RESTORATION OF IMMUNOLOGIC REACTIVITY OF TOLERANT MICE OR MICE TREATED WITH CYCLOPHOSPHAMIDE BY INJECTION OF SYNGENEIC THYMUS CELLS

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Injection of thymus cells into mice, in which tolerance to sheep's red cells (RBC) had been induced by means of cyclophosphamide (CP), abolishes the state of tolerance. The immunologic reactivity of mice treated with CP only could also be restored by injection of syngeneic thymus cells within a certain period. The problem of the lesion affecting different types of immunocompetent cells in tolerance induced by the combined administration of RBC and CP is discussed.

Many investigations, specially those undertaken recently, have shown that the immune response to certain antigens, including sheep's red cells (RBC), is the result of interaction between several types of cells which differ in their origin and function [4, 6, 11, 15]. It was later established that in certain forms of immunologic tolerance cells of different types interacting during the immune response differ in their sensitivity to the tolerance-inducing procedure [3, 14, 16].

The study of the immunologic function of various cells from animals in which tolerance to RBC had been induced by means of CP yielded inconsistent results. According to data in the literature, structures affected in this form of tolerance may be the immunocompetent cells of the bone marrow [17] or thymus [9], circulating cells of thymic origin [12], or, finally, both bone marrow and thymus cells [7]. One reason for these conflicting results could be differences in technique and, in particular, the use of different systems of inducing tolerance.

The writer showed previously [1, 2] that when mice are injected with large doses of RBC and CP with an interval of about 2 days between them, the animals develop immunologic tolerance to this antigen, which lasts more than 3 months and is due, in the initial period (2 weeks), entirely to a deficiency of immunocompetent cells.

The effect of transplantation of various cells of intact animals on the immunologic reactivity of adult mice, subjected to immunodepressive treatment with RBC and CP by accepted scheme or receiving CP only, was studied in this investigation.

EXPERIMENTAL METHOD

Male (CBA × C57BL/6)F_1 mice weighing 18-22 g were used as donors and recipients. Tolerance was induced by intraperitoneal injection of 6.2×10^9 RBC followed, after 42-45 h, by 200 mg/kg CP. The immunologic reactivity of the animals was determined from the number of 19 S antibody-forming (AF) cells in the spleen (reaction of local hemolysis in agar [8]) on the 4th day after the intravenous test injection of 5×10^8 RBC. Suspensions of thymus, spleen, and cervical lymph gland cells were made up in medium No. 199 by carefully pressing the organs in a glass homogenizer and subsequent filtration through 3 or 4 layers

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TABLE 1. Effect of Transplantation of Different Cells on State of Immunologic Tolerance to RBC (M_{geom} and confidence limits)

Animals	Number of mice	Source of transplanted cells	Number of cells injected	Number of AF cells in spleen
Tolerant	4	—	—	91 (37–226)
»	6	Spleen	5×10^{-7}	5 260 (906–30 550)
»	6	Lymph glands	5×10^{-7}	16 790 (4 529–62 230)
»	7	Thymus	5×10^{-7}	2 399 (1 663–3 459)
»	5	Bone marrow	$2{,}5\times10^{-7}$	85 (22–326)
»	5	Thymus + bone marrow	$2{,}5\times10^{-7}$ $+2{,}5\times10^{-7}$	1 910 (141–25 880)
Receiving CP only (control)	6	—	—	2 606 (1 569–4 256)

TABLE 2. Effect of Transplantation of Thymus Cells on Immunologic Reactivity of Mice Treated with CP (M_{geom} for 3 experiments and confidence limits)

Interval between injection of CP and transplantation of thymus cells	Number of AF cells in spleen		P
	control	expt.	
4 h	23 (15–34) $n=6$	36 (18–69) $n=6$	0,165
3 h	29 040 (16 790–50 230) $n=15$	59 840 (44 570–80 350) $n=13$	0,021
7 h	46 030 (24 950–84 920) $n=12$	49 090 (32 730–73 620) $n=13$	>0,05
Intact immunized mice	99 770 (79 070–125 900) $n=11$		0,007*

*Calculated relative to highest index for animals of experimental group (59 840).
Legend: n) number of animals.

of Kapron. Bone marrow cells were obtained by flushing out the medullary cavity of the femoral bones with cold medium No. 199 by means of a syringe, and the cell suspension was then concentrated by centrifugation at 2000 rpm for 5 min. The cell suspensions were injected intravenously in a volume of 0.4-1 ml.

EXPERIMENTAL RESULTS

In the first month the mice received injections of different cells from intact syngeneic mice 3 days after tolerance had been induced in them. A test injection of RBC was given 2 weeks after the transplantation and the number of AF cells in the spleen was studied (Table 1).

It will be clear from Table 1 that cells of the lymph glands, spleen, or thymus, when injected into tolerant mice, abolished the state of tolerance: the animals' response to the antigen either showed no significant difference from the response of the control mice (receiving CP only), or it actually surpassed it (injection of lymph gland cells). Transplantation of bone marrow cells had no effect, while in the case of injection of a mixture of thymocytes and bone marrow cells the number of AF cells in the recipients' spleen was practically the same as after injection of thymocytes only.

In the next experiments in which the tolerant animals were injected with thymus cells, the number of thymocytes injected was increased to 80-100 million per mouse and the transplantation was carried out 24 h after induction of tolerance. Similar results were obtained in all four experiments. The combined results are given in Fig. 1.

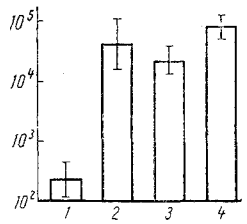

Fig. 1. Effect of transplantation of thymus cells on immunologic reactivity of tolerant mice. Ordinate, number of AF cells in spleen; abscissa: 1) tolerant mice; 2) tolerant mice receiving thymus cells; 3) mice treated with CP; 4) intact immunized mice.

As a result of the injection of thymus cells into tolerant animals, the number of AF cells in their spleen was considerably increased after the test injection of RBC and it did not differ significantly from the control (intact immunized mice). Similar results were obtained in other experiments, the conditions of which were the same as in those described previously except that thymus cells were injected soon after the induction of tolerance (4 h after injection of CP): in response to the test injection of RBC the number of AF cells formed in the spleen of the tolerant animals was 1807 (range from 611 to 5346), while in the tolerant mice receiving thymus cells the number of AF cells formed was 33,110 (range from 14,550 to 75,340) ($P < 0.001$).

Injection of lymphocytes from the spleen, lymph glands, and thymus of intact mice soon after the induction of tolerance thus completely or almost completely restored the immunologic reactivity of the tolerant animals. However, the restoration of immunologic reactivity after transplantation of spleen and lymph gland cells could occur as a result of an immune response of the transplanted cells themselves, but after injection of thymocytes such a mechanism is unlikely. There is evidence in the literature [5, 13, 14] that under the conditions of adaptive transfer thymus cells cannot produce antibodies and, consequently, AF cells found in tolerant animals in the experiments described above were of recipient origin, i.e., after injection of the thymus cells a true "collapse" of tolerance took place.

In the next experiments the effect of transplantation of thymus cells on the state of immunologic reactivity was studied in animals which had received CP only without any preliminary injections of RBC. Mice were injected intraperitoneally with 200 mg/kg CP, and at various times thereafter they were injected intravenously with 50 million thymus cells from intact syngeneic animals, and immunized 1 h later by intravenous injection of 5×10^8 RBC. On the 5th day after immunization the number of 19 S AF cells was determined in the spleen (Table 2).

As Table 2 shows, injection of the thymus cells 4 h after CP did not restore the reactivity of the animals. Transplantation of thymus cells on the 3rd day after injection of CP led to a significant increase in the immune response, while on the 7th day no effect of the thymus cells could be observed. In one of the experiments mice immunized 2 days before the experiment with 6×10^9 RBC also were used as donors of the thymocytes. The thymus cells of these mice restored the reactivity of the recipient mice to the same degree as the thymocytes of intact animals (when transplanted on the 3rd day after injection of CP into the recipients).

The reactivity of animals treated with CP can thus be restored, at least partially, by injection of thymus cells within a certain period. For some hours (possibly days [10]) after injection of CP the function of all cells participating in the immune response is apparently depressed, and for this reason injection of thymus cells had no effect during this period. After partial restoration of immunologic reactivity (3rd day) transplantation of thymus cells gave a definite effect, possibly evidence of their damage in the experimental animals. The absence of stimulation of the immune response if the injection was given on the 7th day after administration of the immunodepressive agent was evidently due to complete restoration of the cells of this type at that time. In all cases the level of the immune response in the experimental animals receiving thymus cells was lower than in intact immunized mice (Table 2).

It can be concluded from the results described in this paper that, in immunologic tolerance arising in mice as the result of combined injection of RBC and CP by the system used, the immunologic function of the thymus cells is disturbed, in agreement with the observations of Many and Schwartz [9]. This disturbance is evidently the result of the direct cytotoxic effect of active metabolites of the CP injected to induce tolerance on the thymus cells, for immunization with a large dose of RBC itself had no effect on the "restorative" function of the thymocytes. The T-cells of the recirculating pool are eliminated (or inactivated) in the same way [12].

At the same time, these results also provide a basis for the hypothesis that the immunodepressive action of CP is not due only to inactivation of the thymus cells (or T-cells), for their injection did not restore immunologic reactivity to its normal level. Presumably CP also damages the B-cells of the bone marrow

and lymphoid organs, in agreement with the immunomorphological data [18]. This factor may be particularly important in the case of induction of immunologic tolerance, for the high proliferative activity of the B-cells (like the T-cells) after injection of the antigen makes them highly sensitive to the action of CP. However, this problem requires further investigation, having regard to the negative results of attempts to restore the immunologic reactivity of tolerant mice by injection of bone marrow cells (Table 1).

It can be postulated on the basis of these results and of data in the literature that, depending on the intensity of the treatment used to induce tolerance, either T-cells or both T- and B-cells of the immunocompetent organs are damaged. This was shown by Mitchison [14] in the case of tolerance of mice to bovine serum albumin: tolerance induced by small doses of antigen was due to inactivation of T-cells, whereas after treatment with large doses of albumin both T- and B-cells were affected. A similar explanation can be given for the results of Jacobs et al. [7], in whose experiments treatment of C57BL mice twice with high doses of RBC and CP together with additional injections of antigen led to inactivation of both types of immunocompetent cells (thymus and bone marrow). The selective damage to immunocompetent bone marrow cells observed by some workers in (NZB × BALB/c)F_1 [17] and (NZB × NZW)F_1 [7] mice is evidently connected with the abnormal state of their lymphoid tissue [19, 20, et al.].

LITERATURE CITED

1. L. A. Pevnitskii, L. N. Fontalin, and T. K. Novikova, Byull. Éksperim. Biol. i Med., No. 7, 70 (1972).
2. L. N. Fontalin, L. A. Pevnitskii, and V. V. Solov'ev, Byull. Éksperim. Biol. i Med., No. 11, 60 (1969).
3. J. M. Chiller, G. S. Habicht, and W. O. Weigle, Proc. Nat. Acad. Sci. (Washington), 65, 551 (1970).
4. H. N. Claman, E. A. Chaperon, and R. F. Triplett, J. Immunol., 97, 828 (1966).
5. A. J. S. Davies, E. Leuchars, et al., Transplantation, 5, 222 (1967).
6. W. E. Davies, L. J. Cole, and W. T. Schaffer, Proc. Soc. Exp. Biol. (New York), 133, 144 (1970).
7. M. E. Jacobs, J. K. Gardon, and M. Talal, J. Immunol., 107, 359 (1971).
8. N. K. Jerne and A. A. Nordin, Science, 140, 405 (1963).
9. A. Many and R. S. Schwartz, Proc. Soc. Exp. Biol. (New York), 133, 754 (1970).
10. J. Marbrook and B. C. Baguley, Internat. Arch. Allergy, 41, 802 (1971).
11. J. F. A. P. Miller and G. F. Mitchell, J. Exp. Med., 128, 801 (1968).
12. J. F. A. P. Miller and G. F. Mitchell, J. Exp. Med., 131, 675 (1970).
13. J. F. A. P. Miller, A. Basten, et al., Cell Immunol., 2, 469 (1971).
14. N. A. Mitchison, in: Cell Interactions and Receptor Antibodies in Immune Responses, London (1971), p. 249.
15. N. A. Mitchison, K. Rajewsky, and R. B. Taylor, in: Developmental Aspects of Antibody Formation and Structure, Prague (1970), p. 547.
16. G. J. Nossal, in: Progress in Immunology, New York (1971), p. 665.
17. J. H. L. Playfair, Nature, 222, 882 (1969).
18. L. W. Poulter and J. L. Turk, Nature, 238, 17 (1972).
19. P. J. Staples, A. D. Steinberg, and N. Talal, J.Exp. Med., 131, 1223 (1970).
20. A. D. Steinberg, T. M. Chused, and N. Talal, Fed. Proc., 29, 308 (1970).