IMMUNOLOGICAL COMPETENCE OF T AND B LYMPHOCYTES IN CYCLOPHOSPHAMIDE-INDUCED TOLERANCE

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Immunological competence of T and B lymphocytes of mice was studied 7 days after induction of tolerance by injection of a massive dose of sheep's red cells and cyclophosphamide. The ability of lymphocytes of the tolerant mice to influence cooperation between normal T and B lymphocytes also was investigated. This particular form of tolerance was shown to be due not to suppressor T cells, but to a true deficiency of "helper" T cells (in both the thymus and spleen) and, to some extent also, of B cells (in the spleen). The very small deficiency of B cells in the bone marrow was connected with the nonspecific action of cyclophosphamide. It is postulated that cyclophosphamide selectively eliminates cells proliferating under the influence of the antigen.

KEY WORDS: immunological tolerance; cyclophosphamide; T and B lymphocytes; suppressor T lymphocytes; blood–thymus barrier.

The status of T and B lymphocytes in various forms of tolerance has not yet been settled. In tolerance induced in adult animals by cyclophosphamide (CP) some workers have observed predominantly depression of the function of the peripheral T cells [9], whereas others have observed total (including the thymus) suppression of the T-cell population [8], and a third group predominantly inhibition of function of the B cells [10]. Meanwhile reports have recently been published of the existence of so-called suppressor T cells in various forms of tolerance [5, 7].

The object of this investigation was to study the ability of T and B cells of the central and peripheral lymphoid organs of mice receiving the complete (sheep's red cells + CP) or incomplete (red cells or CP) tolerogenic treatment to cooperate with normal cells in the immune response, and also to study the possible suppressor properties of the lymphocytes of the tolerant animals.

EXPERIMENTAL METHOD

Strain F_1 (CBA × C57BL/6) mice were used. Tolerance to sheep's red cells was induced by intraperitoneal injection of 6.2×10^9 sheep's red cells followed (after 42-45 h) by injection of CP in a dose of 200 mg/kg [1]. Control animals received one of the ingredients of the complete tolerogenic treatment. The investigation was carried out 7 days after the injection of CP.

To study the status of the T and B lymphocytes, suspensions of thymus, spleen, and femoral bone marrow cells of the experimental and control animals and also of intact animals were injected intravenously into syngeneic irradiated (800 R, cobalt source, 2-4 h before transplantation of the cells) recipients. The cells were injected either separately or in different combinations, in a dose of $5 \cdot 10^7$ thymocytes and spleen cells and $1 \cdot 10^7$ bone marrow cells. Simultaneously with the cells, the recipients received $2 \cdot 10^6$

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TABLE 1. Immunological Activity of T and B Cells from Different Organs of Tolerant and Control Mice

Group No.	Source and number of transplanted cells			Number of recipients	Number of AFCs in spleen	
	thymus ($5 \cdot 10^7$)	bone marrow ($1 \cdot 10^7$)	spleen ($5 \cdot 10^7$)		mean	confidence limits
1	Norm	—	—	82	38	<67
2	—	Norm	—	73	30	<79
3	Norm	Norm	—	87	2729	1905—3908
4	AG	—	—	19	1786	929—3436
5	AG	Norm	—	24	6209	3715—10380
6	CP	—	—	18	5	<10
7	CP	Norm	—	32	1256	476—2698
8	TOL	—	—	7	15	<72
9	TOL	Norm	—	20	179	72—382
10	—	—	AG	8	164400	112 200—241 000
11	—	—	CP	57	7709	5070—11720
12	—	—	TOL	28	34	<84
13	—	Norm	TOL	30	365	116—1023
14	Norm	—	TOL	33	809	472—1387
15	—	AG	—	14	37	<157
16	Norm	AG	—	16	879	227—3304
17	—	CP	—	35	17	<42
18	Norm	CP	—	39	530	243—1002
19	—	TOL	—	8	37	<58
20	Norm	TOL	—	30	280	166—473

Legend. Norm) Intact donors; AG) donors receiving $6.2 \cdot 10^9$ sheep's red cells 9 days previously; CP) donors receiving 200 mg/kg CP 7 days previously; TOL) donors receiving complete tolerogenic treatment (sheep's red cells + CP).

sheep's red cells, with a further injection of $5 \cdot 10^8$ sheep's red cells 4 days later. The recipients were killed 8 days after injection of the cells and the number of antibody-forming cells (AFCs) in their spleen was determined by Jerne's method.

EXPERIMENTAL RESULTS

Data showing the immunological activity of the T and B cells from the central and peripheral lymphoid organs of the various groups of animals are summarized in Table 1. Clearly after injection of thymus and bone marrow cells of intact donors separately into irradiated recipients, no immune response was found in the spleens of the recipient mice (groups 1 and 2), whereas their combined injection led to the formation of many AFCs (group 3).

The next groups (4-9) follow the data showing the presence of immunologically competent lymphocytes in the thymus of the experimental animals. As Table 1 shows, treatment of the animals with CP alone induced a small but not statistically significant reduction in the ability of thymus cells to cooperate with B cells of the intact bone marrow (compare group 7 and group 3). Preliminary injection of a large dose of sheep's red cells ($6.2 \cdot 10^9$) into the donors, on the other hand, strengthened the immunological powers of their thymocytes (group 5), which actually became capable of producing an immune response independently (group 4). This evidently indicated the appearance of B cells in the thymus of donors treated with a large dose of the antigen. Conversely, the immunological competence of the thymocytes of the tolerant mice was sharply reduced. These cells cooperated with the cells of intact bone marrow 8-30 times less successfully than the thymocytes of the other groups (compare group 9 with groups 3, 5, and 7).

A different picture was observed when bone marrow cells were studied (groups 15-20). As Table 1 shows, in the animals of all the experimental groups there was some decrease (by three to ten times) in the ability of the bone marrow cells to cooperate with intact thymocytes. This reduction in cooperative activity was not statistically significant for animals receiving antigen alone, but it was significant for tolerant animals treated with CP. However, the differences between the last two groups (groups 18 and 20) were not statistically significant. It can consequently be concluded that depression of the activity of the bone marrow cells was due to the action of CP and not to the specific state of tolerance.

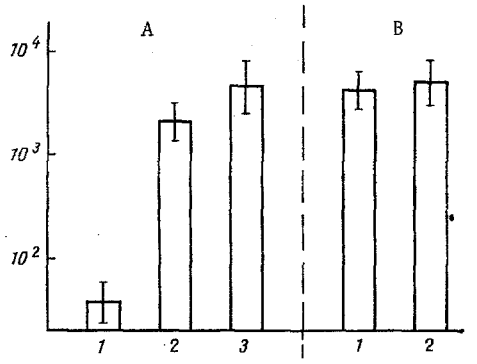

Fig. 1. Absence of suppressor activity of spleen (A) and thymus (B) cells of tolerant mice. In A: 1) spleen cells of tolerant donors; 2) thymus and bone marrow cells of intact donors; 3) spleen cells of tolerant donors together with thymus and bone marrow cells of intact donors. In B: 1) thymus and bone marrow cells of intact donors; 2) thymus cells of tolerant donors together with thymus and bone marrow cells of intact donors. Abscissa, groups of mice receiving cells; ordinate, number of AFCs in recipients' spleens.

Data (groups 12-14) on cooperative activity of T and B cells of the spleen of tolerant animals are given in Table 1. Clearly, judging from the level of the immune response, the spleens of mice receiving a large dose of antigen only (group 10) or CP only (group 11) contained both T and B cells. Conversely, spleen cells of the tolerant mice did not produce antibodies in culture in vivo (group 12). After combined administration of spleen cells of tolerant donors with intact thymocytes (group 14) or bone marrow cells (group 13) an immune response was observed; it was clearer when intact thymocytes were added but in both cases it was far less complete than after combined injection of thymus and bone marrow cells of intact donors (group 3) or transplantation of spleen cells of the control animals (groups 10 and 11). It can accordingly be concluded that in tolerant animals cooperative activity of both B and, in particular, T lymphocytes of the spleen is specifically impaired.

The results of a study of the suppressor activity of the spleen and thymus cells of tolerant animals are given in Fig. 1. It shows that neither spleen cells nor thymocytes of tolerant mice depress cooperation of intact T and B cells. Spleen cells actually intensify this process a little, a result that can be ascribed to antigen persisting in the spleen for 1-2 weeks after the induction of tolerance [3].

It can be concluded from these results that tolerance obtained with the aid of CP is not due to suppressor T cells, but is due to a true deficiency of lymphocytes competent with respect to the given antigen. This conclusion is in agreement both with the writers' previous observations [1, 3] and data in the literature [5].

Damage to the lymphocyte population in tolerant animals, as these observations show, is thus of a combined character. The deficiency of T cells in both the central (thymus) and peripheral (spleen) lymphoid organs is the most marked and specific feature. A specific deficiency of B cells was observed only in in the spleen, whereas in the bone marrow the reduction in the number of B cells was due to the nonspecific action of CP.

When these results are analyzed it must be borne in mind that CP affects actively proliferating cells primarily [4, 6]. The B lymphocytes characteristically have a shorter life cycle [11] and, for that reason, in the absence of antigenic stimulation they should be preferentially damaged by CP. This is in agreement both with the writers' own observations and with data in the literature [12].

During massive antigenic stimulation (tolerogenic treatment) the main targets of CP become clones of T and B lymphocytes induced to proliferate by antigen. This process takes place in the peripheral lymphoid organs and in the thymus (contrary to the widespread view of impermeability of the blood–thymus barrier). In the bone marrow, because of the deficiency of T lymphocytes, the conditions for a proliferative response of the B cells are absent and, consequently, they are not specifically damaged. These circum-

stances, and also the more rapid renewal of the B cell pool than of the T cells [11] and the relative resistance of hematopoietic stem cells to the action of CP [4, 6], lead to rapid recovery of the peripheral B-lymphocyte population. Conversely, the specific deficiency of T lymphocytes can also be detected in the later stages of tolerance [2].

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