RESTRICTED HELPER FUNCTION OF F₁ → 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 F₁ hybrid T cells collaborate with parental
strain B cells. This paper presents an exception to this rule. It will be shown
that F₁ 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-2k), C57BL/6 (B6 H-2b), and (CBA × B6)F₁ mice were obtained
from Cumberland View Farms, Clinton, Tenn. C57BL/10 (B10, H-2b) and B10.Br (H-2k) mice
were obtained from The Jackson Laboratory, Bar Harbor, Maine. (B10 × B10.Br)F₁ 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 x 10⁷ (CBA × B6)F₁ 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 x 10⁶) and B
cells (5-8 x 10⁶ 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)F₁ 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)F₁ marrow → irradiated CBA
chimeras (F₁ → CBA chimeras) and F₁ → B6 chimeras, >97% of spleen and
lymph node (LN) cells from the chimeras were of donor F₁ 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)F₁ mice; this was to
ensure that the first exposure of the F₁ T cells to antigen was in a "normal", i.e.
F₁, environment. 4 x 10⁷ nylon-wool-purified LN T cells (>90% thy 1.2-positive)
(7) from F₁ → CBA chimeras were transferred intravenously with SRC (0.5 ml

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of 25% solution) into irradiated (800 rads 1 day before) normal (CBA × B6)F₁ mice; control groups of these mice received T cells from normal (CBA × B6)F₁ 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)F₁ T cells derived from F₁ → CBA chimeras gave high IgM and IgG anti-SRC PFC responses with B10.Br (H-2k) B cells, but gave no response with B10 (H-2b) B cells. This did not seem to be the result of suppression, since a mixture of chimera F₁ T cells and normal F₁ T cells gave good responses with B10 B cells. Both groups of T cells collaborated well with (B10 × B10.Br)F₁ B cells.

Table II shows that the restriction in helper function was reversed when (CBA × B6)F₁ T cells were derived from F₁ → 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 F₁ → CBA chimera T cells). The restriction mapped to the K end of the H-2 complex since B cells from B10.A(4R) mice (KkI-A⁺I⁻B⁺⁻⁻⁻D⁺) were stimulated by T cells from F₁ → CBA chimeras, but not by T cells from F₁ → 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 F₁ → CBA and F₁ → 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)F₁ mice |
|--------------|--------------------------|---------|-----------------------|-------------------------------------------------------------|
|              |                          |         |                       | K | I-A | I-B | D | IgM  | IgG  |
| 1            | F₁ → CBA chimeras        | B10.Br  | k k k k k k k k k k k k | 79,090 (1.04) | 134,300 (1.12) |
|              |                          | B10     | b b b b b b b b b b b | 47 (2.50)  | 296 (1.97)  |
|              |                          | B10.A4R  | k k k k k k k k k k k | 25,280 (1.10) | 55,605 (1.15) |
| 2            | F₁ → B6 chimeras         | B10.Br  | k k k k k k k k k k k | 2,970 (1.07) | 9,870 (1.21)  |
|              |                          | B10     | b b b b b b b b b b b | 32,030 (1.11) | 50,520 (1.18) |
|              |                          | B10.A4R  | k k k k k k k k k k k | 0 | 0 |
| 3            | Normal (CBA × B6)F₁      | B10.Br  | k k k k k k k k k k k | 53,310 (1.32) | 126,500 (1.33) |
|              |                          | B10     | b b b b b b b b b b b | 27,720 (1.23) | 42,530 (1.23)  |
|              |                          | B10.A4R  | k k k k k k k k k k k | 10,770 (1.09) | 42,320 (1.23)  |
| Groups 1 + 2 |                          | B10.Br  | k k k k k k k k k k k | 86,400 (1.28) | 124,610 (1.36) |
| Groups 1 + 2 |                          | B10     | b b b b b b b 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)F₁ mice as for Table 1. The donor F₁ → CBA chimeras and F₁ → B6 chimeras were reconstituted with marrow 1 yr and 3 mo previously, respectively.
† As for Table 1.
§ 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.A4R 1,140 (1.19) IgM, 1,190 (1.18) IgG. PFC numbers for T cells transferred without T cells all < 200 PFC/spleen.

et al. (8, 9). These workers observed that for T-cell-mediated lympholysis (CML) of virus-infected target cells, F₁ T cells from (a × b)F₁ → a chimeras lysed target cells from strain a and (a × b)F₁, 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 in T-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 × b)F₁ 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 × b)F₁ 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 × b)F₁ mice should resemble normal (a × b)F₁ 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 → F₁ chimera T cells activated to SRC in irradiated parental strain mice support this prediction (J. Sprent, unpublished data).

Summary

F₁ → parent bone marrow chimeras were prepared by transferring F₁ hybrid marrow cells into heavily irradiated parental strain mice. When unprimed, donor-derived F₁ T cells from the chimeras were activated to sheep erythrocytes (SRC) for 5 days in irradiated normal F₁ mice, high IgM and IgG anti-SRC responses were observed with F₁ 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.

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