RESTRICTED HELPER FUNCTION OF F1 HYBRID 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*

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The preceding paper demonstrated that F1 hybrid T cells activated to heterologous erythrocytes for 5 days in irradiated mice of one parental strain cooperated well with B cells derived from either this strain or from F1 mice, but not with B cells of the opposite parental strain. This restricted helper activity could not be accounted for in terms of active suppression. The data implied that F1 T cells contain two discrete subpopulations of antigen-reactive cells, each reactive to antigen presented by radioresistant cells, probably macrophages, of one of the two parental strains. Why the mode of antigen presentation in the parental strain environment affected the capacity of the T cells to collaborate with B cells, however, was not clear. One explanation is that the poor collaboration observed with B cells of the opposite parental strain was simply the result of a failure to reactivate the T cells to antigen on subsequent transfer with B cells. This might occur if, during T-B collaboration, the macrophages presenting antigen to the T cells were of the wrong type, i.e. they did not share major histocompatibility complex (MHC)* determinants with the macrophages responsible for the initial activation of the T cells. Recent studies by Erb et al. (1) provide a precedent for this notion. These workers observed that F1 T cells activated to antigen in vitro in the presence of parental strain macrophages failed to stimulate B cells of the opposite parent, though F1 B cells gave good responses. Significantly, the inability to stimulate B cells of the opposite parental strain could be overcome by supplementing the B cells with macrophages sharing MHC determinants with the macrophages used for initial T-cell activation.

The present paper demonstrates that the addition of appropriate macrophages during T-B collaboration in vivo fails to abrogate the restriction in T helper function. It is concluded, therefore, that the system reflects restrictions imposed not only at the level of helper cell induction, but also during T-B collaboration. Studies with H-2-congenic and recombinant strains suggest that the restrictions at each of these two levels map to the K-end of the H-2 complex.

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1 Abbreviations used in this paper: BM, bone marrow; MHC, major histocompatibility complex; PE, peritoneal exudate; PFC, plaque-forming cells; SRC, sheep red blood cells; TDL, thoracic duct lymphocytes.
Materials and Methods

Mice. CBA Cum (CBA), C57BL/6 Cum (B6) and (CBA × B6)F₁ mice were obtained from Cumberland View Farms, Clinton, Tennessee. C57BL/10J (B10), B10.Br, DBA/2J, B10.D2, (B10 × B10.D2)F₁, and B10.A(6R) mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. B10.A(4R) mice were a gift of Dr. W. Elkins, University of Pennsylvania; these mice were bred at the University of Pennsylvania from lines provided by Dr. F. Bach of the University of Wisconsin at Madison.

Experimental Plan. The details of the experimental plan were reported in full in the preceding paper. Briefly, 5 × 10⁷ nylon-wool-purified (>90% thy 1.2-positive) T cells from lymph nodes of unprimed (CBA × B6)F₁, mice were transferred intravenously with 0.5 ml of 25% sheep erythrocytes (SRC) into intermediate hosts which had received 800 rads the previous day. Thoracic duct fistulas were inserted in the cell recipients 5 days later, and thoracic duct lymphocytes (TDL) were collected overnight. These cells, >95% of which were thy 1.2-positive cells of donor origin, were transferred intravenously with B cells (5 × 10⁶ viable, anti-thy 1.2-serum-treated spleen or TDL from SRC-primed mice) and with 0.1 ml of 5% SRC into irradiated (750 rads) (CBA × B6)F₁, mice. Direct (IgM) and indirect (IgG) anti-SRC plaque-forming cells (PFC) were measured in the spleens of the recipients 7 days later. In some experiments, (DBA/2 × B6)F₁ T cells were used for activation. With these cells, T-B collaboration was measured by transferring the T cells and appropriate B cells into irradiated (DBA/2 × B6)F₁ mice.

Anti-Allotype Antiserum. Anti-Ig₆ allotype was kindly provided by Dr. N. Klinman, University of Pennsylvania. The antiserum was made according to the technique of Herzenberg and Warner (2) by injecting BALB/c mice with anti-pertussis antibodies derived from B6 mice. Testing with the Ouchterlony technique showed that the antiserum was specific for serum from B6, B10, B10.Br, and (CBA × B6)F₁, mice, but not for serum from CBA, C3H, or BALB/c mice. The precise specificity of the antiserum was not determined. Antibodies directed against more than one IgG subclass were probably involved, since the antiserum enhanced a high proportion (60–80%) of the total anti-SRC PFC developed by a polyvalent rabbit anti-mouse Ig antiserum. The enhancing activity of the antiserum was specific for Ig₆ allotypes in that PFC enhancement was observed with B6, B10, and B10.Br mice, but not with CBA or C3H mice.

PFC enhancement was studied by adding 1 drop of the antiserum (diluted 1:10) to the reaction mixture of spleen cells, SRC, and guinea pig serum as a source of complement (see preceding paper). PFC were then counted after transferring the mixture to Cunningham chambers. Numbers of PFC enhanced by the anti-allotype antiserum were calculated by subtracting numbers of direct (IgM) PFC obtained in the absence of the antiserum. Total indirect (IgG) PFC were estimated by subtracting numbers of direct PFC from the numbers of PFC obtained when rabbit anti-mouse Ig antiserum was added to the reaction mixture.

Bone Marrow (BM) Cells. BM cells were obtained from femurs and tibiae of 6- to 8-wk-old mice by flushing with balanced salt solution via a 26-gauge needle attached to a syringe. After counting, the cells were treated with anti-thy 1.2 serum (0.1 ml of undiluted antiserum/2 × 10⁷ cells) and complement in a two-step procedure as described for spleen cells in the preceding paper.

Peritoneal Exudate Cells. Cells from the peritoneal cavity of mice given 1 ml of 4% thioglycolate 4 days earlier were treated with anti-thy 1.2 serum and complement as for spleen cells. Over 80% of these cells had features of macrophages, i.e. they were large cells containing numerous granules.

Results

Failure to Explain Restricted Helper Function of Positively Selected F₁ T Cells as Due to Lack of Appropriate Macrophages during T-B Collaboration. As described in detail in the preceding paper, and as exemplified in Table 1, unprimed (CBA × B6)F₁ T cells activated to SRC for 5 days in irradiated B6 mice (F₁ T⁺SRC-B6) cooperated poorly with CBA B cells, but gave good responses with F₁, B cells. In contrast, F₁, T cells activated to SRC in irradiated F₁ mice (F₁, T⁺SRC-F₁) stimulated both populations of B cells. Conceivably, this failure of F₁, T⁺SRC-B6, cells to provide help for CBA B cells might reflect that the helper cells
**Table I**

**Helper Activity of TDL from Irradiated B6 Mice Given Unprimed (CBA × B6)F₁ T Cells Plus SRC 5 Days Previously: Effect of Supplementing Helper Cells with Macrophage-Containing Cells from (CBA × B6)F₁ Mice***

| Cells transferred | Dose of SRC transferred with T and B cells | Anti-SRC PFC/spleen at 7 days in irradiated (CBA × B6)F₁ mice |
|-------------------|------------------------------------------|----------------------------------------------------------|
|                  |                                          | IgM          | IgG          |
| (CBA × B6)F₁    | 0.1 ml of 5%                            | 12,350 (1.13) | 36,400 (1.14) |
| CBA              | 0.1 ml of 5%                            | 250 (1.57)   | 350 (1.18)   |
| CBA              | 0.1 ml of 50%                           | 170 (1.39)   | 220 (1.20)   |
| F₁ T⁺(SRC-B6)   | F₁, PE cells                            | 0.1 ml of 5% | 750 (1.46)   | 310 (1.37)   |
| CBA              | F₁, BM cells                            | 0.1 ml of 5% | 230 (1.36)   | 870 (1.45)   |
| (CBA × B6)F₁    | 0.1 ml of 5%                            | 48,470 (1.12)| 81,390 (1.14) |
| CBA              | 0.1 ml of 5%                            | 12,310 (1.04)| 49,100 (1.18) |
| CBA              | F₁, PE cells                            | 0.1 ml of 5% | 7,620 (1.04) | 26,630 (1.17) |
| CBA              | F₁, BM cells                            | 0.1 ml of 5% | 6,320 (1.22) | 36,750 (1.18) |

* Cell recipients were exposed to 800 rads 1 day before being injected intravenously with 5 × 10⁷ unprimed nylon-wool-purified (CBA × B6)F₁ T cells (>90% thy 1.2-positive) plus 0.5 ml of 25% SRC.
† Recipients of F₁ T cells and SRC were cannulated at 5 days post-transfer, and the lymph-borne cells were collected overnight. The helper activity of the cells was measured by transferring 0.8 × 10⁶ TDL (nearly all of which were shown by appropriate alloantisera to be T cells of donor F₁ origin) intravenously into irradiated (750 rads) (CBA × B6)F₁ mice plus B cells and 0.1 ml of 5% SRC.
§ Spleen cells from mice primed with SRC 2-4 mo previously and treated with anti-thy 1.2 antiserum plus complement before injection; 5 × 10⁶ viable cells transferred.
‖ 4 × 10⁶ viable cells transferred after treatment with anti-thy 1.2 antiserum plus complement. Cells were mixed with T cells, B cells, and SRC, and were injected intravenously.
¶ Geometric mean of data from 4 mice per group; number in parentheses refers to value by which mean is multiplied or divided to give upper and lower limits, respectively, of SE. Background values given by B cells transferred without T cells have been subtracted. These values (PFC/spleen) were: F₁, 500(1.13) (IgM); 750(1.23) (IgG); CBA, 150(1.30) (IgM); 140(1.22) (IgG); CBA + F₁, PE 90(1.45) (IgM); 190(1.37) (IgG); CBA + F₁, BM 490(1.40) (IgM); 180(1.74) (IgG). Values for T cells transferred without B cells were all <200 PFC/spleen. In the table values in parentheses used to derive SE were calculated before subtraction of background values.

were not reactivated during the stage of T-B collaboration. This could occur if the macrophages presenting the antigen at this stage did not share MHC determinants with the macrophages responsible for the initial activation of the T cells. In considering this possibility, it is essential to emphasize that T-B collaboration was measured in irradiated F₁ mice, i.e. in a situation in which the host macrophages were semi-syngeneic with respect to both the T cells and B cells. Nevertheless, it is possible that when the mixture of T cells, B cells, and SRC were transferred to the irradiated F₁ mice, the antigen was processed not by radioreistant host macrophages, but by the macrophages contaminating the B-cell source (anti-thy 1.2-treated spleen cells). These macrophages would not
TABLE II

| T cells (CBA × B6)F₁ |
|---------------------|
| B cells (anti-thy 1.2-treated) |
| CBA spleen | 370 (1.12) | 740 (1.47) |
| CBA TDL | 130 (1.53) | 120 (1.58) |
| B6 spleen | 15,240 (1.24) | 32,430 (1.17) |
| (CBA × B6)F₁ spleen | 14,080 (1.19) | 47,020 (1.25) |
| CBA spleen | 13,630 (1.09) | 72,050 (1.07) |
| CBA TDL | 12,740 (1.04) | 58,080 (1.06) |
| B6 spleen | 16,710 (1.14) | 38,470 (1.09) |
| (CBA × B6)F₁ spleen | 45,810 (1.16) | 145,080 (1.18) |

* As for Table I. 
† As for Table I. TDL were from first overnight collection of lymph; both spleen and TDL were transferred in a dose of 5 × 10⁶ viable cells after treatment with anti-thy 1.2 serum plus complement; B6 spleen cells were transferred in a dose of 8 × 10⁶ viable cells. 
§ As for footnote ¶ of Table I. Background values for B cells transferred without T cells have been subtracted. These values (PFC/spleen) were: CBA spleen < 100 (IgM), < 100 (IgG); CBA TDL < 100 (IgM), 260 (1.35) (IgG); B6 spleen 660 (1.17) (IgM), 1,770 (1.51) (IgG); F₁ spleen 190 (1.64) (IgM), 640 (1.86) (IgG). Numbers of PFC for T cells transferred without B cells were all < 100 PFC/spleen.

share MHC determinants with the macrophages used for initial T-cell activation, and they would therefore fail to reactivate the T cells.

Four approaches were used to investigate this possibility. The first was simply to increase the dose of antigen adoptively transferred with the T and B cells, thereby making it less likely that macrophages contaminating the B-cell source could preempt antigen-processing by host macrophages. As shown in Table I, increasing the challenge dose 10-fold to 0.1 ml of 50% SRC failed to overcome the poor response obtained with CBA splenic B cells.

The second approach was to supplement the transfer mixture of T and B cells with a cell source enriched for macrophages which shared MHC determinants with the strain used for T-cell activation. As shown in Table I, addition of 4 × 10⁶ anti-thy 1.2-treated peritoneal exudate (PE) cells (>80% macrophages) or BM cells (a source of stem cells and macrophage precursors) obtained from F₁ mice did not influence the poor helper function of F₁ T+(SRC-B6~) cells for B cells prepared from CBA spleen. Similar results were observed when the added PE or BM cells were taken from B6 rather than from F₁ mice (data not shown).

The third approach was to use B cells which were depleted of macrophages. TDL, i.e. cells containing virtually no macrophages (3), were used for this purpose. It can be seen from Table II that the helper function of F₁ T+(SRC-B6~) cells for CBA B cells was very low, irrespective of whether anti-thy 1.2-treated TDL or spleen cells were used as a source of B cells. Both B-cell populations gave good responses with F₁ T+(SRC-F₂) cells.

The final approach was to measure T-B collaboration in F₁ mice which had been irradiated and injected intravenously with F₁ BM cells (3 × 10⁶ anti-thy
Table III
Helper Activity of TDL from Irradiated B6 Mice Given Unprimed (CBA × B6)F1 T Cells Plus SRC 5 Days Previously: Effect of Measuring T-B Collaboration in Irradiated (CBA × B6)F1 Mice Reconstituted with F1 BM 6 Days Earlier*

| T cells‡ (anti-thy 1.2-treated) | B cells§ (anti-thy 1.2-treated) | Pretreatment of F1 recipients of T and B cells|| Anti-SRC PFC/spleen at 7 days in irradiated (CBA × B6)F1 mice |
|---|---|---|---|---|
| | | | IgM | IgG |
| | | | | |
| B6 spleen | — | 32,970 (1.13)|| 59,070 (1.17) |
| (CBA × B6)F1 spleen | — | 43,600 (1.21)|| 85,740 (1.23) |
| CBA spleen | — | 780 (1.46)|| 1,620 (1.11) |
| CBA spleen | F1 BM 6 days before | 260 (1.20)|| 1,340 (1.21) |
| CBA TDL | — | 260 (1.20)|| 2,320 (1.31) |
| CBA TDL | F1 BM 6 days before | 680 (1.95)|| 720 (1.85) |
| B6 spleen | — | 9,280 (1.35)|| 18,010 (1.38) |
| (CBA × B6)F1 spleen | — | 74,700 (1.04)|| 180,360 (1.00) |
| CBA spleen | — | 12,350 (1.41)|| 51,330 (1.21) |
| CBA spleen | F1 BM 6 days before | 2,010 (1.25)|| 11,480 (1.41) |
| CBA TDL | — | 4,510 (1.17)|| 28,470 (1.18) |
| CBA TDL | F1 BM 6 days before | 3,960 (1.24)|| 10,000 (1.56) |

* As for Table I. Background values of B cells transferred without T cells have been subtracted. These values (PFC/spleen) were: B6 spleen 270 (1.78) (IgM), 1,140 (1.69) (IgG); F1 spleen 390 (1.31) (IgM), 1,340 (1.35) (IgG); CBA spleen 160 (1.31) (IgM), 150 (1.50) (IgG); CBA spleen + F1 BM 130 (1.71) (IgM), 210 (1.19) (IgG); CBA TDL <100 (IgM), <100 (IgG); CBA TDL + F1 BM 870 (1.43) (IgM), 1,330 (1.62) (IgG).

† As for Table I.

‡ As for Table I. TDL were from first overnight collection of lymph; both spleen and TDL were transferred in a dose of 5 × 10⁶ viable cells (8 × 10⁶ for B6 spleen) after treatment with anti-thy 1.2 serum plus complement.
§ As for Table I. Mice were given 850 rads followed by intravenous injection of 3 × 10⁶ anti-thy 1.2-treated F1 BM cells 4 h later. These mice were then used as recipients of T and B cells 6 days later. Mice not pretreated received 750 rads 18 h before T- and B-cell transfer.
|| As for Table I. Background values of B cells transferred without T cells have been subtracted. These values (PFC/spleen) were: B6 spleen 270 (1.78) (IgM), 1,140 (1.69) (IgG); F1 spleen 390 (1.31) (IgM), 1,340 (1.35) (IgG); CBA spleen 160 (1.31) (IgM), 150 (1.50) (IgG); CBA spleen + F1 BM 130 (1.71) (IgM), 210 (1.19) (IgG); CBA TDL <100 (IgM), <100 (IgG); CBA TDL + F1 BM 870 (1.43) (IgM), 1,330 (1.62) (IgG).

1.2-treated cells) 6 days previously. At the time of transferring the T and B cells into these mice, the spleens of the recipients were of near normal size and contained large numbers (∼5 × 10⁷/spleen) of newly differentiated hemopoietic cells, including cells with the morphology of macrophages. This approach was designed to answer the objection to the first approach that mature macrophages injected intravenously might not home to the spleen. As shown in Table III, the poor help provided by F1 T+(SRC-B6) cells for CBA B cells (from spleen) was not improved when the irradiated F1 recipients of the T and B cells had been reconstituted with syngeneic BM cells 6 days previously. Similar results were obtained when B6 BM rather than F1 BM was used to reconstitute the irradiated F1 mice (data not shown). A more stringent test was to combine the third and fourth approaches and transfer the F1 T+(SRC-B6) cells into the F1 BM-reconstituted F1 mice along with macrophage-depleted cells as a source of B cells, i.e.
TABLE IV

Helper Activity of TDL from Irradiated CBA and B6 Mice Given (CBA × B6)F₁ T Cells
Plus SRC 5 Days Preceding: Response with B10.Br and B10 B Cells*

| T cells* (CBA × B6)F₁ | B cells (H-2 region, Ig allotype) | Anti-SRC PFC/spleen at 7 days in irradiated F₁ mice |
|-----------------------|---------------------------------|-----------------------------------------------|
|                       |                                 | IgM | IgG | IgG | IgG |
| CBA (H-2k, Igα)       | 12,230 (1.18)                  | 48,800 (1.19) | 0   | 0.00 |
| B10.Br (H-2k, Igβ)    | 41,630 (1.30)                  | 119,790 (1.40) | 88,620 (1.18) | 0.76 |
| B10 (H-2k, Igβ)       | 1,040 (1.17)                   | 2,120 (1.26) | 1,440 (1.16) | 0.68 |
| (CBA × B6)F₁ (H-2k, × H-2k, Igα × Igβ) | 30,640 (1.17) | 99,940 (1.04) | 43,960 (1.13) | 0.44 |
| CBA (H-2k, Igα)       | 1,140 (1.31)                   | 340 (1.94)  | 0   | 0.00 |
| B10.Br (H-2k, Igβ)    | 480 (1.27)                     | 1,080 (1.39) | 830 (1.38) | 0.77 |
| B10 (H-2k, Igβ)       | 27,080 (1.13)                  | 63,700 (1.28) | 48,150 (1.12) | 0.77 |
| (CBA × B6)F₁ (H-2k, × H-2k, Igα × Igβ) | 14,130 (1.28) | 46,960 (1.24) | 15,490 (1.12) | 0.33 |

* As for Table I; § As for Table I. Background values for B cells transferred without B cells have been subtracted. These values (PFC/spleen) were: CBA 430 (1.17) (IgM), <100 (IgG), 0 (Igα); B10.Br 710 (1.27) (IgM), 230 (1.67) (IgG), 230 (1.67) (Igβ); B10 540 (1.21) (IgM), 660 (2.40) (IgG), 500 (1.29) (Igβ); F₁ 510 (1.62) (IgM), 360 (1.36) (IgG), 210 (1.61) (Igβ).

anti-thy 1.2-treated TDL. Again, this failed to overcome the poor helper function of F₁ T⁺(SRC-B6) cells for CBA B cells. It may be noted that responses observed with the control population of T cells (F₁ T⁺(SRC-F₁)) were appreciably lower when BM-reconstituted F₁ mice were used to measure T-B collaboration, possibly because such mice have less available "space" for generating the immune response (4).

Role of the H-2 Complex in the Restricted Helper Function of Positively Selected F₁ T Cells. The data shown in Table IV indicate that the restricted helper function of (CBA × B6)F₁ T cells activated to SRC in parental strain mice is linked to the H-2 complex, but not to immunoglobulin allotype. With respect to the H-2 complex, it is evident that F₁ T⁺(SRC-CBA) cells collaborated well with CBA (H-2k) B cells and B10.Br (H-2k) B cells, but poorly with B10 (H-2b) B cells (B10.Br and B10 differ only with respect to the H-2 complex; B10.Br and CBA have different genetic backgrounds, as do B6 (H-2k) and B10). Conversely, F₁ T⁺(SRC-B6) cells stimulated B10 B cells, but did not cooperate well with either CBA or B10.Br B cells.

The fact that CBA and B10.Br have different Ig allotypes (see Table IV) would seem to rule out the possibility that the restriction in helper function observed with CBA and B6 (or B10) B cells reflected the differing allotypes on these cells (Igα and Igβ, respectively). It can be seen from Table IV that the restriction in helper function as measured by the numbers of IgM and IgG PFC also applied to numbers of PFC enhanced by a polyvalent anti-Igβ anti-allotype serum. It is to be noted that both populations of T cells stimulated (CBA × B6)F₁ B cells to produce Igβ PFC. As expected, the proportion of Igβ PFC (relative to the IgG PFC enhanced by the polyvalent anti-mouse Ig antisera) was appreciably less with allotype-heterozygous (CBA × B6)F₁ B cells (~40%) than with Igβ-homozygous B cells (~75%).

The data shown in Table V indicate that the restricted helper function of positively selected F₁ T cells is controlled by genes mapping in the K-end of the H-2 complex. In this experiment, (CBA × B6)F₁ T cells were activated to SRC...
Table V

Helper Function of TDL from Irradiated B10, B10.Br, and B10.A(4R) Mice Injected with Unprimed (CBA × B6)F₁ T Cells Plus SRC 5 Days Previously: Responses with B10, B10.Br, and B10.A(4R) B Cells

| T cells § (CBA × B6)F₁ | B cells § (anti-thy 1.2 spleen) | 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 |
| F₁ T⁺(SRC-B10)         |   |     |     |   |     |     |
| B10                    | b | b   | b   | b | 33,360 (1.06) | 165,640 (1.09) |
| B10.Br                 | k | k   | k   | k | 2,200 (1.33)  | 7,200 (1.30)   |
| B10.A(4R)              | k | k   | b   | b | 630 (1.43)    | 2,590 (1.48)   |
| F₁ T⁺(SRC-B10.Br)      |   |     |     |   |     |     |
| B10                    | b | b   | b   | b | 1,420 (1.77)  | 4,410 (1.46)   |
| B10.Br                 | k | k   | k   | k | 44,510 (1.03) | 146,420 (1.10) |
| B10.A(4R)              | k | k   | b   | b | 25,410 (1.08) | 134,020 (1.25) |
| F₁ T⁺(SRC-B10.A(4R))   |   |     |     |   |     |     |
| B10                    | b | b   | b   | b | 1,150 (1.27)  | 4,330 (1.13)   |
| B10.Br                 | k | k   | k   | k | 22,950 (1.01) | 77,030 (1.16)  |
| B10.A(4R)              | k | k   | b   | b | 15,590 (1.16) | 69,320 (1.13)  |

* As for Table I.
§ As for Table I. B cells transferred in a dose of 5 × 10⁶ viable cells for B10.Br spleen and 8 × 10⁶ cells for B10 and B10.A(4R) spleen.
|| Vertical lines denote the presumed position of the cross-over in B10.A(4R).
¶ As for Table I. Background values for B cells alone have been subtracted. These values (PFC/spleen) were: B10 330 (1.46) (IgM), 850 (1.42) (IgG); B10.Br 1,270 (1.67) (IgM), 1,200 (1.17) (IgG); B10.A(4R) 220 (1.25) (IgM), 1,740 (1.51) (IgG).

Two points arise from this experiment. First, the helper function of F₁ T cells activated in B10.A(4R) mice resembled that of cells activated in B10.Br mice, in that both T-cell populations gave good responses with B10.Br B cells, but not with B10 B cells; the reverse applied to T cells activated in B10 mice. This suggests that during positive selection in the irradiated intermediate hosts, T-cell recognition of gene products mapping to the left of the I-B subregion determined which subgroup of F₁ T cells was to be activated. Second, it can be seen that T-cell triggering of B10.A(4R) B cells resembled that of B10.Br B cells rather than B10 B cells. Thus, both B10.A(4R) B cells and B10.Br B cells were triggered by F₁ T cells activated in either B10.A(4R) mice or B10.Br mice; B10 B cells were stimulated only by F₁ T cells activated in B10 mice. This implies that, like T-cell activation, collaboration with B cells depends upon T-cell recognition of I-A and/or I-A subregion determinants.

The data shown in Table VI represent a similar study with (DBA/2 × B6)F₁ T cells. When these cells were activated to SRC in irradiated B6 mice (F₁ T⁺(SRC-B6) cells), poor responses were found with both DBA/2 (H-2 d) and B10.D2 (H-2 d on the B10 background) B cells, whereas good cooperation was seen with B10 (H-2 b), B10.A(5R) (Kᵇ I-Aᵇ I-Bᵇ I-Jᵇ I-Eᵇ I-Cᵇ Dᵇ), and (B10.D2 × B10)F₁ B cells (T-B collaboration was measured in irradiated (DBA/2 × B6)F₁ mice). Opposite results were observed with F₁ T⁺(SRC-DBA/2) cells, i.e. poor cooperation was seen...
## Table VI

| T cells (DON × B6)F1 | B cells (anti-thy1.2-spleen) | H-2 region of B cells | Anti-SRC PFC/spleen at 7 days in irradiated (DON × B6)F1 mice |
|----------------------|-----------------------------|----------------------|-------------------------------------------------------------|
|                      |                             | K       | I-A | I-B | I-D | I-E | I-C | D   | IgM | IgG |
| DBA/2                |                             | d       |     |     |     |     |     |     | 430 (1.28) | 1,250 (1.45) |
| B10.D2               |                             | d       |     |     |     |     |     |     | 750 (1.25)  | 660 (1.37)    |
| B10.A(5R)            |                             | b       | b   | b   | k   | k   | d   | d   | 56,190 (1.27) | 87,190 (1.18) |
| B10                  |                             | b       | b   |     |     |     |     |     | 7,180 (1.08)  | 20,870 (1.14) |
| (B10.D2 × B10)(F1)   |                             | b/d     |     |     |     |     |     |     | 14,690 (1.11) | 35,240 (1.22) |
| DBA/2                |                             | d       |     |     |     |     |     |     | 13,010 (1.40) | 55,540 (1.11) |
| B10.D2               |                             | d       |     |     |     |     |     |     | 49,750 (1.15) | 88,420 (1.08) |
| B10.A(5R)            |                             | b       | b   | b   | k   | k   | d   | d   | 920 (1.28)   | 4,780 (1.24)  |
| B10                  |                             | b       | b   |     |     |     |     |     | 190 (1.25)   | 200 (1.28)    |
| (B10.D2 × B10)(F1)   |                             | b/d     |     |     |     |     |     |     | 24,510 (1.13) | 55,850 (1.14) |

* As for Table I.
§ As for Table I. All B cells transferred in a dose of 5 × 10⁶ viable cells.
¶ As for Table I. Background values for B cells transferred alone have been subtracted. These values (PFC/spleen) were: DBA/2 170 (1.19) (IgM), 220 (1.63) (IgG); B10.B2 170 (1.38) (IgM), 180 (1.61) (IgG); B10.A(5R) 950 (1.48) (IgM), 1,940 (IgG); B10 100 (1.29) (IgM), < 100 (IgG); (B10.D2 × B10)(F1) 550 (1.21) (IgM), 180 (1.84) (IgG).

With both B10 and B10.A(5R) B cells. These findings with B10.A(5R) B cells suggest that, as for the (H-2d × H-2d)F1 combination (Table V), activation of (H-2d × H-2d)F1 T cells in a parental strain environment generated helper cells which collaborated specifically with B cells sharing the left-hand region of the H-2 complex with the strain used for activation. Attempts were not made to study the effect of T-cell activation in B10.A(5R) mice, since the H-2k determinants expressed by the I-J and I-E subregions of these mice might in theory have induced allosensitization. D2.GD (Kd I-A d I.B d . . . D b) mice would have been suitable for this purpose, but were not available.

On the Stimulation of F1 B Cells by F1 T Cells Activated to Antigen in Parental Strain Mice. Data presented in the preceding paper demonstrated that (CBA × B6)F1 T cells positively selected to SRC in irradiated parental strain mice invariably gave good responses with F1 B cells. Nevertheless, these responses tended to be lower than the responses observed when F1 B cells were transferred with F1 T cells activated in irradiated F1 mice (F1 T+(SRC-F1), cells). Conversely, parental strain B cells were triggered more effectively by F1 T cells activated in irradiated mice of this parental strain than by F1 T+(SRC-F1), cells. These reciprocal differences, though not dramatic, were seen in 10 of 11 experiments, and they applied to both IgM and IgG PFC (see Table III of this paper, and Tables IV, V, and Figs. 1, 2 of preceding paper); the differences were reflected in titers of serum hemagglutinins as well as in numbers of splenic PFC measured at 5, 7, and 9 days post-transfer. The results of five experiments are tabulated in Table VII. In these experiments the helper functions of F1 T+(SRC-F1), cells and F1 T+(SRC-B6), cells transferred at a dose of 0.8 × 10⁶ cells were compared for their capacity to stimulate F1 B cells and B6 B cells. For simplicity, the results are expressed in terms of the ratios of the responses obtained. In the case of F1 B cells, it can be seen that F1 T+(SRC-F1), cells were more effective as helper cells than were F1 T+(SRC-B6), cells. The ratio of PFC numbers produced by
TABLE VII
Relative Helper Function of (CBA x B6)F1 T Cells Positively Selected to SRC in Irradiated F1 or B6 Mice: Preferential Helper Function for B Cells Syngeneic with the Strain Used for T-Cell Activation

| Ratio* | Number of T cells transferred with constant number of B cells | Anti-SRC PFC with F1 T+(SRC-~,I F1 T+(SRC-B6) | Anti-SRC PFC with F1 T+(SRC-B6) |
|--------|---------------------------------------------------------------|-----------------------------------------------|---------------------------------|
|        |                                                                 | Exp. 1 | Exp. 2 | Exp. 3 | Exp. 4 | Exp. 5 | Mean   |
| 1. Ratio with 0.8 x 10⁶ IgM | 1.72 5.49 5.33 3.25 5.09 4.18 |
| 0.8 x 10⁶ IgG | 2.10 2.59 2.53 3.09 2.89 2.64 |
| 2. Ratio with 0.8 x 10⁶ IgM | 0.28 0.24 0.24 1.10 0.68 0.51 |
| 0.8 x 10⁶ IgG | 0.31 0.30 0.30 1.19 0.45 0.51 |
| Ratio 1 + Ratio 2 | 6.14 22.88 22.21 2.95 7.49 8.20 |
| 6.77 8.63 8.43 2.60 6.42 5.18 |

* The data are expressed in terms of the mean number of PFC/spleen (geometric mean of four mice per group) measured at 7 days post-transfer. B cells (from spleen) transferred in a dose of 5 x 10⁶ viable cells for F1 B cells and 8 x 10⁶ for B6 B cells.

Discussion

A variety of approaches failed to show that the restricted helper function of F1 T cells positively selected to SRC in irradiated parental strain mice reflects a lack of appropriate macrophages during T-B collaboration in vivo. Analogous findings have recently been reported by Swierkosz et al. (5) using an in vitro system. One is thus left with the possibility that the present data reflect restrictions acting not only at the level of helper cell induction, but also during T-B collaboration. With respect to T-B interaction, studies with congenic-resistant strains and recombinant mice suggested that for both (CBA x B6)F1 T cells (Table V) and (DBA/2 x B6)F1 T cells (Table VI), activation to antigen in irradiated mice of one parental strain selected for a population of helper cells...
which collaborated only with B cells sharing the K-end of the H-2 complex with the strain used for T-cell activation. Experiments with (CBA × B6)F₁ (H-2<sup>k</sup> × H-2<sup>b</sup>) T cells and B10.A(4R) (K<sup>b</sup> I-A<sup>k</sup> I-B<sup>b</sup> . . . D<sup>b</sup>) B cells, indicated that the determinants controlling collaboration mapped to the left of the I-B subregion, i.e. in the K or I-A subregions. The determinants controlling T-cell activation (presumably a reflection of T-macrophage interactions, see preceding paper) also appeared to map to this region since (CBA × B6)F₁ T cells collaborated well with both B10.Br and B10.A(4R) B cells, but not with B10 B cells, irrespective of whether the T cells were activated in irradiated B10.Br or in B10.A(4R) mice (Table V).

Before assessing the significance of these findings it is important to consider recent evidence which suggests that, as with F₁, T cells, the response of parental strain T cells to SRC in vivo is subject to H-2 gene control at the level of both T-macrophage interactions and T-B collaboration. This evidence was derived from experiments with T cells which had been depleted of specific alloreactive lymphocytes by an acute procedure, i.e. recirculation from blood to lymph through irradiated H-2-incompatible mice. First, T cells exposed to antigen in H-2-different hosts appeared to be unable to see the antigen in terms of helper
cell induction. Thus, parental strain T cells transferred to irradiated mice in the presence of SRC underwent positive selection to the antigen (i.e., were induced to become activated helper cells) only when the cell recipients shared H-2 determinants with the donor T cells (excellent selection occurred in semiallogeneic [F₁] mice). Second, parental strain T cells failed to stimulate anti-SRC responses by allogeneic B cells unless the T and B cells shared H-2 determinants, in particular K/IA subregion determinants; effective collaboration occurred with F₁ B cells (6, and unpublished observations).

Two predictions follow from these data. Both center on the phenomenon of negative selection—a process occurring within 1–2 days of exposure to antigen which leads to a transient disappearance of specific antigen-reactive lymphocytes from the circulation, e.g. TDL. During negative selection, lymphocytes reactive to the injected antigen become temporarily sequestered in regions such as the spleen before reentering the circulation (7). The first prediction is that if negative selection always precedes positive selection (which is very likely), the failure to observe T-helper cell induction in irradiated H-2-different mice (vide supra) should be paralleled by an equivalent failure to observe negative selection. This has been verified by transferring purified parental-strain T cells with SRC into irradiated mice, and collecting the donor-derived cells from thoracic duct lymph of the recipients 1–2 days later. These T cells were selectively depleted of helper reactivity for the injected antigen but, significantly, only when the donor and host shared H-2 determinants (I-region compatibility was sufficient, and effective selection occurred in semiallogeneic hosts).²³

The second prediction, which follows from the first, is that the restriction in the helper function of F₁ T cells transferred with antigen to irradiated parental strain mice would change reciprocally depending upon whether the cells were harvested from the recipients during the stage of negative selection or during positive selection. Thus, if only one of the two putative subpopulations of F₁ T cells responded to the injected antigen, this subgroup would initially be withdrawn from the circulation to the lymphoid tissues, whereas the other population would ignore the antigen and continue to recirculate. If so, then by analogy with the present data on positive selection of F₁ T cells, F₁ cells taken from the lymph during the stage of negative selection should not collaborate with B cells derived from the strain used for activation, but should act as unprimed helper cells for B cells of the opposite parent and for F₁ B cells. Recent studies have verified this prediction.⁴

The above evidence on the in vivo response to SRC is in line with the observations of other workers indicating that T cells from homozygous mice are

² J. Sprent. 1977. H-2 gene control of homozygous T helper cell induction in vivo. Manuscript in preparation.
³ These data apply to help provided for B cells syngeneic with the donor T cells. It might be suggested that selection to antigen would occur in H-2-different hosts if help were examined for B cells syngeneic with the allogeneic host. In this respect it should be emphasized that under no circumstances (with the exception of raising T cells in a chimeric environment) has the author observed collaboration between H-2-different T and B cells.
⁴ J. Sprent. 1977. Restricted helper function of F₁ hybrid T cells revealed by negative selection through irradiated parental strain mice. Manuscript in preparation.
subject to H-2 gene control both at the level of T-macrophage interactions (8-11) and during T-B collaboration in vivo (12-14). At each of these two levels, the relevant genes mapped in the I-A subregion of the H-2 complex. The fact that, at least with one strain combination, the restrictions observed in the present system both mapped to the left of the I-B subregion is clearly consistent with these data, although further work will be necessary to map the restrictions precisely. If the restrictions observed with F1 T cells do map to the I-A subregion, one is led to the intriguing conclusion that F1 T cells behave functionally as a mixture of (mutually tolerant) T cells derived from the two homozygous parental strains.

A question of obvious importance is whether the restrictions affecting T-macrophage interactions and T-B collaboration are controlled by the same gene (or set of genes) or, alternatively, by two closely linked genes. Although no evidence is available which bears directly on this point, it is conceptually simpler to think in terms of one gene. According to this view, both macrophages
and specifically-reactive B cells present MHC-associated antigen to T cells in an identical or similar fashion. F1 mice contain two subgroups of T cells, each of which is reactive to MHC-associated antigen presented by macrophages or specific B cells of one of the two parental strains. Thus, as illustrated in Fig. 1, positive selection of \((a \times b)F_1\) T cells via macrophage-associated antigen in irradiated parental strain \(a\) induces clonal expansion of the subgroup of T cells destined to help strain \(a\) B cells (and \(F_1\) B cells). The subgroup of T cells reactive to strain \(b\) B cells is not stimulated and remains in an unprimed (nonexpanded) state. This subgroup is activated in strain \(b\) mice, and both subgroups are stimulated in the \(F_1\) environment.

Fig. 1 portrays these restrictive interactions in terms of dual recognition. According to this viewpoint, each of the two subgroups of \((a \times b)F_1\) T cells express identical receptors for conventional antigen X. In addition, the cells carry another set of receptors with specificity for self H-2 (presumably I-A) determinants of either strain \(a\) or strain \(b\); these anti-self receptors are clonally distributed, or if allelic, are allelically excluded (see 10, 13, 15, and 16 for other models of dual recognition). In the case of the \(F_1\) T cells reactive to self H-2\(^a\) determinants, these cells are triggered by a complex of H-2\(^a\)-X presented by (or possibly secreted by; 17) macrophages of strain \(a\). The activated helper cells then stimulate B cells which express self H-2\(^a\) determinants and, in addition, carry antigen X in their Ig receptors. Alternatively, the activated T cell could bind free antigen and focus it onto the appropriate B cell.

An analogous model could be constructed on the basis of altered self recognition (15). Here, one must postulate that altered self determinants are created on the surface of B cells as well as on macrophages; at the B-cell level, the H-2 determinants on the cell would presumably have to be modified by antigen bound to the cell via Ig receptors.

Further genetic mapping studies and experiments with other antigens will be required before the above models can be evaluated critically. It will also be necessary to accommodate the conflicting evidence that T cells release factors which stimulate B cells across H-2 barriers (18, 19), and that according to some groups (20–22), although not others (23), T-B collaboration measured in vitro proceeds across MHC barriers.

Finally, comment should be made on the puzzling finding that \(F_1\) T cells cooperated preferentially with B cells syngeneic with the strain used for activation (Table VII). Thus, \(F_1\) T cells activated to antigen in parental strain mice gave relatively better responses with B cells of that parental strain than with \(F_1\) B cells; conversely, \(F_1\) T cells activated in \(F_1\) mice stimulated \(F_1\) B cells more effectively than parental strain B cells. Significantly, these preferences were observed with small numbers of T cells (\(\leq 0.8 \times 10^6\)) but not with higher doses (Table VIII). This explains the help provided for parental strain B cells by \(F_1\) T cells activated in \(F_1\) mice. Here it can be argued that only one-half of the T cells generated in an \(F_1\) environment provide help for one of the parental B-cell populations. Increasing the dose of helper cells should therefore give maximal help. The situation with the response of \(F_1\) B cells is more complex. A priori, the phenomenon might reflect (a) a mild degree of suppression, (b) allelic exclusion on \(F_1\) B cells of the H-2 determinants controlling collabora-
tion, (c) co-dominant expression of H-2 determinants which, by competition, inhibit collaborative interactions with one of the two subgroups of F1, T cells, or (d) the existence of a third subgroup of F1, T cells which has specificity for hybrid-specific determinants on F1, B cells (24). The fact that the phenomenon was not observed with higher doses of helper cells is against possibilities (a) and (b), unless one argues that for (a), F1, B cells mediate the suppression, or that for (b), increasing help provided for one subgroup of B cells eventually leads indirectly to stimulation of the other subgroup. Various objections can be raised against the third possibility, not the least of which is the unsubstantiated corollary that T-B collaboration should be generally less efficient in F1, mice than in homozygous mice. The fourth possibility is difficult to assess without further information.

Summary

Studies with H-2-congenic and recombinant strains showed that when F1, hybrid T cells were activated to sheep erythrocytes in irradiated mice of parental strain or related strain, a population of helper cells was generated which collaborated only with B cells sharing the K-end of the H-2 complex with the strain used for activation. No evidence was found that the restriction in helper function (a) reflected a deficiency of appropriate macrophages during T-B collaboration, or (b) was influenced by the Ig allotype of the B cells. It was concluded that the results signified restrictions acting at both the level of helper cell induction (presumed to be a reflection of T-macrophage interactions in the irradiated intermediate hosts) and during T-B collaboration. With (CBA × C57BL/6)F1, T cells, the restrictions at each level mapped to the same region, i.e. to the left of the I-B subregion. Consequently, one gene (or set of genes) might control restriction at both levels. If so, T-cell recognition of major histocompatibility complex-associated antigen on macrophages and on specific B cells would be either identical or very similar.

The fact that genes mapping to the K-end of the H-2 complex also control the restrictive interactions of homozygous T cells implies that F1, T cells behave functionally as a mixture of T cells derived from the two parental strains. Positive selection to antigen in parental strain mice appears simply to alter the ratio of these two populations.

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