PROLONGED CHIMERISM OF LYMPHOID TISSUE OBTAINED FROM ADULT MICE WITH THE AID OF CYCLOPHOSPHAMIDE

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UDC 612.42:612.6.02.017.1]
.014.46:615.277.3:547.558.1

Prolonged (for at least 12 weeks) chimerism of lymphoid tissue was obtained as a result of successive injection of cyclophosphamide and spleen cells from (CBA × C57BL/6)F_1 mice into adult mice of line CBA.

It is a very difficult problem to obtain tolerance to allogeneic cells in the immunologically mature organism. If the donor and recipient differ in strong loci of tissue compatibility, it is customary to use preliminary irradiation of the recipient with lethal doses or irradiation preceded by thymectomy for this purpose.

In view of experimental evidence of the particularly high effectiveness of cyclophosphamide as an agent leading to the formation of tolerance to heterogeneic "strong" antigens [3, 7], it was decided to use this compound to produce tolerance to allogeneic cells.

Only a few papers indicating that this is possible can be found in the literature. For instance, Glynn et al. [12] used cyclophosphamide to obtain mouse-chimeras in which the donors and recipients differed in weak loci of tissue. Phillips [14, 15] describes chimerism in a rat–mouse system, but only after the use either of lethal doses of cyclophosphamide [14] or a combination of cyclophosphamide with neonatal thymectomy [15]. Storb et al. [16] observed chimerism in dogs after administration of lethal doses of cyclophosphamide and of bone marrow cells from donors differing from the recipients in weak transplantation antigens.

EXPERIMENTAL METHOD

Experiments were carried out on adult (18-25 g) male CBA mice and on hybrids (CBA × C57BL/6)F_1. The CBA mice received an injection of $6.2 \cdot 10^9$ sheep's erythrocytes, followed after 41-43 h by cyclophosphamide (VEB Ankerwerk, Rudolstadt, East Germany) in a dose of 200 mg/kg. An intravenous injection of a suspension of spleen cells ($0.9 \cdot 10^8$-$1.0 \cdot 10^8$ cells) from (CBA × C57BL/6)F_1 mice, previously (1-8 weeks before the experiment) sensitized with sheep's erythrocytes ($1 \cdot 10^6$ erythrocytes, intravenously) was injected into the experimental mice intravenously 2.5-4.5 h later. Control aminals either received no cells from (CBA × C57BL/6)F_1 mice or underwent no preliminary treatment whatsoever.

At various times (from 3 to 12 weeks) after these procedures the experimental and control animals received an intravenous injection of $5 \cdot 10^8$ sheep erythrocytes or a mixture of $5 \cdot 10^8$ sheep's erythrocytes and $5 \cdot 10^8$ erythrocytes from an August rat. The mice were sacrificed four days later and the spleen cells tested by Jerne's reaction [13] with the modifications described previously [1, 2]. To determine the origin or the antibody-forming cells found in the recipient's spleen, the method described previously by the writers

Laboratory of Immunological Tolerance, N. F. Gamaleya Institute of Epidemiology, and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR O. V. Baroyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 72, No. 8, pp. 71-75, August, 1971. Original article submitted February 26, 1971.

TABLE 1. Restoration of Immunological Reactivity of Line CBA Mice Tolerant to Sheep's Erythrocytes by Spleen Cells of (CBA × C57BL/6)F_1 Mice Preliminarily Sensitized with Sheep Erythrocytes

Group	Animals	Time of investigation			
		3 weeks		10-12 weeks	
		number of mice	number of antibody-forming cells in spleen*	number of mice	number of antibody-forming cells in spleen*
1	Tolerant, of line CBA, receiving cells from (CBA × C57BL/6)F_1 mice	29/30†	71,780 (39,390-128,800)	10/12†	164,800 (68,230-398,100)
2	Tolerant, of line CBA	28	617 (344-1,107)	8	28,250 (16,260-49,090)
3	Intact, of line CBA	23	107,900 (81,470-142,900)	10	219,800 (182,800-264,200)

*Geometric mean and confidence limits of number of antibody-forming cells.
†Numerator gives number of animals differing significantly from tolerant CBA mice in the level of their antibody-forming cells in the spleen; denominator gives total number of animals.

TABLE 2. Antigenic Characteristics of Lymphoid Cells of Mouse-Chimeras Producing Antibodies against Sheep's Erythrocytes ($M_{arith} \pm m$)

Animals	3 weeks*		10-12 weeks*	
	number of mice	percentage of antibody-forming cells sensitive to CBA-anti-C57BL serum	number of mice	percentage of antibody-forming cells sensitive to CBA-anti-C57BL serum
Chimeras (CBA × C57BL/6)F_1 → CBA	27/29†	87.9 ± 3.9	11/11†	89.4 ± 3.8
(CBA × C57BL/6)F_1	23	92.7 ± 1.7	8	95.7 ± 0.5
CBA	31	0.3 ± 6.4	10	6.2 ± 7.1

*Time from moment of creation of chimerism.
†Numerator gives number of animals differing significantly from CBA in their sensitivity to line-specific serum; denominator gives togal number of animals.

[4, 5] was used. Spleen cells were treated in vitro in the presence of rabbit complement with line-specific antiserum obtained in CBA mice after their immunization with spleen cells from C57BL/6 mice. In the control experiments, spleen cells were treated similarly with normal serum from CBA or (CBA × C57BL/6)F_1 mice. The cells were then tested in Jerne's reaction, after which the percentage of antibody-forming cells sensitive to the action of CBA-anti-C57BL serum was calculated.

Two weeks after the transfer of the cells, skin grafts from (CBA × C57BL/6)F_1 mice were transplanted on some of the experimental and control mice by the method of Billingham and Medawar [10]. The times of rejection of the graft were noted, with specific reference to the time of appearance of the first signs of rejection (small hemorrhages and ulcers, slight rigidity of the graft) and of complete rejection (total necrosis).

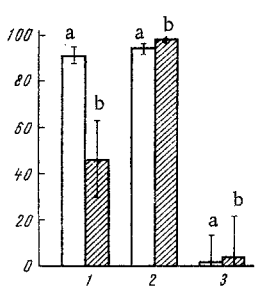

Fig. 1

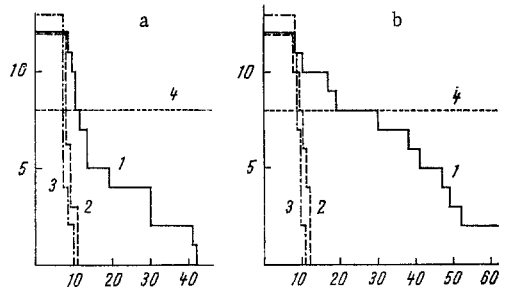

Fig. 2

Fig. 1. Cell composition of population of antibody-forming spleen cells in mouse-chimeras (CBA × C57BL/6)F_1 → CBA 3-12 weeks after formation of chimerism: a) cells forming antibodies against sheep's erythrocytes; b) cells forming antibodies against rat's erythrocytes. Abscissa: 1) mouse-chimeras (9 animals); 2) (CBA × C57BL/6)F_1 mice (9 animals); 3) CBA mice (10 animals). Ordinate; percentage of antibody-forming cells sensitive to the action of CBA-anti-C57BL serum.

Fig. 2. Dynamics of partial (a) and complete (b) rejection of allogeneic skin grafts by mouse-chimeras (CBA × C57BL/6)F_1 → CBA. 1) Mouse-chimeras; 2) mice of line CBA tolerant to sheep's erythrocytes; 3) intact CBA mice; 4) (CBA × C57BL/6)F_1 mice. Abscissa; days of observation; ordinate; number of mice with persistent grafts.

EXPERIMENTAL RESULTS

The results showing the number of cells producing antibodies against sheep's erythrocytes in the spleen of experimental and control animals are given in Table 1. It is clear from these results that the animals receiving a preliminary large dose of sheep's erythrocytes and cyclophosphamide (group 2) partly or completely lost their ability to react to this antigen by antibody production. This is in agreement with results obtained previously by the present writers [6] and others [8, 11]. If, however, soon after receiving cyclophosphamide, these animals were given lymphoid cells from semisyngeneic (F_1) donors, their immunological reactivity was completely restored (Table 1, group 1).

To determine whether these animals were true chimeras or whether their immunological reactivity to sheep's erythrocytes was restored for other reasons, the sensitivity of the antibody-forming cells of the hypothetical chimeras to the cytotoxic action of antidonor sera was investigated (Table 2).

The results given in Table 2 show that, as a rule, the line-specific serum had no effect on the activity of antibody-forming cells of the CBA mice (principal locus of tissue compatibility $H\text{-}2^k$); conversely, cells of the (CBA × C57BL/6)F_1 hybrids loci of tissue compatibility $H\text{-}2^k$ and $H\text{-}2^b$ were inactivated practically completely (by 93-96%). It will also be seen in Table 2 that antibody-forming cells of CBA mice which received an injection of spleen cells from (CBA × C57BL/6)F_1 mice together with cyclophosphamide 3-12 weeks before testing reacted in the overwhelming majority of cases (38 of 40) to line-specific serum in a similar manner to cells from (CBA × C57BL/6)F_1 mice. This indicates the persistent chimerism of the lymphoid tissue, i.e., the prolonged persistence of donor cells foreign with respect to their H-2 tissue compatibility locus, or of their progeny in the lymphoid organs of the recipient. Comparison of the results obtained in the earlier (3 weeks) and later (10-12 weeks) tests on the animals showed no tendency for the donor's cells to be "supplanted."

Analysis of the results in Table 2 gives the impression of the almost complete chimerism of the lymphoid tissue of the experimental animals. However, it must be remembered that for the best manifestation of chimerism, the donor's and recipient's cells were placed from the very beginning in a "dissimilar position" relative to the test antigen: tolerance to sheep's erythrocytes was induced in the recipient mice, whereas the donors were sensitized with this antigen. If the animals were tested for the presence of chimerism by the use of two test antigens (sheep's and rat's erythrocytes) simultaneously,

the results (Fig. 1) showed that chimerism was in fact partial: only half of the total number of cells producing antibodies against rat's erythrocytes were of donor's origin. It must be concluded from the comparison of this fact with the duration of the chimerism that the lymphoid tissue of the recipients regenerated after injection of cyclophosphamide must have acquired immunological tolerance to the donor's transplantation antigens. This hypothesis was confirmed by experiments in which skin grafts from (CBA × C57BL/6)F_1 mice were transplanted to the experimental animals.

As Fig. 2 shows, the allogeneic graft was rejected much later by the mouse-chimeras (group 1) than by the control animals (groups 2 and 3). The final phases of the rejection process were particularly slow (Fig. 2b). The graft on these animals was covered for a long time with tiny ulcers and desquamating scabs, and it gradually lost its hair, but isolated areas of the graft remained unchanged or only slightly changed for quite long periods. This picture of "chronic rejection" has been described after the transplantation of grafts differing from the recipient in weak transplantation antigens [9], but not incompatible relative to the H-2 locus. Chimerism in the experimental animals in the experiments described above was thus accompanied by partial tolerance to the allogeneic skin graft. It is interesting to note that even after total rejection of the skin graft the animals remained chimeras. Rejection of the skin graft by chimeras has been observed by other authors [2, 7].

The general conclusion can thus be drawn that tolerance to allogeneic cells and to skin grafts differing from the recipient in strong H-2 antigens can be obtained in adult mice with the aid of nonlethal doses of cyclophosphamide. This tolerance is accompanied (and, evidently, maintained) by the persistent chimerism of the lymphoid tissue.

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