LYMPHOID FUNCTION IN F1 → PARENT CHIMERAS

Lack of Evidence for Adaptive Differentiation
of B cells or Antigen-presenting Cells*

BY J. SPRENT AND JENNIFER BRUCE

From the Immunobiology Unit, Department of Pathology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104; and the Wistar Institute, Philadelphia, Pennsylvania 19104

It is now well accepted that the specificity of T lymphocytes is directed toward recognition of antigen associated with H-2 determinants. The studies of Zinkernagel et al. (1) strongly suggest that the capacity for T cells to recognize antigen presented in association with a given set of H-2 determinants depends upon the T cells encountering these determinants in the thymus during early differentiation. For example, in normal H-2 heterozygous mice it is argued that confrontation with both sets of parental H-2 determinants on the thymic stroma during ontogeny leads to the production of two discrete subgroups of T cells, each restricted to interact with target cells of only one of the two parental strains. The numerous reports demonstrating such a dichotomy of T cells in F1 mice are consistent with this viewpoint (2).

Strong support for the notion that the thymus determines T-cell specificity has come from studies on the function of heterozygous T cells differentiating from stem cells (marrow cells) in the thymus of irradiated homozygous mice. Despite their F1 origin, the T cells formed in these F1 → parent chimeras interact only with target cells of the host parental strain; responses with cells of the opposite parental strain are low or absent (2). F1 → parent chimeras thus appear to contain only one of the two subgroups of T cells found in normal F1 mice.

Recently Katz et al. (3) have suggested that, like T cells, B cells undergo a process of adaptive differentiation in Fa ~ parent chimeras. Using a hapten-carrier system, these workers observed that normal strain a T cells or T cells from F1 → a chimeras (irradiated strain a mice repopulated with (a × b)Fa marrow cells) collaborated more effectively with homologous F1 → a chimera B cells than with heterologous F1 → b chimera B cells. This finding has important implications and adds a new dimension to the problem of the generation of B-cell diversity. Therefore, it would seem essential to determine whether the phenomenon is a general one. In this respect, the present paper demonstrates that, at least in one situation, i.e., the response to heterologous erythrocytes measured in vivo, B cells from F1 → parent chimeras behave indistinguishably from normal F1 B cells.

Materials and Methods

Preparation of Chimeras. CBA/Cum (CBA) (H-2b) and C57BL/6 (B6) (H-2a) mice were heavily irradiated and reconstituted with 2 × 10^7 anti-Thy 1.2-serum-treated (CBA × B6)F1 bone marrow cells intravenously exactly as described in a previous report (4). Chimeras were used as donors of T and B cells at 3–14 mon after reconstitution.

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Testing Chimerism. Each chimera used to provide T or B cells was tested individually for its content of donor-derived lymphoid cells by the use of appropriate anti-H-2 sera and complement (4). The spleen and pooled lymph nodes (LN) of all chimeras used in the present studies were ≥97% of donor-F1 marrow origin.

T Cells. Nylon-wool-purified LN T cells pooled from peripheral and mesenteric LN of five-eight unprimed chimeras were activated to sheep erythrocytes (SRC) in irradiated (CBA × B6)F1 mice as described elsewhere (4, 5). The SRC-activated T cells were recovered either from thoracic duct lymph or the spleen plus LN of the recipients 6 d later for use as T helper cells.

When using CBA T cells as helper cells, LN cells from CBA mice primed 2 mon before with SRC were first depleted of reactivity to B6 alloantigens by acute recirculation through irradiated (CBA × B6)F1 mice (6). Unprimed (CBA × B6)F1 LN cells were positively selected to SRC in irradiated parental-strain mice as described elsewhere (5).

B Cells. Spleen cells from chimeras or from normal mice (two spleen donors per group) were treated with anti-Thy 1.2 serum and complement to remove T cells (5).

T-B Collaboration. T-B collaboration was measured in vivo by transferring T cells plus B cells and SRC (0.1 ml of 5% solution) intravenously into (CBA × B6)F1 mice given 800 rad 1 d previously (5). IgM (direct) and IgG (indirect) plaque-forming cells (PFC) were measured in the spleen 7 d later.

Results

B Cells from F1 → Parent Chimeras. The approach was to test collaborative interactions between T and B cells from normal and chimeric mice in the PFC response to SRC measured in irradiated F1 hybrid mice (Materials and Methods). T cells taken from unprimed F1 → parent chimeras were first activated to SRC for 6 d in irradiated F1 mice.

Four experiments were performed with T and B cells from F1 → parent chimeras. In three studies, the B cells were taken from SRC-primed chimeras; unprimed chimera B cells were used in the fourth experiment. A representative experiment with primed chimera B cells is shown in Table I. In all experiments, the chimera T cells collaborated well with B cells of the parental strain used for marrow reconstitution, but very poorly with B cells from the opposite parental strain. However, in none of the experiments did the chimera T cells display any obvious restriction in their capacity to stimulate chimera B cells, even with limiting doses of T cells. For example, F1 → CBA chimera T cells collaborated just as effectively with heterologous F1 → B6 chimera B cells as with homologous F1 → CBA chimera B cells (Table I). This point was examined semi-quantitatively by calculating the ratio of the PFC numbers obtained with the homologous chimera B cells divided by the PFC numbers observed with the heterologous chimera B cells. A value of unity would thus signify no preference for the expression of T-cell help. The total mean ratio observed for three experiments with primed B cells plus the one experiment with unprimed B cells was 1.07 (range 0.59–1.51, 14 determinations) for IgM PFC and 0.98 (0.46–2.25, 14 determinations) for IgG PFC.

Analogous data were obtained with two different types of nonchimeric T cells (Table II), viz., normal homozygous T cells (experiment 1) and F1 T cells positively selected to SRC in irradiated parental strain mice (experiment 2). Although these cells showed marked restriction in collaborating with parental strain B cells, no restriction was apparent with F1 → parent chimera B cells.

Expression of Ig Allotype in F1 → Parent Chimeras. PFC in Table II were enhanced with an anti-Ig6 allotype antiserum specific for Ig allotypes of B6 but not of CBA (5). It is evident that both F1 → CBA and F1 → B6 chimera B cells produced approximately equivalent numbers of Ig6-PFC. Therefore, the Ig allotype of the hosts used
### Table I

Collaborative Interactions between T and B Cells from F1 → Parent Chimeras: SRC-primed B Cells

| Donors of SRC-activated T cells* | Dose of T cells | Donors of primed B cells | Anti-SRC PFC/spleen at 7 d in irradiated (CBA × B6)F1 mouse |
|---------------------------------|----------------|--------------------------|-------------------------------------------------------------|
|                                 |                |                          | IgM | IgG |
| F1 → CBA chimeras              | 0.9            | CBA                      | 11,790 (1.21)§ | 50,760 (1.07) |
|                                 | 0.9            | B6                       | 0 | 0 |
|                                 | 0.3            | F1 → CBA chimeras        | 3,250 (1.12) | 20,920 (1.12) |
|                                 | 0.9            | F1 → CBA chimeras        | 10,350 (1.11) | 94,430 (1.16) |
|                                 | 0.3            | F1 → B6 chimeras         | 4,300 (1.12) | 34,270 (1.19) |
|                                 | 0.9            | F1 → B6 chimeras         | 8,800 (1.24) | 133,960 (1.10) |
| F1 → B6 chimeras               | 0.9            | CBA                      | 490 (1.26) | 930 (1.70) |
|                                 | 0.9            | B6                       | 9,780 (1.02) | 21,660 (1.30) |
|                                 | 0.3            | F1 → CBA chimeras        | 2,150 (1.21) | 14,780 (1.43) |
|                                 | 0.9            | F1 → CBA chimeras        | 6,370 (1.09) | 98,510 (1.02) |
|                                 | 0.3            | F1 → B6 chimeras         | 3,240 (1.21) | 14,490 (1.43) |
|                                 | 0.9            | F1 → B6 chimeras         | 8,200 (1.14) | 90,640 (1.06) |

* T cells from LN of unprimed chimeras were activated to SRC in irradiated F1 mice and recovered from the spleen plus LN of the recipients 6 d later.

§ $5 \times 10^6$ viable anti-Thy 1.2-serum-treated spleen cells from mice primed with SRC (0.1 ml of 25% solution) intraperitoneally 6-8 wk previously (two donors per group).

| Donors of primed B cells | Anti-SRC PFC/spleen at 7 d in irradiated (CBA × B6)F1 mouse |
|--------------------------|-------------------------------------------------------------|
|                          | IgM | IgG |
| 0.9 CBA                  | 11,790 (1.21)§ | 50,760 (1.07) |
| 0.9 B6                   | 0 | 0 |
| 0.9 F1 → CBA chimeras    | 3,250 (1.12) | 20,920 (1.12) |
| 0.9 F1 → CBA chimeras    | 10,350 (1.11) | 94,430 (1.16) |
| 0.9 F1 → B6 chimeras     | 4,300 (1.12) | 34,270 (1.19) |
| 0.9 F1 → B6 chimeras     | 8,800 (1.24) | 133,960 (1.10) |

### Table II

Capacity of Parental-Strain T Cells and SRC-selected F1 Hybrid T Cells to Collaborate with F1 → Parent Chimera B Cells

| Experiment Number | T helper cells | Donors of primed B cells* | Anti-SRC PFC/spleen at 7 d in irradiated (CBA × B6)F1 mouse |
|-------------------|----------------|--------------------------|-------------------------------------------------------------|
|                   |                |                          | IgM | IgG | Igb± |
| 1                 | CBA, B6        | CBA                      | 14,770 (1.43)* | 43,700 (1.25) | 0 |
|                   |                | B6                       | 1,250 (1.13) | 910 (1.50) | 660 (1.19) |
|                   |                | F1 → CBA chimeras        | 8,810 (1.09) | 22,000 (1.09) | 4,120 (1.19) |
|                   |                | F1 → B6 chimeras         | 11,610 (1.15) | 29,040 (1.18) | 8,700 (1.46) |
|                   | Normal (CBA × B6)F1 | CBA                      | 9,760 (1.02) | 16,730 (1.46) | 0 |
|                   |                | B6                       | 9,130 (1.27) | 13,040 (1.14) | 4,060 (1.33) |
|                   |                | F1 → CBA chimeras        | 25,020 (1.18) | 36,880 (1.11) | 8,510 (1.26) |
|                   |                | F1 → B6 chimeras         | 17,230 (1.03) | 37,060 (1.10) | 7,630 (1.29) |
| 2                 | F1 T+SRC-CBA, B6 | CBA                      | 24,150 (1.12) | 54,440 (1.28) | 0 |
|                   |                | B6                       | 2,920 (1.26) | 5,360 (1.19) | 3,670 (1.07) |
|                   |                | F1 → CBA chimeras        | 18,990 (1.16) | 28,580 (1.08) | 19,620 (1.14) |
|                   |                | F1 → B6 chimeras         | 39,620 (1.21) | 45,210 (1.17) | 14,064 (1.13) |
|                   | F1 T+SRC-B6, B6 | CBA                      | 1,000 (1.23) | 2,250 (1.22) | 0 |
|                   |                | B6                       | 19,120 (1.17) | 33,880 (1.18) | 18,570 (1.23) |
|                   |                | F1 → CBA chimeras        | 10,170 (1.39) | 19,580 (1.15) | 8,780 (1.18) |
|                   |                | F1 → B6 chimeras         | 15,910 (1.14) | 29,610 (1.05) | 6,400 (1.34) |

* T cells from LN of unprimed chimeras were activated to SRC in irradiated F1 mice and recovered from the spleen plus LN of the recipients 6 d later.

§ In experiment 1, SRC-primed CBA LN cells were depleted of reactivity to B6 alloantigens by acute recirculation through irradiated (CBA × B6)F1 mice (6). A dose of $2.5 \times 10^6$ of these CBA-ε T cells were used as helper cells. Nylon-wool-passed (CBA × B6)F1 LN cells were used as controls.

|| In experiment 2, unprimed (CBA × B6)F1 LN T cells were positively selected to SRC for 5 d in irradiated CBA mice (F1 T+SRC-CBA) or B6 mice (F1 T+SRC-B6) (5). The SRC-selected donor T cells were obtained from thoracic duct lymph of the irradiated hosts and transferred in a dose of $0.8 \times 10^6$ cells as helper cells.

* As in Table I. Subtracted background PFC numbers for B cells transferred without T cells were all <1,000 PFC/spleen for IgM and IgG. PFC numbers for T cells transferred without B cells were <300 PFC/spleen.

| Donors of primed B cells | Anti-SRC PFC/spleen at 7 d in irradiated (CBA × B6)F1 mouse |
|--------------------------|-------------------------------------------------------------|
|                          | IgM | IgG | Igb± |
| 0.9 CBA                  | 14,770 (1.43)* | 43,700 (1.25) | 0 |
| 0.9 B6                   | 1,250 (1.13) | 910 (1.50) | 660 (1.19) |
| 0.9 F1 → CBA chimeras    | 8,810 (1.09) | 22,000 (1.09) | 4,120 (1.19) |
| 0.9 F1 → B6 chimeras     | 11,610 (1.15) | 29,040 (1.18) | 8,700 (1.46) |

| Donors of primed B cells | Anti-SRC PFC/spleen at 7 d in irradiated (CBA × B6)F1 mouse |
|--------------------------|-------------------------------------------------------------|
| 0.9 CBA                  | 14,770 (1.43)* | 43,700 (1.25) | 0 |
| 0.9 B6                   | 1,250 (1.13) | 910 (1.50) | 660 (1.19) |
| 0.9 F1 → CBA chimeras    | 8,810 (1.09) | 22,000 (1.09) | 4,120 (1.19) |
| 0.9 F1 → B6 chimeras     | 11,610 (1.15) | 29,040 (1.18) | 8,700 (1.46) |

$\dagger$ PFC enhanced with a polyvalent anti-Ig b anti-allotype serum (5).
**Table III**

**Antigen Presentation by F<sub>1</sub> → Parent Chimeras: T Helper Specificity of Normal (CBA × B6)F<sub>1</sub> T Cells Activated to SRC for 6 d in Re-irradiated F<sub>1</sub> → Parent Chimeras**

| Irradiated hosts used for activating normal (CBA × B6)F<sub>1</sub> T cells to SRC* | Dose of SRC-activated T helper cells | Anti-SRC PFC/spleen at 7 d in irradiated (CBA × B6)F<sub>1</sub> mice |
| --- | --- | --- |
|  | × 10<sup>6</sup> cells |  | IgM | IgG |
| CBA | 0.9 | CBA | 8,320 (1.21)† | 46,270 (1.11) |
|  | 0.9 | B6 | 630 (1.33) | 1,410 (1.09) |
| B6 | 0.9 | CBA | 870 (1.40) | 890 (1.16) |
|  | 0.9 | B6 | 12,300 (1.15) | 21,540 (1.31) |
| (CBA × B6)F<sub>1</sub> | 0.3 | CBA | 1,530 (1.06) | 11,150 (1.24) |
|  | 0.9 | CBA | 4,180 (1.39) | 22,330 (1.05) |
|  | 0.3 | B6 | 3,020 (1.23) | 5,990 (1.40) |
|  | 0.9 | B6 | 9,460 (1.16) | 13,200 (1.36) |
| (CBA × B6)F<sub>1</sub> → CBA chimeras§ | 0.3 | CBA | 2,270 (1.33) | 15,570 (1.15) |
|  | 0.9 | CBA | 7,980 (1.09) | 30,520 (1.23) |
|  | 0.3 | B6 | 3,010 (1.30) | 4,431 (2.29) |
|  | 0.9 | B6 | 8,240 (1.23) | 13,600 (1.17) |
| (CBA × B6)F<sub>1</sub> → B6 chimeras§ | 0.3 | CBA | 3,590 (1.24) | 13,350 (1.31) |
|  | 0.9 | CBA | 7,420 (1.19) | 25,260 (1.17) |
|  | 0.3 | B6 | 2,820 (1.36) | 3,590 (1.28) |
|  | 0.9 | B6 | 5,280 (1.16) | 16,210 (1.17) |

* 5 × 10<sup>6</sup> normal unprimed (CBA × B6)F<sub>1</sub> LN T cells plus SRC (0.5 ml of 25%) were transferred intravenously into normal and chimeric mice given 850 rad 1 d before and recovered from the spleen plus LN of the irradiated recipients 6 d later. The chimeras had been reconstituted with marrow cells 6 mon previously.
† As in Table I. Subtracted background PFC numbers for B cells transferred without T cells were all <250 PFC/spleen.
§ Testing other chimeras of the same batch with anti-H-2 serum showed that >96% of thyoglycollate-induced peritoneal-exudate cells were of donor-F<sub>1</sub> origin.

for preparing the chimeras did not appear to affect allotype expression by the newly differentiating donor B cells.

**Antigen-presenting Cells in F<sub>1</sub> → Parent Chimeras.** Exposure of normal F<sub>1</sub> T cells to SRC in irradiated mice of one parental strain activates only one of the two T-cell subgroups (5); both subgroups are stimulated in normal F<sub>1</sub> mice. Although the precise identity of the cells presenting antigen in an H-2 restricted fashion to T cells in vivo has yet to be established, the cells concerned are probably of the macrophage lineage (5).

Preliminary work established that macrophage-like cells, e. g., peritoneal exudates, from F<sub>1</sub> → parent chimeras were almost entirely (≥96%) of donor-F<sub>1</sub> origin (footnotes to Table III). This strongly suggested that the antigen-presenting cells in the chimeras were of donor origin. If so, would the antigen-presenting mechanism in F<sub>1</sub> → parent chimeras be similar to that of normal F<sub>1</sub> mice or, because of adaptive differentiation, would it resemble that of parental strain mice?

To study this point, the helper function of normal F<sub>1</sub> T cells was tested after activation to SRC for 6 d in irradiated normal mice or in re-irradiated F<sub>1</sub> → parent chimeras. As shown in Table III, unprimed F<sub>1</sub> T cells activated to SRC in irradiated parental-strain mice collaborated well with B cells of the strain used for activation but poorly with B cells of the opposite parental strain. Significantly, F<sub>1</sub> T cells activated in re-irradiated F<sub>1</sub> → parent chimeras gave good responses with either
population of parental-strain B cells. Thus, as in normal F1 mice and in contrast to parental-strain mice, antigen presentation in F1 → parent chimeras was sufficient to stimulate both subgroups of normal F1 T cells.

Discussion

The theory that H-2 determinants encountered during ontogeny determine T-cell specificity has encountered comparatively little opposition, largely because the decisive role of H-2 determinants on the function of mature T cells is well established. The possibility that B cells undergo an analogous process of adaptive differentiation, however, is less attractive for two reasons. Firstly, in contrast to T cells, there is no evidence that the H-2 complex influences B-cell recognition of antigen. Therefore, it is difficult to envisage a mechanism whereby confrontation with H-2 determinants during early development could affect B-cell specificity. Secondly, the concept of adaptive differentiation of Katz et al. (3) states the H-2-encoded structures on B cells required for interaction with specific T-cell subgroups are clonally distributed. Thus in normal heterozygous mice, Katz et al. envisage two discrete subgroups of B cells, each carrying H-2-encoded determinants of only one parental haplotype. To date there is no precedent for such allelic exclusion of H-2 determinants, at least as detected serologically.

Our failure to detect any evidence for abnormal differentiation of B cells in F1 → parent chimeras clearly contrasts with the findings of Katz et al. Attempting to reconcile these conflicting findings is difficult because the mouse strains, antigens and assay systems used in the two studies were different. Hence the present data cannot be said to contradict the findings of Katz et al. Nevertheless, the data do imply that H-2-dependent adaptive differentiation of B cells may not be a general phenomenon.

Because H-2 restriction applies to T-macrophage interactions as well as to T-B collaboration, we thought it also of interest to determine whether macrophages (accessory cells) undergo abnormal differentiation in F1 → parent chimeras. Studies on the helper specificity of normal F1 T cells activated to SRC in re-irradiated F1 → parent chimeras (Table III) suggest in fact that, as for B cells, the antigen-presenting mechanism in these chimeras is not discernably different from that in normal F1 mice.

The present data thus favor the conservative view that the H-2 determinants on B cells and macrophages which restrict T-cell function are expressed codominantly on F1 hybrid cells and that the expression of these determinants is not influenced by the environment in which cell differentiation occurs.

Summary

Information was sought on whether B cells undergo abnormal differentiation in F1 → parent chimeras (irradiated parental-strain mice reconstituted with F1-hybrid bone marrow cells). As assessed by collaborative responses to sheep erythrocytes in vivo, three different types of T cells restricted to interaction with strain a H-2 determinants were shown to collaborate as effectively with heterologous F1 → b chimera B cells as with homologous F1 → a chimera B cells. This applied to both primed and unprimed B cells, to IgM- and IgG-antibody formation and to production of Ig allotype. Thus, unlike T cells, B cells from F1 → parent chimeras behaved indistinguishably from normal F1 B cells.

F1 → parent chimeras were also examined for their capacity to present antigen to
normal F1 T cells in vivo. The results suggested that the antigen-presenting cells in these chimeras were no different than in normal F1 mice.

Collectively these data imply that, at least in the situation studied, raising F1 stem cells in a parental-strain environment has a marked effect on T-cell specificity but does not discernably influence the differentiation of B cells or macrophage-like cells.

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