SYNGENEITY AS A FACTOR IN COOPERATION BETWEEN T- AND B-LYMPHOCYTES

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Effectiveness of cooperation between (CBA × C57BL/6)F_1 thymocytes and CBA bone-marrow cells in the immune response to sheep's red cells was compared with syngeneic combinations of the same cells in culture *in vivo*. The selectiveness of cooperation between T- and B-lymphocytes of different origin also was investigated in incomplete (CBA × C57BL/6)F_1→CBA chimeras obtained with the aid of cyclophosphamide, in which the donors were primed with sheep's red cells and the recipients were either intact or were tolerant to that antigen. F_1 T-cells were shown to cooperate with CBA B-cells 10-50 times less effectively than with syngeneic B-cells. It is postulated that the similar antigenic structure of the cell membrane of T- and B-lymphocytes, acting in conjunction with their physical contact, increases the effectiveness of action of the T-mediator on the B-cell
KEY WORDS: *T- and B-lymphocytes; intercellular interaction; genetic compatibility; chimerism.*

The role of genetic identity of the T- and B-lymphocytes in their cooperation during the immune response to different antigens has recently been a topic for frequent discussion. According to some workers [9-11] allogeneic T- and B-cells cannot cooperate. This conclusion is disputed by other workers, [8, 12]. The solution to the problem of the role of syngeneity of T- and B-lymphocytes in their effective interaction is important to the understanding of the mechanism of cell cooperation [9].

Effectiveness of cooperation of syngeneic and semisyngeneic T- and B-lymphocytes was compared under conditions which excluded immune conflict between them, and also between these cells and their microenvironment. The experiments were planned so as to exclude

TABLE 1. Interaction between Syngeneic and Nonsyngeneic T- and B-Cells in Immune Response to Sheep's Red Cells

Group	Cells injected		Number of recipients	Number of cells in spleen ($\times 10^6$)	Number of APC in spleen $\bar{x}_g$ and confidence limits for $P < 0.05$)
	thymocytes (5×10^7)	bone marrow cells (1×10^7)			
1	—	—	5	7	3 (<6)
2	(CBA × C57BL/6)F_1	—	6	18	20 (7–53)
3	CBA	—	5	19	21 (10–44)
4	—	(CBA × C57BL/6)F_1	14	71	168 (48–446)
5	—	CBA	14	78	71 (25–158)
6	(CBA × C57B/6)F_1	(CBA × C57BL/6)F_1	15	125	6053 (3228–11 350)
7	CBA	CBA	13	118	6427 (3365–12 270)
8	(CBA × C57B/6)F_1	CBA	16	99	346 (155–771)

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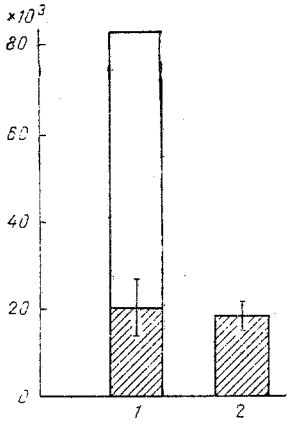

Fig. 1

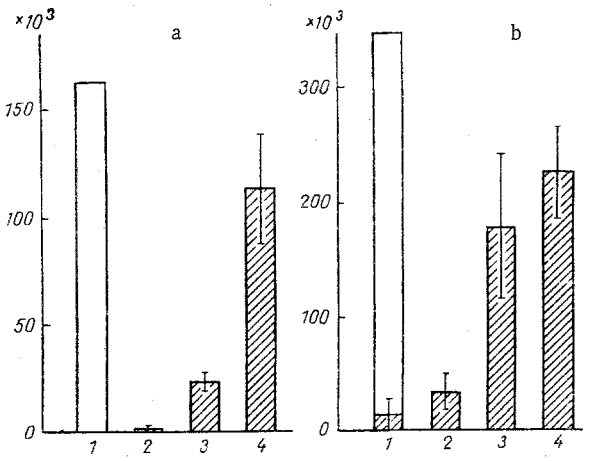

Fig. 2

Fig. 1. Autonomy of immune response of nonsyngeneic populations of lymphoid cells in incomplete chimeras (duration of chimerism 3 weeks). Abscissa: 1)(CBA × C57BL/6)F_1→CBA chimeras; 2) control CBA mice receiving CP alone. Ordinate, number of cells forming antibodies (AFC) against sheep's red cells (RBC) in spleen. Shaded columns — AFC with CBA (H-2^k) antigenic characteristics; unshaded columns — AFC with (CBA × C57BL/6)F_1 (H-$2^{b/k}$) antigenic characteristic.

Fig. 2. Autonomy of immune response of nonsyngeneic lymphocyte populations in chimeras in which recipients are tolerant to RBC. Duration of chimerism: a) 3 weeks, b) 2-3 months. Abscissa: 1) (CBA × C57BL/6)F_1→CBA chimeras; 2) CBA mice tolerant to RBC; 3) CBA mice receiving cyclophosphamide only; 4) intact CBA mice. Ordinate, number of AFC against RBC in spleen. Shaded columns — AFC with CBA (H-2^k) antigenic characteristic; unshaded columns — AFC with (CBA × C57BL/6)F_1 (H-$2^{b/k}$) antigenic characteristic.

other factors which might have distorted the results, specifically the effect of allogeneic inhibition and nonsyngeneity of the stromal cells on the effectiveness of T—B cooperation.

EXPERIMENTAL METHOD

In the experiments of series I cooperation between nonsyngeneic T- and B-cells was investigated in short-term experiments under optimal conditions for the manifestation of the cooperative effect [17]. CBA mice, irradiated in a dose of 850 R, were given an intravenous injection of 5×10^7 thymus cells, 1×10^7 bone marrow cells, or a mixture of both. Sheep's red cells (2×10^6) were injected along with these cells. A further injection of 5×10^8 RBC was given 4 days later. The mice were killed 8 days after the beginning of the experiments and the number of antibody-forming cells (AFC) in the spleen was determined by Jerne's method. Intact CBA or (CBA × C57BL/6)F_1 males were used as donors. Besides the syngeneic controls, a semiallogeneic combination also was used: F_1 thymocytes were injected together with CBA bone marrow cells.

In two other series of experiments the selectivity of cooperation was studied in mixed populations of T- and B-cells kept together for a long time. Long-living chimeras were obtained as described previously [3]. Cyclophosphamide (CP) was injected intraperitoneally into CBA mice in a dose of 200 mg/kg body weight, and 3-5 h later an injection of 1×10^8 spleen cells of (CBA × C57BL/6)F_1 mice, previously (1-4 weeks beforehand) immunized with 1×10^6 RBC intravenously, was given to the animals. In some experiments the recipients received 6.2×10^9 RBC intraperitoneally 42-45 h before the injection of CP. A few weeks later, 5×10^8 RBC were injected intravenously into both experimental and control mice; the animals were killed 4 days later, and the number of AFC of donor and recipient origin was determined in the spleen. The method described earlier [5], with certain modifications, was used for this purpose. A mixture of $3\text{-}5 \times 10^6$ spleen cells with 0.1 ml CBA anti-C57BL/6

antiserum and 0.1 ml rabbit complement was incubated in a total volume of 0.5 ml for 45 min at 37°C. In parallel tests the cells were incubated with normal mouse serum and complement. Both series of samples were examined in Jerne's test and the origin of the AFCs was determined by comparing the results.

EXPERIMENTAL RESULTS

The results of the experiments of series I are given in Table 1. They show that a mixture of syngeneic thymocytes and bone marrow cells produces a much greater immune response to injection of RBC than the response of each of these populations separately or their arithmetical sum. Nonsyngeneity of the irradiated recipient (CBA) was no obstacle to effective cooperation between (CBA × C57BL/6)F_1 T- and B-cells, and that fully effective survival of F_1 cells could also be demonstrated with respect to another criterion, namely repopulation of the donors' cells in the recipients' spleen. Injection of F_1 thymocytes with CBA bone marrow cells gave a much weaker immune response than injection of syngeneic combinations of thymocytes and bone marrow cells (F_1 + F_1 or CBA + CBA); nevertheless, this response was rather higher than the response of CBA bone marrow cells or F_1 thymocytes alone.

The results of the experiments of series II are shown in Fig. 1. Clearly in CBA mice receiving CP and (CBA × C57BL/6)F_1 spleen cells 3 weeks previously persistent chimerism of the lymphoid tissue was observed. This chimerism was partial in character, i.e., immunocompetent cells of donor origin and cells of recipient origin tolerant to them coexisted for a long time in the animals (both conclusions are in agreement with the writers' previous observations [3, 6]). The number of AFC of recipient origin in the chimeras did not exceed the number of AFC in the control animals (not receiving F_1 cells). Since the AFC were the direct progeny of the B-cells, this means absence of cross interaction between primed T-cells of donor origin and the recipient's B-cells under conditions when the F_1 T-cells had "freedom of choice" between syngeneic (F_1) and nonsyngeneic (CBA) B-cells.

This conclusion is confirmed by analysis of the results of the experiments of series III (Fig. 2), which differ from those of the previous series in that a state of tolerance to RBC was first induced in the recipients. In tolerance of this type, a specific deficiency of T-lymphocytes is observed in the animals, while the B-cells remain relatively intact (2, 4, 13, 15]. However, as is clear from Fig. 2, even under these specially created conditions, cross-cooperation between T-cells of donor origin and B-cells of recipient origin was absent. The large number of AFC of donor origin was evidence of effective cooperation between the T- and B-cells of the donors (F_1), but no cooperation was present between T-cells of the donors (F_1) and B-cells of the recipient (CBA).

In all three series of experiments it was thus observed that the process of cooperation between T- and B-lymphocytes was substantially worsened if these cells did not possess the identical genotype. Incompatibility between T- and B-cells and the stroma of the recipient's lymphoid organs played no significant role in these experiments.

If the results now obtained are compared with data in the literature it will be seen that most workers observed cooperation between F_1 T-lymphocytes and B-cells of the parental strain (differing in the H-2 tissue incompatibility locus [10, 14 etc.]). Meanwhile, many papers indicate that this semisyngeneic system was less effective than the completely syngeneic system [1, 11, 16]. One reason for the inconsistency of data in the literature could be that insufficient attention was paid to artefacts. In particular, the use of F_1 hybrids as recipients, when the T- or B-cells belonged to the parental strain, could be a cause either of nonspecific stimulation of the immune response (the so-called allogeneic effect), or of its inhibition (the phenomenon of allogeneic inhibition). The possibility likewise cannot be ruled out that with some combinations of strains, incompatibility for A-cells, the main source of which is the stroma of the recipient's lymphoid organs, could be reflected in the cooperation effect. Finally, in some investigations the workers concerned do no more than state that cooperation existed between nonsyngeneic cells, without attempting to make comparison with the cooperation effect of syngeneic lymphocytes. In the present investigation an attempt was made to avoid these shortcomings.

Defectiveness of interaction between nonsyngeneic T- and B-lymphocytes is explained by Katz [9, 10] in terms of genetic differences in the structure of the mediator produced by the T-lymphocytes. Katz postulates that the same gene (in the I sublocus) codes the

structure both of the mediator of the T-lymphocytes and of its receptor (stereochemically complementary) on the membrane of the B-cell.

In the present writers' view it is more logical to suppose that the similar antigenic structure of the cell membranes of the T- and B-lymphocytes acts in conjunction with their physical contact. Direct contact between T- and B-lymphocytes must, in turn, enhance the effectiveness of action of the corresponding mediator on the B-cell. Suggestions that under natural conditions the T-factor is a short-distance mediator has been put forward previously be several workers [7].

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