

COLLABORATION OF HISTOINCOMPATIBLE T AND B LYMPHOCYTES USING CELLS FROM TETRAPARENTAL BONE MARROW CHIMERAS

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Experiments have been reported suggesting that under conditions where an allogeneic effect could not be demonstrated, primed histoincompatible T and B cells did not cooperate in a humoral immune response (1, 2). By contrast semiallogeneic T and B cells were found to cooperate. The authors therefore concluded that in order to obtain efficient T-B collaboration the cells concerned must share determinants coded for by the *H-2*-gene complex, most likely the *I* region (3).

In different experiments Bechthol et al. (4, 5) demonstrated that allophenic mice, produced by the fusion of embryos of strains giving a high and low response to the synthetic antigen poly-L(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys (TGAL),¹ produced TGAL-specific antibody of low responder allotype. The authors interpreted this finding as an indication of collaboration between histoincompatible high responder T cells and low responder B cells. While this interpretation may be correct, direct evidence has not been presented to support the notion. It could be argued, for example, that in allophenic mice low responder T cells show a heightened response to TGAL. While the mechanism of such a conversion is unknown the possibility should be considered until disproven by experimental data.

It is obviously important to know whether the presence of certain determinants on allogeneic T and B cells prevent T-B collaboration under all circumstances, or whether this is only so when the cells are not mutually tolerant. We have studied the collaboration of histoincompatible T and B lymphocytes in response to sheep erythrocytes (SRBC) in an adoptive transfer system using primed T cells from tetraparental mice and primed B cells from both normal and chimeric mice. In previous reports we have shown that T cells from tetraparental bone marrow chimeras (TBMC) are specifically unresponsive to the determinants of the *H-2* complex of the host in the mixed leukocyte reaction (MLR) as well as in cell-mediated lympholysis (CML) (6, 7). The data presented here suggests that primed T cells are able to cooperate with allogeneic B cells to which they have been tolerized in a chimeric environment.

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¹ Abbreviations used in this paper: CML, cell-mediated lympholysis; LN, lymph node; MLR, mixed leukocyte reaction; PFC, plaque-forming cell; TBMC, tetraparental bone marrow chimeras; TDL, thoracic duct lymphocytes; TGAL, poly-L(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys.

Materials and Methods

Mice. (CBA/J × DBA-2/J) F_1 and (C3H/J × SJL) F_1 hybrids, as well as the corresponding parental strains were used.

Cells. Bone marrow, spleen, lymph node (LN), and thoracic duct lymphocytes (TDL) were prepared as described previously (6).

Immunization. Mice were given a single intraperitoneal injection of 0.1 ml of 25% SRBC in saline and used 1–3 mo later.

Anti-H-2 and Anti- θ (Thy 1^b) Sera. The sera were prepared by cross-immunizing mice as described previously (references 6 and 8, respectively).

TBMC. TBMC were obtained by injecting lethally irradiated (950 R) F_1 hybrids with 10^7 anti- θ -treated bone marrow cells from both parental strains (described in detail in reference 6).

Preparation of CBA Helper T Cells from TBMC. TBMC were given an intraperitoneal injection of 0.1 ml of 25% SRBC suspension 4 mo after irradiation and injection with bone marrow cells. 1–2 mo after immunization suspensions of TDL, spleen, and LN cells were prepared and Ig-positive lymphocytes removed either by passage through a column of Sephadex G100 to which anti-immunoglobulin antibodies had been covalently bound (modified from Schlossman and Hudson, reference 9) or by passage through nylon wool columns (10). In some experiments the filtered suspension containing lymphocytes of both parental strains was treated with anti-H-2 sera to obtain helper T cells of a single H-2 type. The damaged cells were removed by a simple filtration method (11) and the proportion of the remaining viable cells carrying alloantigens of the other parental strain was determined by a cytotoxicity test.

Preparation of B Cells Primed to SRBC. 1–4 mo after priming with SRBC, spleens were removed and cell suspensions prepared. The suspensions were treated with anti- θ serum plus complement (C) as described elsewhere (8).

Adoptive Transfer System for a Secondary Plaque-Forming Cell (PFC) Response against SRBC. F_1 recipient mice were X irradiated (850 R). The mice were injected intravenously with a mixture of primed B cells and varying numbers of primed T cells from TBMC, and other recipients were injected with the separate cell populations. In control experiments X-irradiated parental strain mice received unfractionated spleen cells from mice which served as donors for the B cells. At the same time, each mouse in the experimental and control groups received an intravenous injection of 5×10^8 SRBC. 7 days after cell transfer, the spleens were taken out and the number of direct (19S) and indirect (7S) PFC determined.

Assay for PFC. PFC were detected using the technique described by Cunningham and Szenberg (12). Indirect (7S) PFC were developed by the addition of rabbit antimouse-Ig serum to the cell mixture. This antiserum, at the concentration used, did not suppress the PFC response (mainly IgM) of mice primed for 4 days with SRBC. Indirect PFC were calculated by subtracting the number of PFC obtained in the absence of developing serum from the numbers obtained after addition of the serum.

Treatment of PFC with Anti-H-2 Sera. Spleen cell suspensions were incubated with anti-H-2 sera or normal mouse serum for 30 min at 4°C, washed once, and then incubated with C for 30 min at 37°C. After further washing, the suspension was tested in the plaque assay.

Results

Lymphoid Cell Chimerism of TBMC used as Donors of Helper T Cells. For the first set of experiments a total of 8 TBMC were used; these were prepared by injecting (CBA/J × DBA-2/J) F_1 hybrids with bone marrow cells from both parental strains. Each mouse was tested for lymphoid cell chimerism using either TDL or LN cells from which the damaged cells had been removed. Each mouse showed a lymphoid cell chimerism corresponding to roughly equal proportions of CBA and DBA/2 lymphocytes (Table I). As with previous experiments (6) no significant number of lymphocytes of host origin were detected.

Purity of CBA T Cells Prepared from TBMC. Mixtures of TDL and LN cells prepared from TBMC originally contained approximately 30% of immunoglobu-

TABLE I
Lymphoid Cell Chimerism in TBMC used as Donors for Helper T Cells

Cells	Dead cells after incubation with:			
	Anti-CBA + C	Anti-DBA + C	Saline + C	Anti-CBA + anti-DBA + C
	%	%	%	%
CBA LN	100	11	15	99
DBA LN	12	99	14	95
F ₁ (CBA × DBA) LN	97	99	11	98
Chimera 1 TDL	55	50	0	99
Chimera 2 LN	49	56	15	99
Chimera 3 LN	70	36	20	100
Chimera 4 LN	67	40	22	98
Chimera 5 LN	49	56	11	ND*
Chimera 6 LN	49	63	14	ND
Chimera 7 LN	62	50	18	ND
Chimera 8 TDL	54	48	9	ND

* ND, not determined.

TABLE II
Purity of CBA T Cells Prepared from TBMC

Exp.	Cells	Ig-positive cells		Dead* CBA cells after incubation with:		
		Original population	Anti-Ig column effluent population	Saline + C'	Anti DBA + C'	Anti CBA + C'
		%	%	%	%	%
1	LN + TDL	31	0.8	12	11	100
2		27	0.4	14	15	100

* After anti-Ig column filtration the CBA/DBA/2 cell mixture was incubated with anti-DBA/2 plus C and damaged cells were removed. The number of CBA cells in the remaining suspension was then estimated as shown.

lin-bearing (B) lymphocytes. This proportion was reduced to less than 1% after anti-Ig column filtration in two separate experiments (Table II). The column-filtered cells were then treated with an anti-DBA/2 serum and C. After filtration through cotton wool under special conditions to remove the damaged cells (11), up to 100% of the remaining cells could be killed with an anti-CBA serum plus C, showing that a suspension of T cells had been obtained, the vast majority of which carried CBA alloantigens.

The Helper Effect of Primed CBA T Cells from TBMC for Primed Syngeneic or Allogeneic B Cells. In a preliminary experiment, 10^7 anti- θ -treated CBA spleen cells primed to SRBC were transferred together with varying numbers of primed anti-Ig column-passed LN cells from CBA into lethally irradiated syngeneic recipients. At the same time the mice were given an intraperitoneal injection of SRBC and the PFC response measured 7 days after cell transfer.

This experiment was carried out to determine the minimum number of T cells required to give a detectable helper effect. As shown in Fig. 1, 5×10^5 helper cells increased the number of direct and indirect PFC, and a 10-fold increase in PFC was obtained with $1-2 \times 10^6$ helper cells. Transfer of 2×10^6 CBA T cells alone, produced no increase in the background PFC observed in X-irradiated mice not receiving B cells (<150 PFC/spleen). Transfer of 10^7 primed unfractionated CBA spleen cells produced 40,000 IgG PFC, suggesting that in the titration experiments T cells were the limiting cell population.

SRBC-primed CBA T cells derived from TBMC, and therefore tolerant to DBA/2 alloantigens (6), were transferred with primed CBA or DBA/2 anti- θ -plus C-treated spleen cells into lethally irradiated (CBA \times DBA/2) F_1 hybrids. These CBA T cells augmented the IgG PFC response of the primed syngeneic CBA or allogeneic DBA/2 B cells: with 2×10^6 helper CBA T cells a 10-fold increase in IgG PFC was observed with both syngeneic and allogeneic anti- θ -treated primed spleen cells (Fig. 2).

When 10^7 primed CBA or DBA/2 spleen cells from the donors used for the T-B collaboration experiments were transferred to syngeneic CBA or DBA/2 recipients, a response of 35,000 IgG PFC was observed with CBA spleen cells and a response of 60,000 IgG PFC with DBA/2 spleen cells. The titration experiment using CBA T cells from TBMC therefore suggests that those helper cells, tolerant to *H-2* determinants of DBA/2, could effectively augment the secondary response of primed syngeneic CBA or allogeneic DBA/2 cells. Moreover, the data suggest that a similar number of helper T cells was required to achieve a 10-fold "background" response with either syngeneic or allogeneic anti- θ -treated primed cells.

This finding was confirmed in a further experiment where only one dose of CBA helper T cells from TBMC was tested (Table III). In this experiment, CBA anti- θ -treated SRBC-primed spleen plus 2×10^6 T cells gave a higher absolute response than DBA/2 cells plus T cells in contrast to the preceding experiment.

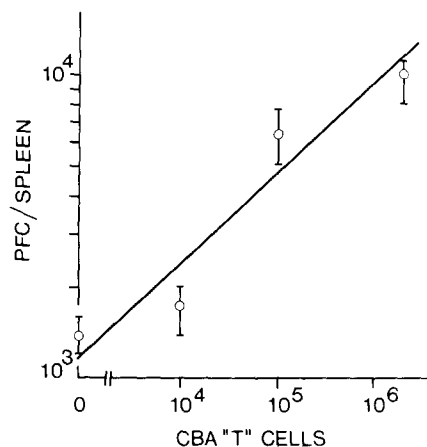


FIG. 1. The helper effect of SRBC-primed CBA T cells from LN with 10^7 syngeneic SRBC-primed anti- θ -treated spleen cells in an adoptive transfer system. The geometric mean and upper and lower limits of PFC found in five recipients per experimental group is given.

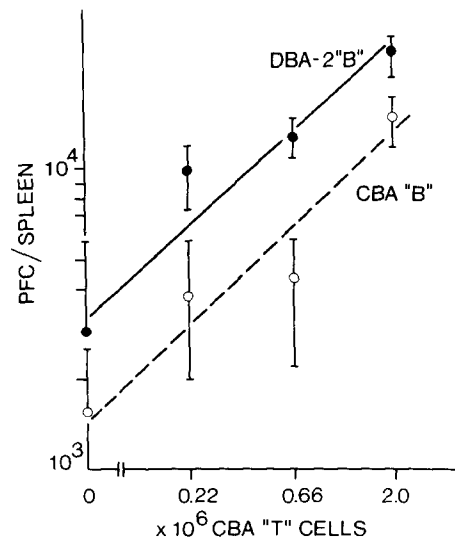


FIG. 2. The helper effect of SRBC-primed CBA T cells obtained from TDL and LN of TBMC with 10^7 syngeneic (○) or allogeneic (●) SRBC-primed anti- θ -treated spleen cells transferred into X-irradiated (CBA \times DBA) F_1 recipients. The geometric mean and upper and lower limits of IgG PFC found in four to five recipients per experimental group is given.

TABLE III
Helper Effect of SRBC-Primed CBA T Cells from TBMC for Syngeneic and Allogeneic-Primed B Cells

Cells transferred	IgG plaques
2×10^6 CBA T cells from TBMC	964 (1,292-719)*
10^7 CBA B cells	2,668 (3,941-1,8096)
10^7 DBA B cells	907 (1,146-717)
2×10^6 CBA T _{TBMC} + 10^7 CBA B	35,107 (44,679-27,585)
2×10^6 CBA T _{TBMC} + 10^7 DBA B	17,763 (18,977-16,627)

* Geometric mean; upper and lower limits are given in parentheses.

Normal primed spleen cells were not tested in this experiment. It is again apparent that CBA helper T cells from TBMC significantly increased the PFC response of both syngeneic and allogeneic anti- θ -treated spleen cells.

The Helper Effect of T Cells from TBMC on a Mixed Population of B Cells from TBMC. To test whether T cells when transferred with a mixture of syngeneic and allogeneic B cells would preferentially help syngeneic B cells the following experiments were conducted: TBMC were prepared by injecting (C3H \times SJL) F_1 hybrids with bone marrow cells from both parental strains. After 2 mo the mice were primed with SRBC and 1 mo later T and B cells were prepared. One group of X-irradiated (750 R) (C3H \times SJL) F_1 recipients received 2×10^6 T cells from TBMC together with 6×10^6 B cells from TBMC. (Control groups received either T or B cells alone.) A second group of recipients received 2×10^6 C3H T cells from TBMC, prepared by treating the TBMC with anti-SJL serum

(45% lysis), together with 6×10^6 C3H and SJL B cells from TBMC. 7 days after priming with SRBC, PFC were determined and their origin was tested with anti-C3H and anti-SJL sera as described in the Materials and Methods. It would be expected that if the C3H T cells cooperated more efficiently with syngeneic than with allogeneic B cells then the proportion of C3H PFC would be higher in the recipients receiving C3H helper T cells compared with those recipients receiving a mixed population of C3H and SJL helper T cells. As shown in Table IV this was not observed. 2×10^6 T cells, consisting of approximately equal proportions of C3H and SJL T cells, had a similar helper effect as 2×10^6 C3H T cells, obtained after treatment of the cells with anti-SJL serum. In addition, irrespective of whether a mixture of C3H and SJL T cells or C3H T cells alone were injected, approximately 30% of the PFC were C3H and 70% of SJL origin. This suggests that under conditions where mutually tolerant populations of T and B cells are used the T cells can help the allogeneic B cells as efficiently as syngeneic B cells.

Discussion

TBMC seem to provide an experimental model which allows a detailed study of the regulation of the immune response. For this purpose it is important to know whether T and B cells from different strains will cooperate in TBMC since regulatory influences of T cells may alter the immune response pattern of B cells. The design of the experiments reported here is in many aspects different from that used by Katz and his colleagues (1-3). We have used not only a different protocol for the preparation of tolerant T cells but also different mouse strains and antigens. In our system T cells were generated in vivo in a histoincompatible environment, and so were specifically tolerant to H-2 determinants of the allogeneic B cells with which they were transferred in the adoptive

TABLE IV
Helper Effect of SRBC-Primed T Cells from TBMC for a Mixture of Allogeneic and Syngeneic B Cells from TBMC

Cells injected		Proportion of T cells killed by:		PFC IgG	Proportion of:	
T cells	B cells	α C3H serum	α SJL serum		C3H-PFC	SJL-PFC
		%	%		%	%
2×10^6	6×10^6	58	45	3,124 (3,845-2,539)	29.8	64.0
2×10^6	—	58	45	36 (54-24)	ND	ND
—	6×10^6	—	—	305 (373-244)	ND	ND
2×10^6 anti-SJL treated	6×10^6	98	—	3,044 (3,939-2,352)	30.4	70.1

All cells were from TBMC. The proportion of C3H and SJL PFC was determined as described in the Materials and Methods: 4 samples of a suspension containing PFC were treated either with normal mouse serum, anti-C3H, anti-SJL, or both antisera together and C. Numbers obtained after treatment with normal mouse serum and C were 100%. Both antisera together killed more than 95% of all PFC.

transfer experiments. In some experiments the B cells were also derived from tolerant donors. In addition, the T cells had been primed to SRBC in the presence of allogeneic T and B cells.

There are other experiments by Toivanen and Toivanen (13) and by Kindred (14) demonstrating the failure of effective T-B collaboration in either chickens reconstituted with allogeneic bursa cells or mice grafted with allogeneic thymus cells. These experiments differ from the experiments described here since the reconstitution was in both instances performed in animals in which the development of the hemopoietic system was well advanced. In the present experiments stem cell-rich allogeneic bone marrow cells were transferred into lethally irradiated hosts, thus allowing the stem cells of both populations to differentiate into mature lymphocytes at the same time. It might be argued that T cells can only cooperate with allogeneic B cells if they are fully tolerant to those B cells, or when the T cells recognizing determinants on allogeneic B cells are absent.

While the effect of the circumstances mentioned above on the generation of helper T cells is unknown, the results presented in this communication are compatible with conclusions drawn by Bechthol et al. (4, 5) from experiments in allophenic mice. These authors concluded that collaboration between high responder T cells and histoincompatible low responder B cells could occur in these mice.

It might be argued that the collaboration across *H-2* barriers reported here and postulated by Bechthol et al. (4, 5) in allophenic mice is due to an allogeneic effect. However, this explanation seems unlikely for three reasons: (a) Bechthol et al. (4, 5) could not demonstrate an allogeneic effect in allophenic mice produced by embryo fusion of two histoincompatible low responder strains, i.e., in this situation no elevated levels of TGAL antibody compared to the low responder strains could be demonstrated (5); (b) in contrast to a report by Phillips et al. (15), we could not demonstrate elevated "background" of thymidine incorporation by thymus and LN cells in vitro as an indication of allogeneic stimulation in vivo. To the contrary, our results showed a specific deletion of MLR as well as CML-reactive cells in TBMC and no evidence was obtained for either suppressor cells or soluble blocking factors (6), and (c) the same number of helper T cells were required for a significant helper effect with syngeneic as well as allogeneic B cells.

Although the data presented here clearly indicate that allogeneic T-B collaboration can occur as readily as syngeneic collaboration in this system, we cannot, as yet, explain the conflict of our findings with those published by Katz et al. (1-3). One could speculate that either allogeneic T or anti- θ -treated spleen cell populations may recognize each other and as a consequence produce some factors interfering with T-B collaboration. This may depend on the cell numbers used, the degree of removal of T cells by anti- θ serum, and the method used to inactivate the T cells in their response to the allogeneic B cells. It seems that all these complicating factors are eliminated in an experimental situation where both allogeneic T and B cells are mutually tolerant. In this situation T helper cells do not seem to have a preference for syngeneic B cells as compared to allogeneic B cells. Since allogeneic T and B cells in TBMC retain their surface *H-2* antigens and their ability to stimulate in MLR, it is possible to justify the

argument that the differing surface antigens on allogeneic T and B cells do not alone inhibit T-B collaboration. If our speculation is correct one can postulate that some other determinants involved in T-B collaboration must be easily recognized by allogeneic nontolerant cells.

Summary

T-B collaboration has been studied in a secondary response to sheep erythrocytes using either syngeneic or allogeneic T- and B-cell combinations. T cells prepared from tetraparental bone marrow chimeras (TBMC), carrying H-2 determinants of one parental strain only, cooperated with syngeneic, as well as with allogeneic B cells carrying the alloantigens to which the T cells had been tolerized in the chimeric environment. When TBMC-derived cells of a single H-2 specificity were transferred with a mixture of TBMC-derived B cells of both H-2 types of the parental strains, no preference for syngeneic cooperation was found.

The data therefore suggest that the presence of differing H-2-complex determinants on the allogeneic T- and B-cell populations of the two different strain combinations tested do not interfere with T-B collaboration when the cell populations studied are mutually tolerant.

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