Brief Definitive Report

RESTRICTED HELPER FUNCTION OF F_1 → PARENT BONE MARROW CHIMERAS CONTROLLED BY *K*-END OF *H-2* COMPLEX

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Although the question of whether T and B lymphocytes collaborate across major histocompatibility complex (MHC) barriers remains controversial (1-5), there is general agreement that F_1 hybrid T cells collaborate with parental strain B cells. This paper presents an exception to this rule. It will be shown that F_1 T cells differentiating from stem cells in mice of one parental strain collaborate well with B cells from this strain, but lose their capacity to stimulate B cells of the opposite strain.

Materials and Methods

Mice. CBA/Cum (CBA, *H-2*^{k}), C57BL/6 (B6 *H-2*^{b}), and (CBA × B6)F_1 mice were obtained from Cumberland View Farms, Clinton, Tenn. C57BL/10 (B10, *H-2*^{b}) and B10.Br (*H-2*^{k}) mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. (B10 × B10.Br)F_1 mice were bred in our laboratory. B10.A (4R) mice were a gift from Dr. W. L. Elkins, University of Pennsylvania.

Chimeras. Split-dose irradiation was used to prepare the chimeras. CBA and B6 mice were exposed to 600 rads (6), left for 2 wk, and then given 850 rads. 4 h later the mice received an intravenous injection of 3×10^7 (CBA × B6)F_1 bone marrow cells treated with anti-thy 1.2 antiserum plus complement (3).

Assay for T-B Collaboration. As described in detail elsewhere (6), T cells (0.8×10^6) and B cells ($5\text{-}8 \times 10^6$ anti-thy 1.2-treated spleen cells from mice primed with sheep erythrocytes [SRC] 2 mo before) were transferred with SRC (0.1 ml of 5% solution) into irradiated (750 rads) (CBA × B6)F_1 mice. Direct (IgM) and indirect (IgG) plaque-forming cells (PFC) were then measured in the spleen 7 days later.

Results

Cytotoxic indices with CBA anti-B6 and B6 anti-CBA alloantisera plus complement (6) showed that, for both (CBA × B6)F_1 marrow → irradiated CBA chimeras (F_1 → CBA chimeras) and F_1 → B6 chimeras, >97% of spleen and lymph node (LN) cells from the chimers were of donor F_1 origin. This applied to 10 of 10 chimeras tested 3-12 mo after marrow reconstitution.

To test the helper function of the chimeras, unprimed T cells prepared from LN were first activated to SRC in irradiated (CBA × B6)F_1 mice; this was to ensure that the first exposure of the F_1 T cells to antigen was in a "normal", i.e. F_1, environment. 4×10^7 nylon-wool-purified LN T cells (>90% thy 1.2-positive) (7) from F_1 → CBA chimeras were transferred intravenously with SRC (0.5 ml

* Supported by grants AI-10961 and CA-15822 from the U. S. Public Health Service.

TABLE I

Restricted Helper Function of (CBA × B6)F_1 → CBA Bone Marrow Chimeras Linked to H-2 Complex

T-cell group	Donor of helper T cells*	B cells‡	Anti-SRC PFC/spleen at 7 days in irradiated (CBA × B6)F_1 mice	
			IgM	IgG
1	F_1 → CBA chimeras	B10.Br	62,120 (1.11)§	123,250 (1.29)
		B10	0	0
		(B10 × B10.Br)F_1	75,600 (1.16)	200,940 (1.05)
2	Normal (CBA × B6)F_1	B10.Br	47,290 (1.10)	126,000 (1.29)
		B10	35,540 (1.38)	70,750 (1.24)
		(B10 × B10.Br)F_1	95,250 (1.38)	226,120 (1.24)
Groups 1 + 2 (0.8×10^6 of each)		B10	29,250 (1.21)	64,940 (1.08)

* Unprimed T cells pooled from five chimeras reconstituted with marrow 1 yr previously were activated to SRC in irradiated normal (CBA × B6)F_1 mice before use as helper cells (see text); activated helper cells were recovered from thoracic duct lymph of the recipients at 5 days post-transfer.

‡ Anti-thy 1.2-serum-treated spleen cells from SRC-primed mice were transferred intravenously in a dose of 5×10^6 viable cells (8×10^6 for B10 B cells) with T cells (0.8×10^6) and SRC (0.1 ml of 5%) into irradiated (750 rads 1 day before) (CBA × B6)F_1 mice.

§ Geometric mean of data from four mice per group. Values in parentheses refer to the numbers by which the means are multiplied or divided to give upper and lower limits, respectively, of SE. Background values obtained when B cells were transferred without T cells have been subtracted. These values (PFC/spleen) were: B10.Br 440(1.48) (IgM), 1,630(1.05) (IgG); B10 670(1.24) (IgM), 980(1.27) (IgG); (B10 × B10.Br)F_1 2,580(1.30) (IgM), 4,670(1.79) (IgG). Numbers of PFC when T cells were transferred without B cells were < 100 PFC/spleen.

of 25% solution) into irradiated (800 rads 1 day before) normal (CBA × B6)F_1 mice; control groups of these mice received T cells from normal (CBA × B6)F_1 mice plus SRC. Donor cells were recovered from thoracic duct lymph of both groups of recipients 5 days later (6).

As shown in Table I, SRC-activated (CBA × B6)F_1 T cells derived from F_1 → CBA chimeras gave high IgM and IgG anti-SRC PFC responses with B10.Br (*H-2*^{k}) B cells, but gave no response with B10 (*H-2*^{b}) B cells. This did not seem to be the result of suppression, since a mixture of chimera F_1 T cells and normal F_1 T cells gave good responses with B10 B cells. Both groups of T cells collaborated well with (B10 × B10.Br)F_1 B cells.

Table II shows that the restriction in helper function was reversed when (CBA × B6)F_1 T cells were derived from F_1 → B6 chimeras, i.e. good collaboration occurred with B10 B cells, whereas only a poor response was seen with B10.Br B cells (the latter response was significant but represented <8% of the response observed when B10.Br B cells were transferred with F_1 → CBA chimera T cells). The restriction mapped to the *K* end of the *H-2* complex since B cells from B10.A(4R) mice (*K*^{k}*I-A*^{k}*I-B*^{b}- - -*D*^{b}) were stimulated by T cells from F_1 → CBA chimeras, but not by T cells from F_1 → B6 chimeras.

Discussion

Previous work has shown that although homozygous T cells from normal (nonchimeric mice) fail to collaborate with *H-2*-different B cells in vivo (2, 5), T cells taken from tetraparental bone marrow chimeras stimulate B cells derived from either of the two parental strains involved (3). To explain this discrepancy Katz et al. (2) suggested that T cells differentiating from stem cells in an *H-2*-different environment develop abnormal "cell-interaction determinants", enabling these cells to stimulate B cells of the opposite parental strain. This "adaptive differentiation" hypothesis has recently been refined by Zinkernagel

TABLE II

Helper Function of T Cells from $F_1 \rightarrow$ CBA and $F_1 \rightarrow$ B6 Chimeras Controlled by K-End of H-2 Complex

T-cell group	Donor of helper T cells*	B cells‡	*H-2* region of B cells				Anti-SRC PFC/spleen at 7 days in irradiated (CBA × B6)F_1 mice	
			K	*I-A*	*I-B*	*D*	IgM	IgG
1	$F_1 \rightarrow$ CBA chimeras	B10.Br	k	k	k	k	79,090 (1.04)§	134,300 (1.12)
		B10	b	b	b	b	470 (2.50)‖	200 (1.97)‖
		B10.A(4R)	k	k	b	b	25,260 (1.10)	55,605 (1.15)
2	$F_1 \rightarrow$ B6 chimeras	B10.Br	k	k	k	k	2,970 (1.07)	9,870 (1.21)
		B10	b	b	b	b	32,030 (1.11)	50,520 (1.18)
		B10.A(4R)	k	k	b	b	0	0
3	Normal (CBA × B6)F_1	B10.Br	k	k	k	k	53,310 (1.32)	126,500 (1.33)
		B10	b	b	b	b	27,720 (1.20)	42,530 (1.23)
		B10.A(4R)	k	k	b	b	10,770 (1.09)	42,320 (1.23)
Groups 1 + 2		B10.Br	k	k	k	k	86,400 (1.28)	134,610 (1.26)
Groups 1 + 2		B10	b	b	b	b	31,060 (1.22)	46,250 (1.40)

* Unprimed T cells pooled from three chimeras per group activated to SRC for 5 days in irradiated (CBA × B6)F_1 mice as for Table I The donor $F_1 \rightarrow$ CBA chimeras and $F_1 \rightarrow$ B6 chimeras were reconstituted with marrow 1 yr and 3 mo previously, respectively.

‡ As for Table I.

§ As for Table I. Background numbers of PFC obtained when T cells were transferred without T cells were: B10.Br 1,810(1.29) (IgM), 10,320(1.03) (IgG); B10 950(1.13) (IgM), 1,330(1.35) (IgG); B10.A(4R) 1,140(1.69) (IgM), 1,190(1.18) (IgG). PFC numbers for T cells transferred without T cells all < 200 PFC/spleen.

‖ Not significantly above values of B cells transferred without T cells ($P > 0.05$).

et al. (8, 9). These workers observed that for T-cell-mediated lympholysis (CML) of virus-infected target cells, F_1 T cells from $(a \times b)F_1 \rightarrow a$ chimeras lysed target cells from strain a and $(a \times b)F_1$, but did not lyse strain b targets. From this and other evidence it was concluded that CML occurred only with targets which shared *H-2* determinants with the thymus in which the T cells differentiated from stem cells.

The data in the present paper are consistent with this hypothesis and suggest that the thymus controls the specificity of not only T cells responsible for CML, but also of T helper cells involved inT-B collaboration. It should be mentioned that although there is clear evidence that the thymus per se rather than other microenvironments controls T-cell specificity for CML (9), this has yet to be proved for T-helper function.

Recent studies in this laboratory have suggested that T cells from normal $(a \times b)F_1$ mice behave functionally as a 50:50 mixture of (mutually tolerant) T cells derived from the two parental strains; each subgroup of T cells appears to be able to collaborate with B cells derived from only one of the two parental strains (6, 10). By analogy with the data of Zinkernagel et al., one can suggest that these two subgroups of T helper cells are generated as the result of their stem cell precursors encountering *H-2* determinants of both strain a and strain b on thymic epithelial cells during early differentiation. The progeny of these T-cell precursors then collaborate in a restricted fashion with B cells of strain a and b, respectively. A prediction from this notion which is confirmed in the present paper, is that when $(a \times b)F_1$ T cells differentiate from stem cells in strain a mice, only one of the two subgroups of T cells is generated, namely the subgroup able to collaborate with B cells from strain a.

A further prediction is that homozygous T cells of strain a differentiating from stem cells in $(a \times b)F_1$ mice should resemble normal $(a \times b)F_1$ T cells in

function. One subgroup of cells should collaborate with syngeneic (strain *a*) B cells, but not with allogeneic (strain *b*) B cells; the other subgroup should stimulate only allogeneic and not syngeneic B cells. Preliminary studies on the helper function of parent → F_1 chimera T cells activated to SRC in irradiated parental strain mice support this prediction (J. Sprent, unpublished data).

Summary

F_1 → parent bone marrow chimeras were prepared by transferring F_1 hybrid marrow cells into heavily irradiated parental strain mice. When unprimed, donor-derived F_1 T cells from the chimeras were activated to sheep erythrocytes (SRC) for 5 days in irradiated normal F_1 mice, high IgM and IgG anti-SRC responses were observed with F_1 B cells, and with B cells *H-2*-compatible with the strain in which the T cells were raised from stem cells. Significantly, however, responses with B cells of the opposite parental strain were either absent or very low. The restriction in T-helper function mapped to the *K*-end of the *H-2* complex and could not be attributed to active suppression.

Stimulating discussion with D. B. Wilson and the skillful typing of Miss K. Nowell are gratefully acknowledged.

Received for publication 27 February 1978.

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