T-HELPER FUNCTION OF PARENT \( \rightarrow \) F\(_1\) CHIMERAS

Presence of a Separate T-Cell Subgroup Able to
Stimulate Allogeneic B Cells but not Syngeneic B Cells

BY J. SPRENT AND H. VON BOEHMER

From the Immunology Unit, Department of Pathology, University of Pennsylvania School of Medicine and
The Wistar Institute, Philadelphia, Pennsylvania 19104, and the Basel Institute for Immunology, 487
Grenzacherstrasse, CH4058 Basel, Switzerland

Previous work demonstrated that T cells from irradiated F\(_1\) hybrid mice reconstituted with parental strain marrow cells (parent \( \rightarrow \) F\(_1\) chimeras) showed apparent unrestricted T-helper function in vivo (1). Thus, T cells from these mice collaborated as effectively with H-2-different B cells of the opposite parental strain as with syngeneic B cells. Such findings provided a sharp contrast to the marked H-2 restriction reported by Katz et al. (2) and ourselves (3) for nonchimeric T cells.

To reconcile these differing observations, it was suggested that homozygous T cells differentiating in a heterozygous environment undergo a process of adaptive differentiation which enables the cells to interact with B cells of the opposite parental strain (1, 4). Zinkernagel et al. (5, 6) have refined this theory by proposing that, at least for lysis of virus-infected target cells, adaptive differentiation is controlled at the level of the thymus. These workers conclude that the capacity for T cells to interact with target cells bearing a particular set of H-2 determinants depends upon the T cells encountering these determinants in the thymus during early differentiation.

Recently it has become apparent that, as for T-killer cells, T-helper cells in F\(_1\) hybrid mice consist of two separate subgroups of T cells, each restricted to H-2 determinants of only one parental strain (7-9). According to Zinkernagel et al. (5), these T-cell subgroups are formed as the result of intrathymic exposure of stem cells to each set of parental-strain H-2 determinants during ontogeny. For T-killer cells, two subgroups of T cells have also been found in parent \( \rightarrow \) F\(_1\) chimeras, i.e., where differentiation of homozygous stem cells is driven by a heterozygous thymus (5). Whether such a dichotomy also applies to T-helper cells in these mice has yet to be studied. If this were the case, the apparent unrestricted T-helper function of parent \( \rightarrow \) F\(_1\) chimera T cells could thus be explained as reflecting the combined action of two subgroups of H-2-restricted T cells, each able to interact with B cells of only one parental strain.

The present paper demonstrates that homozygous T cells from unprimed parent \( \rightarrow \) F\(_1\) chimeras do indeed contain two discrete subgroups of T-helper cells, each restricted to K-end H-2 determinants of one of the two parental strains.

Materials and Methods

Mice. CBA/Cum (CBA) (H-2\(^b\)), C57BL/6 Cum (B6) (H-2\(^b\)), and (CBA X B6)F\(_1\) mice were obtained from Cumberland View Farms, Clinton, Tenn. B10.BR (H-2\(^b\)) and C57BL/10
(B10) (H-2\(^b\)) mice were obtained from The Jackson Laboratories, Bar Harbor, Maine. (B10.BR
T-HELPER FUNCTION OF PARENT → F1 CHIMERAS

× B10)F1 mice were bred in our colony at the University of Pennsylvania. B10.A(4R) mice were a gift of Dr. W. L. Elkins, University of Pennsylvania.

Media. RPMI-1640 (Microbiological Associates, Walkersville, Md.) supplemented with 10% fetal calf serum was used.

Injections. All suspensions of lymphoid cells and sheep erythrocytes (SRC) were given intravenously.

Cells. Thoracic duct lymphocytes were obtained as described elsewhere (10). Suspensions of lymph node (LN) cells were prepared by teasing the organs with fine forceps through an 80-mesh stainless steel sieve in cold medium.

Irradiation. Mice were exposed to 106 R by 137Cs γ-irradiation at a dose rate of 106 rads/min.

Antisera. Anti-Thy 1.2 antiserum, and CBA anti-B6 and B6 anti-CBA H-2 alloantisera were prepared as described elsewhere (8).

Preparation of Chimeras. (CBA × B6)F1 mice 10-14 wk old were given sublethal irradiation (700 rads) and then, 4-6 h later, an intravenous injection of 2 × 10⁷ CBA or B6 marrow cells; marrow cells were pretreated with anti-Thy 1.2 serum and complement before injection as described elsewhere (1).

Purification of Chimera Cells. LN suspensions pooled from cervical, inguinal, axillary, and mesenteric nodes were depleted of B cells (and presumably most macrophages) by nylon-wool filtration (11). The effluent T cells (≥90% Thy 1.2-positive) were depleted of host cells by treatment with appropriate anti-H-2 serum and complement, e.g. CBA anti-B6 serum for CBA → (CBA × B6)F1 chimeras. Cells were incubated with antiserum (1 ml of undiluted serum/2 × 10⁸ cells) for 30 min on ice, washed twice, incubated with complement (1 ml of 1:6 dilution guinea pig serum/2 × 10⁷ cells) for 30 min at 37°C, and then washed once before counting. This treatment killed > 98% of (CBA × B6)F1 LN cells.

Selection of Chimera Cells to SRC in Irradiated Mice

Positive selection. Chimera LN cells depleted of B cells and host cells (see above) were mixed with sheep erythrocytes (SRC) (0.5 ml of 25% solution) and injected intravenously in a dose of 5 × 10⁶ viable cells into irradiated mice (900 rads 1 d before) of the appropriate strain (8). Thoracic duct fistulas were inserted in the recipients 5 d later and the lymph-borne cells were collected overnight. Where the donor and host were H-2-incompatible, testing with appropriate anti-H-2 sera and complement (8) showed that 89-95% of the lymph-borne cells were of donor origin.

Negative selection. For negative selection, the irradiated recipients of purified chimera T cells plus SRC were cannulated at 1 d posttransfer and the lymph-borne cells were collected over the following 24 h (12). Because cell yields are low during negative selection (≤10% of the number injected), T cells and SRC were injected in twice the dose used above for positive selection.

Preparation of B cells. As described in detail elsewhere (8), B cells were prepared by treating spleen cells with anti-Thy 1.2 serum and complement by a two-step procedure. The B-cell donors were primed with SRC 2-4 mo previously.

T-B Collaboration. T and B cells were mixed with SRC (0.1 ml of 5% solution) and transferred intravenously into irradiated (800 rads 1 d before) (CBA × B6)F1 mice. Direct (19s, IgM) and indirect (7s, IgG) plaque-forming cells (PFC) to SRC were measured in the spleen 7 d later (8).

Results

Experimental Design. Chimeras were prepared by transferring 2 × 10⁶ anti-Thy 1.2-serum-treated CBA or B6 marrow cells into sublethally irradiated (700 rads) (CBA × B6)F1 mice (Materials and Methods). Under these conditions, the lymphohematopoietic system is formed from both donor and host stem cells in a ratio of 50:50. Thus, in the case of (CBA × B6)F1 mice given CBA marrow cells (CBA → F1 chimeras), testing with CBA anti-B6 H-2 alloantiserum gave cytotoxic indices of

Abbreviations used in this paper: LN, lymph nodes; MHC, major histocompatibility complex; PFC, plaque-forming cells; SRC, sheep erythrocytes.
30–60% with spleen and/or LN cells removed at 1–18 mo postreconstitution; the proportion of host cells was slightly higher with B6 → F1 chimeras, presumably because of the strong Hh barrier in this situation. Both types of chimeras survived in excellent health and had normal sized spleen and LN. Chimeras were used as T-cell donors at 4–12-mo reconstitution.

LN-cell suspensions from the chimeras were passed through nylon-wool columns and treated with anti-host alloantiserum and complement to leave a purified population of T cells of donor parental strain origin (Materials and Methods); these cells gave no detectable response to host-type determinants in mixed-lymphocyte culture (data not shown). For detecting subgroups of T-helper cells, purified chimera T cells were subjected to positive and negative selection to SRC in irradiated parental strain mice. For reasons discussed elsewhere (9), selection to SRC in vivo appears to reflect H-2-restricted contact of T cells with antigen presented by radioresistant macrophages (or related cells) of the host. The responding T cells initially become sequestered in the spleen and at this stage (day 1–2 posttransfer) are absent from thoracic duct lymph (negative selection). After extensive proliferation, the responding cells then re-enter the circulation in large numbers, e.g., at day 5–6 posttransfer (positive selection). Nonresponsive T cells (e.g., cells restricted to H-2 determinants of the opposite parent) fail to undergo either negative or positive selection. Hence, the lymph at day 1–2 posttransfer contains only nonresponsive T cells, whereas at day 5–6 the clonally expanded responding T cells far outnumber other cells.

T-B collaboration was measured in vivo by transferring T plus B cells (anti-Thy 1.2-serum-treated SRC-primed spleen) and SRC into irradiated (CBA × B6)F1 mice and measuring splenic PFC 1 wk later.

**Helper function of T cells from primed chimeras.** CBA T cells were purified from CBA → F1 chimeras primed with SRC 3 mo previously. As shown in Table I, these cells showed apparent unrestricted T-helper function, i.e., they collaborated as effectively with allogenic B6 B cells as with CBA and (CBA × B6)F1 B cells.

**Helper Function of Unprimed Chimera T Cells Positively Selected to SRC in Irradiated Parental Strain Mice.** Purified CBA T cells from unprimed CBA → F1 chimeras were transferred intravenously into heavily irradiated (900 rads) CBA, B6, or (CBA × B6)F1 mice, together with 0.5 ml of 25% SRC (Materials and Methods). The donor CBA cells were recovered from thoracic duct lymph of the recipients 5–6 d later, i.e., during the stage of positive selection. (Testing with appropriate alloantiserum showed that >90% of the lymph-borne cells were indeed of donor origin.) For measuring T-helper function, the lymph-borne cells were transferred with B cells derived from mice of the B10 congenic lines (these mice have identical genetic backgrounds and differ only at the H-2 complex).

As shown in Table II, CBA (H-2k) chimera T cells positively selected to SRC in irradiated (CBA × B6 (H-2k))F1 mice collaborated well with B cells from B10.BR (H-2k), B10 (H-2k), and (B10.BR × B10)F1 mice. With selection in irradiated parental strain mice, by contrast, the cells showed marked H-2 restriction in their helper function. Thus, after selection in CBA mice, the cells collaborated well with H-2-compatible B10.BR B cells, but poorly with H-2-different B10 B cells. Conversely, selection in B6 mice generated helper cells able to collaborate well with H-2-incompatible B10 B cells but not with H-2-compatible B10.BR B cells. Suppression did not appear to be involved because (a) good responses were observed with (B10.BR ×
Table I
Unrestricted T-Helper Activity of CBA T Cells from CBA → (CBA × B6)F1 Chimeras Primed with SRC 3 Mo Previously

| SRC-primed T-cell donors* | Dose of T cells (x 10^-6) | B cells§ | Anti-SRC PFC/spleen at 7 d in irradiated (CBA × B6)F1 mice§ |
|---------------------------|--------------------------|---------|---------------------------------------------|
|                           |                          |         | IgM                                         | IgG           |
| CBA → (CBA × B6)F1 chimera |                          |         |                                             |               |
|                           | 2.1                      | CBA     | 3,710 (1.23)                               | 24,630 (1.04) |
|                           | 0.7                      | B6      | 2,580 (1.10)                               | 6,950 (1.18)  |
|                           | 2.1                      | B6      | 7,310 (1.15)                               | 23,880 (1.25) |
|                           | 2.1                      | (CBA × B6)F1 | 10,790 (1.50)            | 49,220 (1.21) |
| Normal (CBA × B6)F1      | 2.1                      | CBA     | 8,970 (1.28)                               | 35,960 (1.08) |
|                           | 0.7                      | B6      | 2,600 (1.24)                               | 5,210 (1.13)  |
|                           | 2.1                      | B6      | 5,830 (1.36)                               | 14,380 (1.24) |
|                           | 2.1                      | (CBA × B6)F1 | 9,860 (1.45)            | 39,580 (1.11) |

* Cell donors were primed with 0.1 ml of 25% SRC intraperitoneally 3 mo before. LN cells pooled from four mice per group were passed through two consecutive nylon-wool columns. The chimera cells were then treated with CBA anti-B6 H-2 alloantiserum and complement (Materials and Methods).

§ 5 × 10^6 viable anti-Thy 1.2-serum-treated spleen cells from mice primed with SRC 2-4 mo. previously (8 × 10^6 for B6 B cells).

§ T and B cells were transferred intravenously together with SRC (0.1 ml of 5% solution) into (CBA × B6)F1 mice given 800 rads 1 d before.

I Geometric mean of data from three mice per group. Number in parenthesis refers to value by which mean is multiplied or divided to give upper and lower limits, respectively, of SE.

II Background values given by B cells transferred without T cells have been subtracted. These values (PFC/spleen) were: CBA 470 (2.22) (IgM), 500 (1.35) (IgG); B6 1,380 (1.21) (IgM), 1,270 (1.37) (IgG); F1 1,430 (1.22) (IgM), 1,510 (1.29) (IgG). T cells transferred without B cells gave very low numbers of PFC (<300 PFC/spleen).

B10)F1 B cells, and (b) the chimera cells failed to inhibit the capacity of (CBA × B6)F1 T cells to stimulate B10.BR or B10 B cells.

B10.A(4R) (K^b I-A^4 I-B^8 --- D^b) mice were used to map the restrictions observed in the above experiment. It is evident from Table III that chimera CBA T cells selected to SRC in irradiated B10.A(4R) mice exhibited the same type of restriction in helper function as cells selected in CBA mice, i.e., both groups of activated T cells collaborated well with B10.BR and B10.A(4R) B cells, but gave only low responses with B10 B cells. Chimera cells selected in B6 mice, by contrast, failed to stimulate either B10.BR or B10.A(4R) B cells. Again, appropriate addition experiments showed no evidence that the restrictions resulted from suppression. These data imply that (a) confrontation with K, I-A-subregion determinants controlled T-cell activation (positive selection) in the irradiated intermediate hosts, and (b) the activated T cells collaborated only with B cells which shared these same determinants with the hosts used for selection.

Table IV shows a similar experiment with B6 T cells derived from unprimed B6 → F1 chimeras. The results closely resemble those obtained above with CBA chimera T cells. Thus, selection in irradiated B6 mice stimulated helper cells which collaborated well with B10 B cells but not with B10.BR or B10.A(4R) B cells. The reverse applied to cells selected in irradiated CBA mice.
### Table II

**Helper Activity of T Cells from Unprimed CBA → (CBA × B6)F1 Chimeras after Positive Selection to SRC in Irradiated Parental Strain Mice. Restriction in T-Helper Function Linked to H-2 Complex**

| T-cell group          | Positively selected T-helper cells (0.8 × 10^6)* | B cells‡ | H-2 haplotype of B cells | Anti-SRC PFC/spleen at 7 d in irradiated (CBA × B6)F1§ |
|-----------------------|-----------------------------------------------|----------|--------------------------|-------------------------------------------------------|
|                       |                                               |          |                          | IgM                          | IgG                          |
| A Chim.CBA T+(SRC-CBA) | B10.BR k                                      | 30,020 (1.10) | 42,580 (1.12)            |
|                       | B10 b                                          | 29,990 (1.21) | 40,870 (1.11)            |
|                       | (B10.BR × B10) F1 k x b                       | 53,730 (1.16) | 67,940 (1.11)            |
| B Chim.CBA T+(SRC-CBA)| B10.BR k                                      | 43,370 (1.13) | 111,580 (1.16)           |
|                       | B10 b                                          | 1,170 (1.16)  | 200 (1.48)               |
|                       | (B10.BR × B10) F1 k x b                       | 37,620 (1.10) | 59,000 (1.06)            |
| C Chim.CBA T+(SRC-B6) | B10.BR k                                      | 1,590 (1.29)  | 510 (1.34)               |
|                       | B10 b                                          | 35,460 (1.14) | 57,170 (1.16)            |
|                       | (B10.BR × B10) F1 k x b                       | 30,900 (1.28) | 42,310 (1.08)            |
| D F1 T+(SRC-F1)       | B10.BR k                                      | 53,520 (1.16) | 127,200 (1.16)           |
|                       | B10 b                                          | 23,090 (1.03) | 48,240 (1.10)            |
|                       | (B10.BR × B10) F1 k x b                       | 68,130 (1.25) | 136,390 (1.26)           |
| Groups B + D (0.8 × 10^6 of each) | B10 b  | 32,700 (1.15)  | 58,420 (1.33)            |
| Groups C + D (0.8 × 10^6 of each) | B10.BR k | 49,780 (1.31) | 139,290 (1.11)           |

* LN suspensions from 25 unprimed CBA → (CBA × B6)F1 chimeras were depleted of B and host cells as described in Materials and Methods (≈ 40% of the cells were of host origin). The resulting chimera CBA T cells were transferred intravenously in a dose of 5 × 10^7 viable cells plus 0.5 ml of 25% SRC into irradiated (900 rads 1 d before) (CBA × B6)F1, CBA, or B6 mice (three mice per group). Thoracic duct fistulas were established in the recipients 5 d later and the lymph-borne cells were collected overnight. Testing with anti-Thy 1.2 serum and appropriate H-2 alloantisem (8) showed that >90% of the lymph-borne cells (pooled from three mice per group) were T cells of donor CBA origin; cell yields compared with the numbers originally injected were 20-40%. Abbreviations: Chim.CBA+SRC-F0 = Chimera CBA T cells positively selected to SRC for 5 d in irradiated (CBA × B6)F1 mice, etc. Unprimed (CBA × B6)F1 T cells positively selected to SRC in irradiated F1 mice (F1+SRC-F1) were used as control cells.

§§ Same as for Table I.

Because positively selected T-cell populations are contaminated with small numbers (5-10%) of radioresistant host cells it could be argued that these latter cells accounted for the collaboration observed with H-2 different B cells in the above experiments. To exclude this possibility, the B6 chimera T cells positively selected to SRC in irradiated CBA mice (Table IV) were treated with B6 anti-CBA H-2 serum both before and after selection. As shown in Table IV, this additional treatment with anti-host alloantisem did not diminish collaboration with B10.BR B cells.

**Helper Function of Unprimed Chimera T Cells Negatively Selected to SRC in Irradiated Parental Strain Mice**

The simplest explanation for the above findings is that H-2-restricted T cells in parent → F1 chimeras exist as a dichotomous population before first contact with antigen. If so, negative selection of unprimed chimera T cells to
Helper Activity of T Cells from Unprimed CBA → (CBA × B6)F1 Chimeras after Positive Selection to SRC in Irradiated Parental Strain Mice: Restriction in T-Helper Function Linked to K/I-A End of H-2 Complex

| T-cell group | Positively selected T-helper cells (0.8 × 10⁶)* | B cells | H-2 region of B cells | Anti-SRC PFC/spleen at 7 d in irradiated (CBA × B6)F1 mice§ |
|--------------|-----------------------------------------------|---------|----------------------|-------------------------------------------------------------|
|              |                                               |         |                      | K    | I-A | I-B | D      | IgM   | IgG   |
| A Chim.CBA T+SRC/CBA | B10.BR  k  k  -  -  -  k |          |                      | 33,550 (1.02) | 52,780 (1.05) |
|               | B10  b  b  -  -  -  b |          |                      | 1,169 (1.34) | 420 (1.15) |
|               | B10.A(4R)  k  k  b  -  -  b |          |                      | 15,220 (1.39) | 22,250 (1.34) |
| B Chim.CBA T+SRC/B6 | B10.BR  k  k  -  -  -  k |          |                      | 240 (1.22) | 380 (1.62) |
|               | B10  b  b  -  -  -  b |          |                      | 27,020 (1.30) | 52,310 (1.25) |
|               | B10.A(4R)  k  k  b  -  -  b |          |                      | 470 (2.32) | 900 (1.83) |
| C Chim.CBA T+SRC/B10.A(4R) | B10.BR  k  k  -  -  -  k |          |                      | 33,690 (1.18) | 56,090 (1.24) |
|               | B10  b  b  -  -  -  b |          |                      | 1,200 (1.29) | 350 (1.44) |
|               | B10.A(4R)  k  k  b  -  -  b |          |                      | 19,880 (1.18) | 18,940 (1.15) |
| Groups A + B (0.8 × 10⁶ of each) | B10.BR  k  k  -  -  -  k |          |                      | 38,620 (1.01) | 55,700 (1.13) |
|               | B10  b  b  -  -  -  b |          |                      | 33,540 (1.34) | 45,390 (1.08) |

* Same as for Table II, i.e. the chimera CBA T cells were positively selected to SRC for 5 d in either irradiated CBA, B6, or B10.A(4R) mice.
§§ Same as for Table II, B10.A(4R) B cells transferred in a dose of 8 × 10⁶ viable cells. Vertical line shows position of chromosomal crossover.
I Subtracted background values of B cells transferred without T cells were: B10.BR 880 (2.02) (IgM), 1,800 (1.50) (IgG); B10 1,420 (1.45) (IgM), 1,500 (1.82) (IgG); B10.A(4R) 110 (1.59) (IgM), 110 (1.59) (IgG). PFC with T cells alone were <200 PFC/spleen.

SRC in irradiated mice of one parental strain should remove T-helper function for B cells of this strain but not affect help provided for B cells of the opposite parental haplotype.

To examine this question, B6 T cells from unprimed B6 → F1 chimeras were negatively selected to SRC in irradiated B6 mice, i.e., recovered from thoracic duct lymph of the recipients at day 1–2 posttransfer (Materials and Methods and footnotes to Table V). These cells (group A, Table V) and control cells filtered in the absence of SRC (group B) were then positively selected to SRC in irradiated (CBA × B6)F1 mice. As shown in Table V, the group A T cells failed to collaborate with B10 B cells, i.e., cells that were H-2 compatible with the strain used for negative selection. Significantly, excellent collaboration occurred with B10.BR B cells, i.e., cells carrying H-2 determinants of the opposite parental strain. The group B T cells stimulated both B-cell populations and addition studies showed no evidence for suppression. (The main reason for positively selecting the T cells in irradiated F1 mice after negative selection was to enhance T-helper function [unprimed T cells give poor responses except in high doses]. In addition, positive selection provided a stringent test for the specificity of negative selection.)

Discussion

Selection to antigen in irradiated mice provides a useful tool for isolating H-2 restricted T-helper cells. Based on previous findings with heterozygous T cells, selection appears to be controlled by H-2 determinants on host macrophages or related cells (8). From a variety of approaches it has been concluded that H-2 restriction affects T-helper cells in vivo at two different levels: first, during T-cell activation (T-macrophage interaction) and second, during T-B collaboration. Expo-
Table IV

**Helper Activity of T Cells from Unprimed B6 → (CBA × B6)F1 Chimeras after Positive Selection to SRC in Irradiated Parental Strain Mice: Restriction in T-Helper Function Linked to K/I-A End of H-2 Complex**

| Positively selected T cells (0.8 × 10^6)* | B cells‡ | H-2 region of B cells | Anti-SRC PFC/spleen at 7 d in irradiated (CBA × B6)F1 mice§ |
|------------------------------------------|----------|-----------------------|--------------------------------------------------------|
|                                          |          | K | I-A | I-B | --- | D | IgM | IgG |
| Chim. B6 T+(SRC-B6)                    | B10      | b | b | b | --- | b | 26,010 (1.23) | 78,770 (1.37) |
|                                          | B10.BR   | k | k | k | --- | k | 3,220 (1.31) | 8,670 (1.19) |
|                                          | B10.A(4R)| k | k | k | --- | k | 2,310 (1.30) | 3,390 (1.16) |
|                                          | (B10.BR × B10)F1 | k/b | k/b | k/b | --- | k/b | 26,780 (1.18) | 48,550 (1.29) |
| Chim. B6 T+(SRC-CBA)                   | B10      | b | b | b | --- | b | 1,730 (1.27) | 1,510 (1.35) |
|                                          | B10.BR   | k | k | k | --- | k | 35,860 (1.15) | 80,750 (1.16) |
|                                          | B10.A(4R)| k | k | k | --- | k | 70,730 (1.06) | 135,660 (1.08) |
|                                          | (B10.BR × B10)F1 | k/b | k/b | k/b | --- | k/b | 30,570 (1.13) | 58,350 (1.22) |
| Chim. B6 T+(SRC-CBA)                   | B10.BR   | k | k | k | --- | k | 56,790 (1.22) | 119,090 (1.34) |

* The same as for Table II, except B6 → (CBA × B6)F1 chimeras were used. LN cells pooled from these contained ~55% host cells. The latter were removed by treatment with B6 anti-CBA serum and complement after the cells had first been passed through nylon-wool columns. After positive selection of the B6 T cells in irradiated CBA mice, 89% of the lymph-borne cells were of donor B6 origin, i.e., resistant to lysis with B6 anti-CBA serum.

‡§ Same as for Table I. To reduce Hh resistance, the (CBA × B6)F1 recipients used for measuring T-B collaboration were given split dose irradiation, i.e., 600 rads followed 2 wk later by 750 rads (the latter dose being given 1 d before cell transfer).

¶ Same as for Table I. Subtracted background values for B cells transferred without T cells were: B10 500 (1.54) (IgM), 1,520 (1.09) (IgG); B10.BR 1,600 (1.16) (IgM), 5,670 (1.11) (IgG); B10.A(4R) 1,710 (1.32) (IgM), 9,040 (1.43) (IgG); (B10.BR × B10)F1 500 (2.00) (IgM), 6,306 (1.43) (IgG).

¶ 0.8 × 10^6 viable cells transferred after treating the lymph-borne cells with B6 anti-CBA serum and complement. The higher response given by these cells than by cells not treated with anti-CBA serum (after selection) probably reflects the slight enrichment for donor B6 T cells (100 vs. 89%).

**Discussion**

The present studies indicate that homozygous cells from parent → F1 chimeras contain two discrete subgroups of H-2 restricted T cells. Stimulation of a subgroup to self H-2 determinants occurred when T cells from unprimed chimeras were positively selected to SRC in syngeneic irradiated mice. Selection in mice of the opposite parental strain revealed a second subgroup restricted to allo H-2 determinants. Functionally, these two T-cell subgroups appear to be identical to those observed previously in normal heterozygous mice (9). In both situations the restrictions map to the K, I-A, region of the H-2 complex. Analogous experiments with double (tetraparental) chimeras have given similar results (unpublished data).

Before discussing the significance of these findings, it is first necessary to consider...
Table V

| T-cell group | Irradiated hosts used for selection of B6 chimera T cells* | B cells‡ | H-2 haplo-type of B cells | Anti-SRC PFC/spleen at 7 d in irradiated (CBA × B6)F1 mice§ |
|--------------|----------------------------------------------------------|----------|--------------------------|----------------------------------------------------------|
| A            | B6 plus SRC (CBA × B6)F1 plus SRC | B10       | b                        | 740 (1.40)                                               |
|              | (CBA × B6)F1 plus SRC | B10.BR    | k                        | 63,380 (1.45)                                             |
| B            | B6 without SRC (CBA × B6)F1 plus SRC | B10       | b                        | 20,150 (1.17)                                             |
|              | (CBA × B6)F1 plus SRC | B10.BR    | k                        | 92,630 (1.19)                                             |
| Groups A + B | (10⁶ of each)                                             | B10       | b                        | 16,760 (1.18)                                             |

* B6 T cells were purified from unprimed B6 → (CBA × B6)F1 chimeras (see footnote to Table IV) and transferred intravenously in a dose of 10⁶ viable cells into irradiated (900 rads 6 h before) B6 mice together with 0.5 ml of 50% SRC (group A) or without SRC (group B). Thoracic duct fistulas were inserted in the recipients (two mice per group) 1 d later and the lymph-borne cells were collected for the following 24 h. Each group yielded ~ 3 × 10⁷ lymph-borne cells. These cells were transferred intravenously with SRC (0.5 ml of 25%) into irradiated (900 rads 6 h before) (CBA × B6)F1 mice. 6 d later the cells were recovered from the spleens of the second hosts and used as helper cells in a dose of 10⁶ viable cells. Testing with appropriate alloantiserum showed that >90% of the cells from the spleen were Thy 1.2-positive cells of donor B6 origin.

‡‡ The same as for Tables I and IV.

‖ The same as for Table I. Subtracted background values for B cells transferred without T cells were: B10 340 (1.31) (IgM), 280 (1.80) (IgG); B10.BR 1,470 (2.21) (IgM), 3,260 (1.52) (IgG). T cells alone gave < 200 PFC/spleen.

The question of whether parent → F₁ chimera T cells are indeed functionally different from normal homozygous T cells. In other words, are normal nonchimeric T cells totally restricted to self H-2 determinants? The literature on this point is confusing. Thus, whereas certain groups claim that normal T cells have no detectable capacity to stimulate major histocompatibility complex (MHC)-different B cells (2, 3), others report good collaboration across MHC barriers (13). Similarly, normal T cells lyse haptenated or virus-infected target cells across MHC barriers in some situations (14; J. Bennink and P. Doherty, unpublished data) but not in others (15–18). Despite this controversy, in our hands normal and chimeric T cells clearly behave differently in the response to SRC in vivo. With the same strain combinations used in the present study, we have found no evidence that normal homozygous T cells depleted of alloreactivity by an acute procedure can collaborate with H-2-different B cells (3). This applies even with prior exposure of unprimed cells to antigen in irradiated F₁ mice (19). From such findings one is thus led to the conclusion that, at least for the response to SRC in vivo, normal homozygous T cells behave as a single population restricted to self H-2 determinants.

From the work of Zinkernagel et al. (5) and Fink and Bevan (20) for T-killer cells, it seems highly probable that H-2 restriction is imposed in the thymus. With respect to T-helper function, two pieces of evidence provide strong, if indirect, support for this viewpoint. First, induction of long-term tolerance to MHC determinants by a
process which does not involve differentiation in a foreign thymus, i.e. classic neonatal
tolerance, does not abrogate restriction to self H-2 determinants (21, 22). Second, F₁
T cells differentiating from stem cells in irradiated parental strain mice (F₁ → parent
chimeras) are restricted to H-2 determinants of the parental strain used for reconsti-
tution (23).

It appears therefore that the capacity for T cells to collaborate with B cells bearing
a particular set of H-2 determinants depends upon the T cells encountering these H-
2 determinants in the thymus (presumably on epithelial cells) during early differen-
tiation. Thus, differentiation of strain a or (a × b)F₁ stem cells in a strain a thymus
produces an anti-a population of T cells which interacts only with target cells bearing
strain a H-2 determinants. By the same token, strain a or (a × b)F₁ stem cells processed
in an (a × b)F₁ thymus generate a mixture of anti-a and anti-b cells. As mentioned
earlier, H-2 determinants (presumably I-A determinants) restrict the function of these
cells both during T-macrophage interactions and during T-B collaboration (4, 8, 9,
24). It seems very likely that the restriction(s) applying first in the thymus and second
on macrophages and B cells are controlled by the same H-2 gene products. Although
appealing from the point of view of symmetry, this notion remains to be proved.

A key assumption in the above model is that H-2 restriction is imposed before
contact with antigen. Hence, the dichotomy of T cells in parent → F₁ chimeras should
be apparent before antigen-priming. Perhaps the best support for this notion comes
from the finding that negative selection of unprimed parent → F₁ chimera T cells to
antigen in syngeneic irradiated mice leaves a T-cell subgroup restricted to B cells of
the opposite parental strain (Table V); analogous data have been reported for
unprimed heterozygous T cells (12, 25). These findings, unlike data based on positive
selection, would seem to exclude the possibility that contact with H-2-associated
antigen imposes restriction on uncommitted virgin precursor cells, i.e., cells with
specificity for both parental haplotypes.

Katz et al. (26) have recently reported that T cells from parent → F₁ chimeras do
not collaborate with B cells of the opposite parental strain. It is important to
emphasize that this finding was based on studies with single chimeras, i.e., F₁ mice
totally repopulated with marrow cells of one parental strain. The essential difference
between these mice and the parent → sublethally irradiated F₁ chimeras used in the
present study is that the antigen-presenting cells in single chimeras are virtually all of
donor strain origin (J. S. unpublished data). Hence, priming single chimeras with
antigen in situ would be expected to stimulate only one of the two subgroups of T
cells, i.e., the subgroup restricted to self H-2 determinants. According to this viewpoint,
both T-cell subgroups would be stimulated if unprimed cells were activated to antigen
in the presence of normal heterozygous macrophages, i.e., by transfer to normal
irradiated F₁ mice. Indeed, both Waldmann et al. (22) and ourselves (J. S. unpub-
lished) have shown that single chimera T cells primed under these circumstances give
excellent responses with B cells of the opposite parental strain. Analogous findings
have been reported for T killer cells from single chimeras (5).

Finally, it should be stressed that although the role of the thymus in determining
H-2 restriction is clearly important, it may not necessarily be absolute. Indeed, as
mentioned earlier, there are an increasing number of reports that in certain situations
T cells do interact with target cells bearing MHC determinants not encountered in
the thymus (13, 14, 27–29). It is perhaps tempting to dismiss these anomalous findings
as having little physiological significance. Conversely, one could argue that those
groups who have failed to see interaction with MHC determinants not encountered in the thymus have simply failed to push their systems hard enough (29).

Summary

Parent → F1 chimeras were prepared by reconstituting sublethally irradiated H-2 heterozygous mice with marrow cells from one parental strain. Purified parental strain T cells prepared from unprimed chimeras were exposed to sheep erythrocytes in heavily irradiated mice of each of the two parental strains and recovered from thoracic duct lymph of the recipients at either day 1 or day 5 posttransfer. The lymph-borne cells were then tested for their capacity to collaborate in vivo with B cells of the two parental strains. From this approach it was concluded that parent → F1 chimera T cells contain two discrete subgroups of T-helper cells, one specific for self H-2 determinants and the other restricted to H-2 determinants of the opposite parental strain. The restrictions mapped to the K-end of the H-2 complex.

The expert typing skills of Mrs. K. King is gratefully acknowledged.

Received for publication 2 October 1978.

References

1. von Boehmer, H., and J. Sprent. 1976. T cell function in bone marrow chimeras: absence of host-reactive T cells and cooperation of helper T cells across allogeneic barriers. Transplant. Rev. 29:3.
2. Katz, D. H., T. Hamaoka, and B. Benacerraf. 1973. Cell interactions between histo-incompatible T and B lymphocytes. II. Failure of physiologic cooperative interactions between T and B lymphocytes from allogeneic donor strains in humoral response to hapten-protein conjugates. J. Exp. Med. 137:1405.
3. Sprent, J., and H. von Boehmer. 1976. Helper function of T cells depleted of alloantigen-reactive lymphocytes by filtration through irradiated F1 hybrid recipients. I. Failure to collaborate with allogeneic B cells in a secondary response to sheep erythrocytes measured in vivo. J. Exp. Med. 144:617.
4. Katz, D. H., and B. Benacerraf. 1976. Genetic control of lymphocyte interactions and differentiation. In The Role of Products of the Histocompatibility Gene Complex in Immune Responses. D. H. Katz and B. Benacerraf, editors. Academic Press, Inc., N. Y. 355.
5. Zinkernagel, R. M., G. N. Callahan, A. Althage, S. Cooper, P. A. Klein, and J. Klein. 1978. On the thymus in the differentiation of “H-2 self-recognition” by T cells. Evidence for dual recognition? J. Exp. Med. 147:882.
6. Zinkernagel, R. M., G. N. Callahan, A. Althage, S. Cooper, J. W. Streilein, and J. Klein. 1978. The lymphoreticular system in triggering virus plus self-specific cytotoxic T cells. Evidence for T help. J. Exp. Med. 147:897.
7. Swierkosz, J. E., K. Rock, P. Marrack, and J. W. Kappler. 1977. The role of H-2-linked genes in helper T-cell function. II. Isolation on antigen-pulsed macrophages of two separate populations of F1 helper T cells each specific for antigen and one set of parental H-2 products. J. Exp. Med. 147:554.
8. Sprent, J. 1978. Restricted helper function of F1 hybrid T cells positively selected to heterologous erythrocytes in irradiated parental strain mice. I. Failure to collaborate with B cells of the opposite parental strain not associated with active suppression. J. Exp. Med. 137:1142.
9. Sprent, J. 1978. Restricted helper function of F1 T cells positively selected to heterologous
erythrocytes in irradiated parental strain mice. II. Evidence for restrictions affecting helper cell induction and T-B collaboration, both mapping to the K-end of the H-2 complex. J. Exp. Med. 147:1159.

10. Sprent, J. 1973. Circulating T and B lymphocytes of the mouse I. Migratory properties. Cell Immunol. 7:10.

11. Julius, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of thymus-derived murine lymphocytes. Eur. J. Immunol. 3:645.

12. Sprent, J. 1978. Two subgroups of T helper cells in F(1) hybrid mice revealed by negative selection in vivo. J. Immunol. 121:1691.

13. Heber-Katz, E., and D. B. Wilson. 1975. Collaboration of allogeneic T and B lymphocytes in the primary antibody response to sheep erythrocytes in vitro. J. Exp. Med. 142:928.

14. Wilson, D. B., K. F. Lindahl, D. H. Wilson, and J. Sprent. 1977. The generation of killer cells to trinitrophenyl-modified allogeneic targets by lymphocyte populations negatively selected to strong alloantigens. J. Exp. Med. 146:361.

15. von Boehmer, H., W. Haas, and H. Pohlit. 1978. Cytotoxic T cells recognize male antigen and H-2 as distinct entities. J. Exp. Med. 147:1291.

16. Janeway, C. A., P. D. Murphy, J. Kemp, and H. Wigzell. 1978. T cells specific for hapten-modified self are precommitted for self major histocompatibility complex antigens before encounter with the hapten. J. Exp. Med. 147:1065.

17. Schmitt-Verhulst, A. M., and G. M. Shearer. 1977. Specificity of CML and MLR clones responding to chemically modified syngeneic and allogeneic cells. J. Supramol. Struct. 1(Suppl.):206.

18. Bennink, J. R., and P. C. Doherty. 1978. T-cell populations specifically depleted of alloreactive potential cannot be induced to lyse H-2-different virus-infected target cells. J. Exp. Med. 148:128.

19. Sprent, J. 1978. Role of H-2 gene products in the function of T helper cells from normal and chimeric mice measured in vivo. Immunol. Rev. In press.

20. Fink, P. J., and M. J. Bevan. 1978. H-2 antigens of the thymus determine lymphocyte specificity. J. Exp. Med. 148:766.

21. Kindred, B. 1975. Can tolerant allogeneic cells restore nude mice? Cell Immunol. 20:241.

22. Waldmann, H., H. Pope, L. Brent, and K. Bighouse. 1978. Influence of the major histocompatibility complex on lymphocyte interactions in antibody formation. Nature (Lond.). 274:166.

23. Sprent, J. 1978. Restricted helper function of F(1)→ parent bone marrow chimeras controlled by K-end of H-2 complex. J. Exp. Med. 167:1838.

24. Erb, P., and M. Feldmann. 1975. The role of macrophages in the generation of T-helper cells. II. The genetic control of the macrophage-T cell interaction for helper cell induction with soluble antigens. J. Exp. Med. 142:160.

25. Thomas, D. W., and E. M. Shevach. 1978. Nature of the antigenic complex recognized by T lymphocytes. V. Genetic predisposition of independent F(1) T cell subpopulations responsive to antigen-pulsed parental macrophages. J. Immunol. 120:638.

26. Katz, D. H., B. J. Skidmore, L. R. Katz, and C. A. Bogowitz. 1978. Adaptive differentiation of murine lymphocytes. I. Both T and B lymphocytes differentiating in F(1)→ parental chimeras manifest preferential cooperative activity for partner lymphocytes derived from the same parental strain type corresponding to the chimeric host. J. Exp. Med. 168:727.

27. Pierce, S. K., and N. R. Klinman. 1976. Allogeneic carrier-specific enhancement of hapten-specific secondary B-cell responses. J. Exp. Med. 144:1254.

28. Thomas, D. W., and E. M. Shevach. 1977. Nature of the antigenic complex recognized by T lymphocytes. III. Specific sensitization by antigens associated with allogeneic macrophages. Proc. Natl. Acad. Sci. U.S.A. 74:2104.

29. Matzinger, P. and G. Mirkwood. 1978. In a fully H-2 incompatible chimera, T cells of donor origin can respond to minor histocompatibility antigens in association with either host or donor H-2 type. J. Exp. Med. 148:84.