FACTORS DETERMINING LONG-TERM CHIMERISM OF LYMPHOID TISSUE IN CYCLOPHOSPHAMIDE TREATED MICE

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The conditions of origin of long-term chimerism were investigated in adult CBA mice receiving cyclophosphamide and F_1(CBA × C57BL/6) spleen cells. Essential conditions are a high dose of cells (more than 50 × 10^6), a dose of cyclophosphamide of not less than 200 mg/kg, and short intervals (3-6 h) between their injections. The results are interpreted from the standpoint of the hypothesis that cyclophosphamide can induce reversible injuries in lymphocyte DNA; these injuries become fixed after contact between lymphocytes and antigen and they lead to death of the corresponding cell alone.

KEY WORDS: chimerism; cyclophosphamide; immunologic tolerance.

The writers showed previously that by combined injections of nonlethal doses of cyclophosphamide (CP) and hematopoietic cells tolerance to allogeneic donors' tissues differing from those of the recipient in having strong H-2 antigens, can be induced in adult mice. This tolerance is accompanied and, evidently, supported by long-term (for 12 weeks) chimerism of the lymphoid tissue [2]. The possibility of long (for 5 months and more) survival of an allografted heart in such mice has been demonstrated [6].

The object of this investigation was to study the optimal conditions necessary for the creation of long-term chimerism in this sytem. The dependence of survival of the donors' cells on their dose, the dose of CP, and the interval between their injections were studied.

TABLE 1. Dependence of Long-Term Chimerism of Lymphoid Tissue on Number of Semisyngeneic Cells Injected

Group No.	Number of cells injected (× 10^6)	Time of investigation					
		5th day			18–25th day		
		number of animals	number of chimeras	number of AFC	number of animals	number of chimeras	number of AFC
1	0 (control)	15	—	⩽18 (10—30)	55	—	374 (270—519)
2	20	5	5/5	11 480 (5 260—25 060)	23	—	406 (184—897)
3	50	22	17/17	25 290 (12 820—49 890)	23	4/15	5 200 (2 377—11 380)
4	100	21	21/21	147 600 (107 900—201 400)	47	33/33	44 360 (28 180—69 820)

*Numerator gives number of mice in which the presence of chimerism was confirmed by the results of discriminative analysis; denominator gives total number of animals tested by this method.

†Geometric mean values and confidence limits for number of AFC in spleen given.

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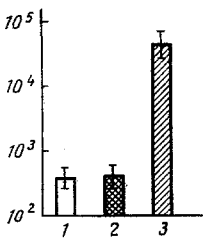

Fig. 1

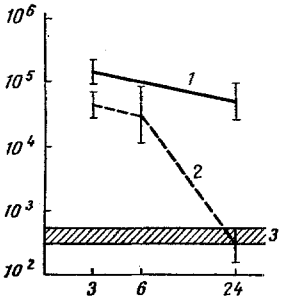

Fig. 2

Fig. 1. Dependence of survival of semisyngeneic cells on dose of CP. Abscissa: 1) mice not receiving cells (55 animals), 2) mice receiving F_1 cells and 100 mg/kg CP (18 animals), 3) mice receiving F_1 cells and 200 mg/kg CP (47 animals); ordinate, number of AFC against RBC in spleens of CBA mice on 18th day after injection of CP.

Fig. 2. Survival of semisyngeneic cells as a function of time of injection of CP. Abscissa, interval between injections of CP and F_1 cells into CBA mice (in h); ordinate, number of AFC against RBC in recipients' spleens 5 days (1) and 18 days (2) after injection of cells and level of immune response in animals not receiving cells (3).

EXPERIMENTAL METHOD

Experiments were carried out on adult (18-25 g) male CBA mice and F_1 (CBA × C57BL/6) hybrids. The CBA mice (recipients) received an intraperitoneal injection of $6.2 \cdot 10^9$ sheep's red cells (RBC) followed by CP 41-43 h later in doses of 100 or 200 mg/kg. This treatment induced tolerance to RBC in the recipients [5]. At various times (3, 6, or 24 h) after CP the experimental mice were given an intravenous injection of a suspension of spleen cells from F_1 (CBA × C57BL/6) mice in a dose of $2 \cdot 10^7$, $5 \cdot 10^7$, or $1 \cdot 10^8$ cells per mouse. The donors had previously (1-4 weeks before the experiment) been sensitized with RBC ($1 \cdot 10^6$ red cells intravenously). CBA mice receiving the same injections of RBC and CP but no injections of cells, F_1 hybrids presensitized to RBC, and also intact mice of both strains were used as the control.

The number of antibody-forming cells (AFC) in the spleen of the chimera mice and control animals was determined in Jerne's local hemolysis in gel test on the fifth, 18th, and 25th days after injection of the cells. For the investigations on the 18th and 25th days, 4 days before the test the animals received an intravenous injection of $5 \cdot 10^8$ RBC. The presence of an immune response to RBC was one of the criteria of persistence of the lymphocytes of donor origin, for the recipients were tolerant to that antigen.

The direct test for the presence of chimerism was the method of discriminative analysis of cell suspensions by means of strain-specific antiserum suggested previously [4]. The following modifications were made to the method as described: Three million spleen cells were incubated for 45 min at 37°C with 0.05-0.1 ml anti-C57BL/6 CBA serum in the presence of 0.1-0.25 ml rabbit complement in a total volume of 0.5 ml. Control suspensions were treated with normal mouse serum and complement and incubated under the same conditions. The decrease in the number of AFC as a result of treatment with anti-C57BL/6 CBA serum indicated their donor (F_1) origin.

EXPERIMENTAL RESULTS

The results of investigation of the spleens of CBA mice receiving different numbers of cells from semisyngeneic donors (20, 50, and 100 million cells per mouse) are given in Table 1. In this series of experiments CP was injected in a dose of 200 mg/kg 3 h before injection of the cells. The spleens were tested 5, 18, and 25 days after injection of the cells.

The results given in Table 1 show that on the fifth day chimerism existed in all the experimental animals. The presence of chimerism was confirmed both by the absolute level of the immune response to RBC and by the results of discriminative analysis. The intensity of antibody-formation in the 5-day chimeras depended on the number of donors' cells injected. The spleens of the control mice which did not receive F_1 cells contained no AFC.

On the 18th-25th day persistent chimerism of the lymphoid tissue was observed only in those recipients which had received 100 million donors' cells (in 33 of the 33 animals tested). In the group of animals receiving 50 million cells per mouse, AFC of donor origin were present in four of the 15 mice tested. With a decrease in the number of cells injected to 20 million, the chimerism was lost. The magnitude of the immune response to RBC in this group was within the control limits recorded in CBA mice not receiving donors' cells.

The effectiveness of survival of the semisyngeneic cells also depended on the dose of CP used to induce tolerance in the recipients (Fig. 1). In this series of experiments CP was injected in a dose of 100 or 200 mg/kg, and the F_1 cells were injected in a dose of 100 million per mouse 3 h after CP. The number of AFC was determined 18 days later. As Fig. 1 shows, in mice receiving F_1 cells after an injection of 100 mg/kg CP, no immune response was present to RBC, just as in control animals tolerant to RBC and not receiving the cells. By contrast, CP in a dose of 200 mg/kg produced a high level of immune response in all mice of this group, as a result of chimerism of the lymphoid tissue (more than 100 times higher than the control).

The results of experiments to study survival of the donors' cells depending on the interval between injections of CP and cells are given in Fig. 2. CP was injected in a dose of 200 mg/kg and the cells in a dose of 100 million, 3, 6, or 24 h after CP. As Fig. 2 shows, after 5 days, irrespective of the time of injection of CP, survival of the donors' immunocompetent cells was observed in all the recipients. After 18 days AFC of donor origin were present only in the spleens of mice receiving the cells 3-6 h after the injection of CP. If the cells were injected 24 h after the CP chimerism was lost.

Examination of the results leads to the following conclusions. The dependence of the duration of chimerism on the dose of cells injected and on the intensity of immunodepressive action was established previously by various workers both for radiation chimeras (see [3] etc.) and for chimeras obtained with CP [8, 9]. However, the fact must be emphasized that chimerism and tolerance to H-2-incompatible donors' cells can be obtained by the use of nonlethal doses of CP, whereas irradiation for the same purpose must be given in lethal or even superlethal doses. In chimerism obtained with CP the recipient's lymphocytes competent toward other antigens are preserved [2]. CP evidently leads to induction of tolerance without killing the recipient's lymphocytes totally, merely and modifying their response to the antigen. Conversely, to obtain radiation chimerism, practically all the recipient's immunocompetent cells must be killed.

The temporal relationships of the co-tolerogenic action of moderate doses of CP deserve special attention. In experiments in which irradiation [11] or near-lethal doses of CP [10] were used, long-term chimerism was observed even when the allogeneic cells were injected 1-2 days after immunodepressive action. Conversely, in the present investigation, the effective interval between injection of a moderate dose of CP (200 mg/kg) and the cells was under 1 day (3-6 h). Similar relations were observed by the writers previously in the case of induction of tolerance to xenogeneic red cells [5].*

The shortness of the effective interval between injections of CP and antigen suggests that changes in resting lymphocytes induced by CP are reversible in character. If the lymphocytes at or shortly after the time of action of CP start to proliferate as a result of contact with antigen or for other reasons, reversible injuries induced by CP in DNA are fixed and lead to death of the corresponding cell clone. This hypothesis is in good agreement with the characteristics of the action of CP on tumor cells [12], hematopoiesis and myelopoiesis [7], and the formation and realization of the immunologic memory [1].

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* In some experimental models an optimal tolerogenic effect has been obtained by injection of CP after the antigen [5, 9, etc.]. In the present system this method was unsuitable because of the toxic action of CP on transplantable lymphocytes.

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