No. 2, 83 (1971).
3. L. A. Pevnitskii et al., Byull. Éksperim. Biol. i Med., No. 10, 59 (1969).
4. L. A. Pevnitskii et al., Byull. Éksperim. Biol. i Med., No. 2, 56 (1970).
5. L. N. Fontalin et al., Byull. Éksperim. Biol. i Med., No. 11, 60 (1969).
6. L. N. Fontalin et al., Vestn. Akad. Med. Nauk SSSR, No. 7, 75 (1970).

7. A. C. Aisenberg, J. Exp. Med., 125, 833 (1967).
8. R. F. Barth, J. Immunol., 103, 648 (1969).
9. M. C. Berenbaum, Nature, 215, 1481 (1967).
10. H. N. Claman et al., Proc. Soc. Exp. Biol. (New York), 122, 1167 (1966).
11. F. M. Dietrich and P. Dukor, Path. et Microbiol. (Basel), 30, 909 (1967).
12. K. James, Clin. Exp. Immunol., 2, 685 (1967).
13. N. K. Jerne and A. A. Nordin, Science, 140, 405 (1963).
14. E. M. Lance and P. B. Medawar, Proc. Roy. Soc. B, 173, 447 (1969).
15. E. M. Lance and P. B. Medawar, Transplant. Proc., 1, 429 (1969).
16. A. Many and R. S. Schwartz, Clin. Exp. Immunol., 6, 87 (1970).
17. A. Many and R. S. Schwartz, Proc. Soc. Exp. Biol. (New York), 133, 754 (1970).
18. W. J. Martin and J. F. A. P. Miller, J. Exp. Med., 128, 855 (1968).
19. J. F. A. P. Miller and G. F. Mitchell, J. Exp. Med., 128, 801 (1968).
20. J. F. A. P. Miller and G. F. Mitchell, J. Exp. Med., 131, 675 (1970).