CELLULAR AND GENETIC CONTROL OF ANTIBODY RESPONSES

V. Helper T-Cell Recognition of H-2 Determinants on Accessory Cells but Not B Cells

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Antibody responses to most antigens require interactions among T lymphocytes, B lymphocytes, and adherent accessory cells. The mechanisms by which genes within the major histocompatibility complex (MHC)[1] regulate the interactions among these cell populations and, hence, regulate immune responsiveness, remain controversial. The controversy derives, in part, from conflicting results that have been obtained as the experimental approaches to examining this question have become increasingly sophisticated. Initial studies examining the role of MHC-region genes in cell interactions indicated a requirement for MHC identity between adoptively transferred antigen-primed helper T cells and B cells (1), and between antigen-primed proliferating T cells and accessory cells (2). In contrast, subsequent studies demonstrated that strain$_A$ T cells, which had differentiated in an (A × B)F$_1$ chimeric environment, could collaborate equally well with either strain$_A$ or strain$_B$ B cells (3). This indicated that genotypic identity between helper T cells and B cells was not necessary for effective collaboration to occur. Also, recent experiments demonstrated that (A × B)F$_1$ T cells, that had been primed to antigen in a parent$_A$ host, only provided primed help to parent$_A$ B cells (4, 5), indicating that genotypic identity between helper T cells and B cells was also not sufficient for effective collaboration to occur. Thus, rather than a requirement for MHC homology between T cells and B or accessory cells, the role of MHC-region genes in cell interactions might be better understood as a requirement for T-cell recognition of MHC determinants expressed on the surface of accessory and/or B cells. Indeed, recent studies have suggested that antigen-specific antibody responses require that helper T cells recognize the identical MHC determinants on both accessory cells and B cells (5–8).

One difficulty with accepting the conclusions of these earlier studies (4–7) is that separated populations of T cells, B cells, and accessory cells were not used. In the present report, the requirements for helper T-cell recognition of murine MHC (H-2) determinants have been analyzed by separating all three interacting cell types into

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highly purified and functionally distinct subpopulations of T cells, B cells, and accessory cells. In this way, the requirements for helper T-cell recognition of H-2 determinants expressed on accessory cells and on B cells were individually assessed. Complicating allogeneic effects were minimized by utilizing $(A \times B)F_1 \rightarrow parent_A$ chimeric helper T cells which are genotypically F_1 yet, are restricted to recognizing the H-2 determinants of $parent_A$ and not $parent_B$ (9–11). This study demonstrates that for the generation of anti-hapten responses to trinitrophenyl conjugates of keyhole limpet hemocyanin: (a) a requirement for helper T-cell recognition of identical H-2 determinants on both accessory cells and B cells does not exist, either before or after antigen priming; (b) there exists a strict requirement for helper T-cell recognition of K or I-A region-encoded H-2 determinants expressed on accessory cells, and that such a requirement exists before antigen priming; and (c) no requirement was observed either in vitro or in vivo for helper T-cell recognition of H-2 determinants expressed on B cells.

Materials and Methods

Animals. C57BL/10Sn (B10), C57BL/6 (B6), B10.A, B10.A(5R), B10.A(4R), B10.BR, B10.D2, DBA/2, (B10 × B10.A)F_1, and (C57BL/6 × DBA/2)F_1 (BDF$_1$) mice were either obtained from The Jackson Laboratory, Bar Harbor, Maine, or were provided by Dr. David H. Sachs, National Institutes of Health, Bethesda, Md. 2- to 5-mo-old adult males were used in all experiments.

Chimeras. Recipient mice were irradiated with 850 rads x-ray and reconstituted 4–6 h later with 1–2 × 10^7 bone marrow cells that had been pretreated with rabbit anti-mouse brain serum (RαMB) + complement (C'). The RαMB reagent employed has been extensively characterized and is a cytotoxic reagent that is specific for T cells (12) with no detectable anti-stem-cell activity. The absence of anti-stem-cell activity of this RαMB was ascertained by in vivo pulse labeling with ^{125}iododeoxyuridine of proliferating donor cells in the spleens of irradiated (850 rads) mice that had been repopulated with treated or untreated syngeneic bone marrow (13) (unpublished data). Chimeras are designated as bone marrow donor → irradiated recipient. Spleen cells were obtained from each chimera no earlier than 3 mo after irradiation, and were individually typed by indirect immunofluorescence and/or complement-mediated cytotoxicity using H-2-specific alloantisera that was generously provided by Dr. David Sachs, National Institutes of Health, Bethesda, Md. Fluorescein isothiocyanate-conjugated rabbit anti-mouse IgG$_2$ reagent was used as the indirect fluorescent reagent and was kindly provided by Dr. Howard Dickler, National Institutes of Health, Bethesda, Md. The alloantisera used for typing the spleen cells from each chimera were: (a) (B10 × B10.A)F_1 → B10: positive with B10 anti-B10.A; (b) (B10 × B10.A)F_1 → B10.A: positive with B10.A anti-B10; (c) BDF$_1$ → B6: positive with (B10 × A/J)F_1 anti-B10.D2; and (d) B6 → BDF$_1$: positive with (B10.A × A/J)F_1 anti-B10; negative with (B10 × A/J) anti-B10.D2. All the chimera spleen cells used in these studies were >95% of donor origin.

Antigens. Keyhole limpet hemocyanin (KLH) (lot 530195, Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.) and ovalbumin (OVA) (lot A5503, Sigma Chemical Co., St. Louis, Mo.) were conjugated with 2,4,6-trinitrobenzene sulfonate (Pierce Chemical Co., Rockford, Ill.) as previously described (12). The degree of trinitrophenyl (TNP) substitution was 20 TNP groups/100,000 daltons KLH (TNP-KLH) and 12 TNP groups/100,000 daltons OVA (TNP-OVA). TNP-KLH was used in culture at a final concentration of 10 µg/ml.

In Vivo Immunization. Mice were primed with 50 µg KLH or 200 µg TNP-OVA in complete Freund's adjuvant intraperitoneally 4–10 wk and 3 mo, respectively, before use. However, all spleen cell populations were obtained from unimmunized animals unless specifically stated otherwise.

Preparation of Cells

ADHERENT CELL-DEPLETED SPLEEN CELLS.   Spleen cells were depleted of adherent accessory cells by passage over Sephadex G-10 columns (Pharmacia Fine Chemicals, Div. of Pharmacia

Inc., Piscataway, N. J.) as previously described (14). This procedure markedly reduces the percentage of latex-ingesting cells although it does not significantly alter the percentage of T cells or B cells (15).

T CELLS. T cells were prepared by passage of spleen cells over nylon fiber columns and collecting the nylon nonadherent (NNA) eluate. The purity of each T-cell population was functionally determined by the fact that none of the T-cell populations used generated any plaque-forming cells (PFC), either when cultured alone or when cultured with spleen adherent accessory cells. Cell-surface markers and mitogen-response characteristics of this population have previously been described (12).

(B + ACCESSORY) CELLS. (B + accessory) cells were prepared by depleting spleen cells of T cells by pretreatment with a T-cell-specific cytotoxic RαMB reagent + C'. The T-cell specificity of this reagent has been detailed in previous reports (12).

B CELLS. B cells were prepared by first depleting spleen cells of adherent cells by passage over Sephadex G-10 columns and by then depleting the Sephadex G-10-passed spleen cells of T cells by treatment with RαMB + C'.

SPLEEN-ADHERENT CELLS (SAC). 2-h glass-adherent spleen cells (SAC) were prepared by the method described by Cowing et al. (16). All SAC populations were pretreated with RαMB + C', irradiated with 1,000 rads, and precultured at 10^7/ml on a roller drum overnight before addition to the antibody cultures. Such populations have been characterized (15) and consist of 50–80% latex-ingesting cells, 8–15% nonphagocytic sIg$^+$ cells, <0.3% Thy 1.2$^+$ cells, and 15–25% cells negative for all markers. The accessory activity of spleen cell populations has been shown to reside in radiation resistant, non-T-, non-B-, glass-adherent cells (15) which express both I-A and I-E/C region-encoded cell-surface determinants (14), and which have recently been shown to phagocytose latex,[2] thus representing an Ia$^+$ subpopulation of spleen phagocytes.

TNP-KLH-PULSED SAC. SAC were prepared and treated as described above except that the SAC were cultured overnight on a roller drum in the presence of 100 μg/ml of TNP-KLH. Before the addition to culture, these cells were extensively washed to remove any soluble TNP-KLH.

Culture Conditions. All cultures were performed in a volume of 200 μl per flat-bottom well of microtiter plates (Linbro Chemical Co. Hamden, Conn.) and were incubated for 4 d at 37°C in a 5% CO_2-humidified air atmosphere. The medium employed was Eagle's minimum essential medium supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 1 mM sodium pyruvate, 1X nonessential amino acids, 15 mM Hepes buffer solution, 5 × 10^{-5} M 2-mercaptoethanol, and 10% fetal calf serum, as previously described (12). Cells were harvested by repeated pipetting, washed, resuspended in Hanks' balanced salt solution, and individual cultures assayed for hemolytic PFC. The optimal cell density for obtaining primary responses to TNP-KLH is 5 × 10^5 cells/200 μl culture (12) although the optimal cell density for obtaining secondary responses to TNP-KLH is 2 × 10^5 cells/200 μl culture (unpublished data). The cell number of each individual cell population added to each culture is indicated for each experiment.

In Vivo Assay for Cell Collaboration. 12- to 16-wk-old recipient mice were lethally irradiated with 850 rads. 4–6 h later, these animals were intravenously injected with unprimed spleen cells or spleen cell subpopulations and 50 μg TNP-KLH. 6 d after transfer, the spleens from these mice were removed and assayed for anti-TNP PFC.

PFC Assay. Sheep erythrocytes (SRBC) were conjugated with TNP (TNP-SRBC) by the method of Rittenberg and Pratt (17). Direct (IgM) PFC to TNP-SRBC were assayed by the slide modification of the Jerne hemolytic plaque technique (18). IgG anti-TNP PFC were assayed by first blocking all IgM PFC with a 1:250 dilution of goat anti-mouse μ serum and then developing with a 1:200 dilution of a 1:1 mixture of rabbit anti-mouse IgG₁ and IgG₂ sera (the generous gifts of Dr. Richard Asofsky, National Institutes of Health, Bethesda, Md.). The TNP specificity of the PFC generated under these culture conditions has been demonstrated by inhibition with a TNP-conjugated bovine serum albumin reagent which, in low concentration, inhibits anti-TNP PFC specifically (12). All points shown in each experiment represent the

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[2] Cowing, C., S. Sharrow, K. S. Hathcock, R. J. Hodes, A. Singer, and H. B. Dickler. Manuscript in preparation.
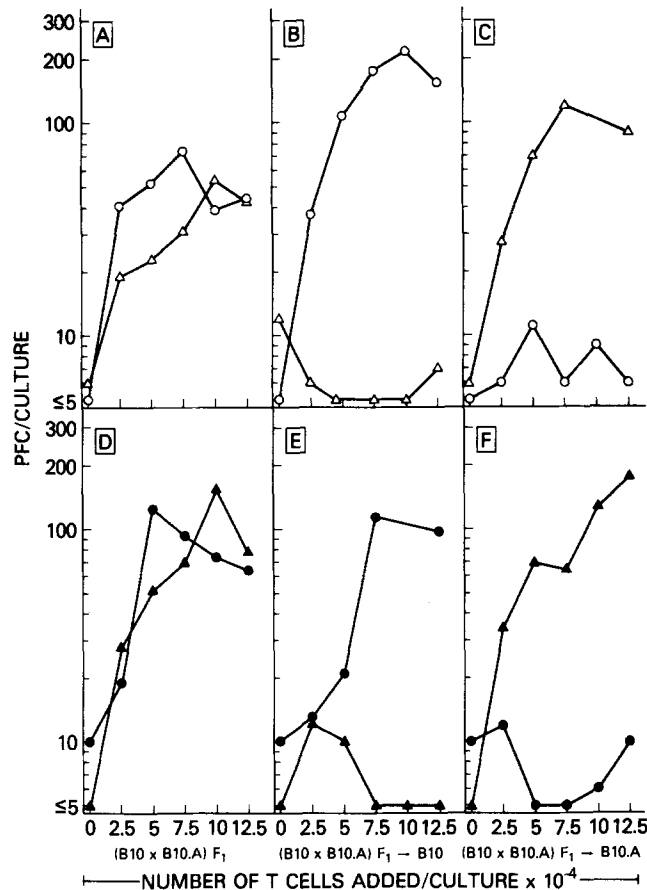
FIG. 1.   (A × B)F₁ → parentₐ chimeric helper T cells are restricted to recognizing K or I-A region-encoded H-2 determinants of parentₐ. Graded numbers of either normal or chimeric T cells were added to cultures containing 10 μg/ml TNP-KLH and 4 × 10⁵ (B + accessory) cells from B10 (○), B10.A (△), B10.A(5R) (●), or B10.A(4R) (▲). <5 PFC/culture were observed either in the absence of antigen or in cultures containing TNP-KLH and T cells alone.

mean responses of three replicate cultures or the mean responses of at least four adoptively transferred mice.

Results

(A × B)F₁ → Parentₐ Chimeric Helper T Cells are Restricted in Their Recognition of Parental H-2 Determinants Before Antigen Exposure.   To determine whether unprimed helper T cells are restricted in their recognition of H-2 determinants expressed by (B + accessory) cells, naive helper T cells from normal (B10 × B10.A)F₁ mice, (B10 × B10.A)F₁ → B10 chimeras, and (B10 × B10.A)F₁ → B10.A chimeras were assayed for their ability to cooperate with unprimed (B + accessory) cells from either parental (B10 and B10.A) or parental recombinant strains (B10.A[5R] and B10.A[4R]) for responses to TNP-KLH. Essentially, no responses were obtained in the absence of added T cells (Fig. 1); responses were obtained upon the addition of normal F₁ T cells to (B + accessory) cells from either parental (Fig. 1 A) or parental recombinant strain

(Fig. 1 D). In contrast, $F_1 \rightarrow$ B10 chimeric T cells only cooperated with (B + accessory) cells from B10 (Fig. 1 B) and B10.A(5R) (Fig. 1 E); $F_1 \rightarrow$ B10.A chimeric T cells only cooperated with (B + accessory) cells from B10.A (Fig. 1 C) and B10.A(4R) (Fig. 1 F). These data demonstrate in reciprocal $F_1 \rightarrow$ parent chimeric situations that unprimed (A × B)$F_1 \rightarrow$ parent$_A$ chimeric T cells, although genotypically F_1 and expressing both A and B parental H-2 determinants, are only able to recognize and cooperate with (B + accessory) cells that express the K or I-A region-encoded determinants of parent$_A$.

(A × B)$F_1 \rightarrow$ Parent$_A$ Chimeric Helper T cells are Restricted in Their Recognition of H-2 Determinants Expressed by Accessory Cells.   To eventually distinguish among the possibilities that helper T cells recognize the H-2 determinants expressed by accessory cells, B cells, or both, it was first determined whether helper T cells recognize the H-2 determinants expressed by accessory cells.

Spleen cells were depleted of adherent accessory cells by Sephadex G-10 passage, a procedure which both significantly diminishes responsiveness to TNP-KLH and diminishes the percentage of phagocytic cells without significantly altering the percentage of T cells and B cells (15). Responses that are diminished or abolished by Sephadex G-10 passage can be fully reconstituted by the addition to culture of SAC as accessory cells (12, 14, 15). The cells within the SAC population which mediate accessory function have been shown to be an Ia^+ subpopulation of spleen phagocytes (Materials and Methods).

The responses of BDF$_1 \rightarrow$ B6, BDF$_1 \rightarrow$ BDF$_1$, and B10.BR spleen cells were significantly diminished ($P < 0.01$) by Sephadex G-10 passage (Table I). These adherent cell-depleted spleen cell populations were then assayed for their ability to cooperate with SAC from H-2^b, H-2^d, and H-2^k mice. The number of SAC added to each culture (2–4 × 10^4) has previously been shown to be optimal for cooperation between syngeneic or semisyngeneic lymphocytes and accessory cells (12, 14, 15). It should be noted that under these conditions, allogeneic accessory cells did not reconstitute Sephadex G-10-depleted responses (Table I). The responses of both B10.BR and BDF$_1 \rightarrow$ BDF$_1$ lymphocytes were fully reconstituted (percent reconstitution >100%) by the addition of syngeneic or semisyngeneic SAC. In contrast, BDF$_1 \rightarrow$ B6 chimeric lymphocytes were only reconstituted by H-2^b (parent$_A$) SAC and not by H-2^d (parent$_B$) SAC. Thus, it appeared that (A × B)$F_1 \rightarrow$ parent$_A$ lymphocytes were restricted to only recognizing parent$_A$ H-2 determinants expressed by accessory cells. However, it was possible that the inability of H-2^d SAC to reconstitute the response of BDF$_1 \rightarrow$ B6 lymphocytes was not a result of an inability of these cells to collaborate with one another, but rather, a result of active suppression that was somehow induced by the presence of H-2^d (parent$_B$) SAC. Indeed, active suppression is induced by the presence in culture of allogeneic SAC as evidenced by the fact that the addition of allogeneic H-2^k SAC to culture suppressed the ability of H-2^b SAC to reconstitute the responses of BDF$_1 \rightarrow$ B6 lymphocytes (Table I). In contrast, the presence of semisyngeneic but nonreconstituting H-2^d SAC had no effect on the ability of H-2^b SAC to reconstitute the responses of BDF$_1 \rightarrow$ B6 lymphocytes (Table I).

To demonstrate that the observed H-2 restrictions on lymphocyte-accessory cell interactions were actually restrictions on helper T-cell-accessory-cell interactions, experiments were performed in which spleen cells were first separated into three

TABLE I

$F_1 \to$ Parent$_A$ Chimera Lymphocytes Only Cooperate with Parent$_A$ Accessory Cells

No. of SAC per culture	Strain	H-2	Percent reconstitution* of responses depleted by Sephadex G-10 passage‡		
			$BDF_1 \to$ BDF_1§	B10.BR‖	$BDF_1 \to$ B6¶
4×10^4	B10	b	137	−56	114
	B10.D2	d	175	−67	16
	B10.BR	k	−28	153	−38
2×10^4	B10	b	180	−57	141
	B10.D2	d	110	−66	10
	B10.BR	k	7	121	9
2×10^4	B10 + B10.D2	b + d	ND**	ND	138
$2 \times 10^{4+}$	B10 + B10.BR	b + k	ND	ND	−25

* Percent reconstitution

(PFC response of Sephadex G-10 passed spleen cells + SAC)

$$= \frac{- \text{(PFC response of Sephadex G-10 passed cells alone)}}{\text{(PFC response of normal spleen cells)} - \text{(PFC response of Sephadex G-10 passed cells alone)}}.$$

‡ Spleen cells were depleted of adherent accessory cells by Sephadex G-10 passage and cultured at 5 × 10^5 cells/200 μl culture.

§ Pre-Sephadex G-10 response, 210 (1.08) PFC/culture; Post Sephadex G-10 response, 71 (1.05) PFC/culture.

‖ Pre-Sephadex G-10 response, 151 (1.22) PFC/culture; Post Sephadex G-10 response, 61 (1.21) PFC/culture.

¶ Pre-Sephadex G-10 response, 210 (1.01) PFC/culture; Post Sephadex G-10 response, 112 (1.16) PFC/culture.

** ND, not done.

functional subpopulations: T cells (NNA spleen cells), B cells (spleen cells depleted both of adherent cells and T cells), and accessory cells (SAC). The T cells used in these experiments were obtained from unprimed (B10 × B10.A)$F_1 \to$ B10 and (B10 × B10.A)$F_1 \to$ B10.A chimeric spleens; the B cells were obtained from unprimed normal (B10 × B10.A)F_1 spleens (rather than parental spleens to avoid any possible H-2-restrictions on cell collaboration imposed by the B-cell haplotype); the accessory-cell populations were SAC from normal B10, B10.A, or B10.A(4R) spleens. Neither chimeric T-cell population was able to cooperate with F_1 B cells in the absence of added accessory cells (Fig. 2). However, $F_1 \to$ B10 T cells did cooperate with F_1 B cells in the presence of SAC from B10, but not B10.A or B10.A(4R) (Fig. 2A); conversely, $F_1 \to$ B10.A T cells cooperated with F_1 B cells in the presence of SAC from both B10.A and B10.A(4R), but not B10 (Fig. 2B). Thus, it can be concluded that a requirement exists for helper T-cell recognition of H-2 determinants expressed on the surface of accessory cells, and that the determinants recognized are encoded in the K or I-A region of H-2. Furthermore, it should be noted that the requirement for helper T-cell recognition of K or I-A region-encoded determinants on (B + accessory) cells could be fully accounted for by required recognition of those determinants on accessory cells alone.

No Requirement for Unprimed Helper T Cells to Recognize the Identical H-2 Determinants on Both Accessory Cells and B Cells.   Because it has been demonstrated that helper T cells are required to recognize the H-2 determinants expressed by accessory cells, it was next determined whether helper T cells are required to recognize the identical H-2
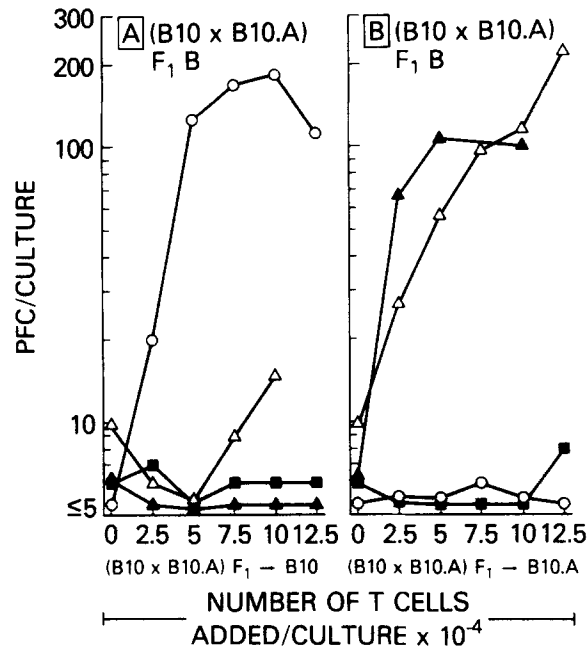
Fig. 2. (A × B)F₁ → parentₐ chimeric helper T cells cooperate with accessory cells expressing parentₐ but not parentв K or I-A region-encoded H-2 determinants. Graded numbers of chimeric T cells were added to a culture containing 10 μg/ml TNP-KLH, 4 × 10⁵ F₁ B cells (Sephadex G-10 passed and R MB + C′-treated spleen cells), and either no SAC (■) or 4 × 10⁴ SAC from B10 (○), B10.A (△), or B10.A(4R) (▲). Less than 5 PFC/culture were obtained either in the absence of antigen or in culture devoid of F₁ B cells, i.e., containing only TNP-KLH, T cells, and/or SAC.

determinants on both accessory cells and B cells. For these experiments, spleen cells were again separated into three subpopulations of T cells, B cells, and accessory cells. Normal F₁ T cells were unable to cooperate with B cells from either parent in the absence of added accessory cells (Fig. 3). As expected, normal F₁ T cells did cooperate with B10 B cells in the presence of B10 accessory cells (Fig. 3 A) and did cooperate with B10.A B cells in the presence of B10.A accessory cells (Fig. 3 B). If a requirement exists for helper T-cell recognition of the identical H-2 determinants on both accessory cells and B cells, no response should be observed either when the B cells are H-2^b and the accessory cells are H-2^a, or when the B cells are H-2^a and the accessory cells are H-2^b. However, such unresponsiveness was not observed (Fig. 3 A, B). Essentially equivalent responses were induced by (B10 × B10.A)F₁ T cells when the B cells were either B10 (Fig. 3 A) or B10.A (Fig. 3 B) and the accessory cells were either B10 or B10.A.

A second experimental approach to this same question involved the use of parentₐ → (A × B)F₁ chimera spleen T and B cells which were tolerant to both A and B H-2 determinants as measured by specific nonreactivity in mixed lymphocyte reactions (data not shown). B6 → BDF₁ chimeric spleen cells were depleted of adherent accessory cells by Sephadex G-10 passage, resulting in significantly diminished responses to TNP-KLH ($P < 0.01$) (Fig. 4). The ability of these Sephadex G-10-passed H-2^b → H-2$^{b/d}$ chimeric T and B cells to cooperate with H-2^b, H-2^d, or H-2^k accessory cells was then examined (Fig. 4). In contrast to normal B6 (H-2^b) lymphocytes, B6
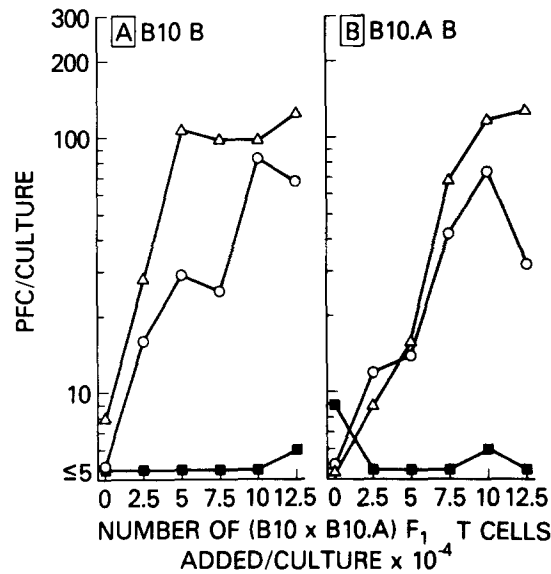
Fig. 3. No requirement for helper T-cell recognition of identical H-2 determinants expressed on both accessory cells and B cells. Graded numbers of normal F_1 T cells were added to cultures containing 10 μg/ml TNP-KLH, either 4 × 10^6 B10 or B10.A B cells (Sephadex G-10 passed and RαMB + C′ treated spleen cells), and either no SAC (■), or 4 × 10^4 SAC from B10 (○) or B10.A (△). Less than 5 PFC/culture were observed either in the absence of antigen or in cultures devoid of B cells, i.e., containing only TNP-KLH, T cells, and/or SAC.

→ BDF_1 chimera lymphocytes (which were <5% $H-2^d$ and consequently >95% $H-2^b$) cooperated with both B6 ($H-2^b$) and DBA/2 ($H-2^d$) SAC, but not with third party allogeneic B10.BR ($H-2^k$) SAC. Because the B6 → BDF_1 chimera T cells simultaneously cooperated with B6 → BDF_1 chimeric B cells (which were $H-2^b$) and accessory cells which were $H-2^d$, this experiment also demonstrates that helper T-cell function does not require recognition of the identical determinants on both accessory and B cells. In addition, this experiment demonstrates that cooperation between T and accessory cells does not require H-2 syngeny but rather, is consistent with a requirement for accessory cells to express H-2 determinants syngeneic to that of the environment in which the T cells differentiated.

In Vivo Antigen Priming Does Not Induce a Requirement for Helper T-Cell Recognition of Identical H-2 Determinants on Both Accessory Cells and B Cells. Because no requirement was observed for unprimed helper T cells to interact with accessory cells and B cells that express identical H-2 determinants, it was important to determine whether such a requirement could be induced by antigen priming the T cells and B cells in vivo. KLH-primed (B10 × B10.A)F_1 T cells were assayed for their ability to cooperate with both TNP-OVA-primed B10 and B10.A B cells in the presence of B10-accessory cells for the generation of secondary IgM and IgG anti-TNP PFC. The culture conditions for obtaining such in vitro secondary responses are identical to those for obtaining in vitro primary responses with the important exception that optimal secondary responses are obtained with 2 × 10^5 (rather than 5 × 10^5) cells/culture. To allow comparisons of the magnitude of carrier-primed secondary responses with those of primary responses, the results obtained in secondary cultures are expressed as
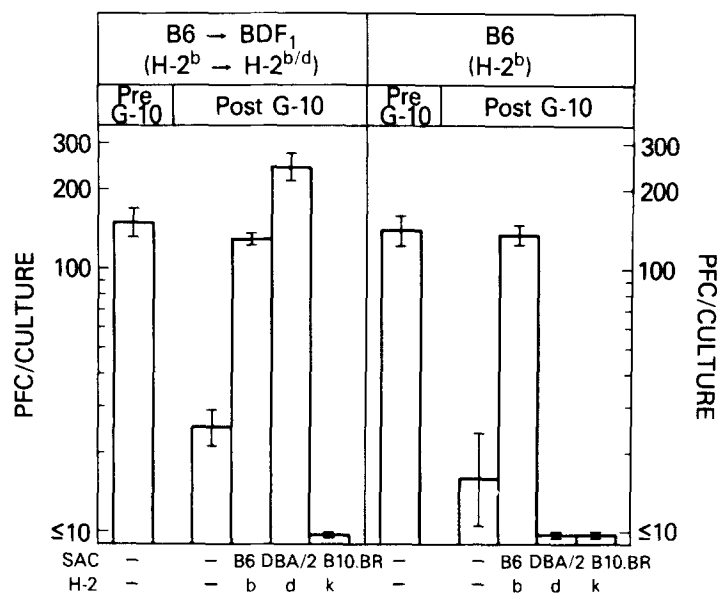
B6 → BDF₁
(H-2^b → H-2^{b/d})

B6
(H-2^b)

Pre G-10 | Post G-10

Pre G-10 | Post G-10

PFC/CULTURE

300
200
100
≤10

300
200
100
≤10

PFC/CULTURE

SAC   −   − B6 DBA/2 B10.BR −   − B6 DBA/2 B10.BR
H-2   −   − b   d   k   −   − b   d   k

Fig. 4. Responses to TNP-KLH of parent$_A$ → (A × B)F₁ chimeric spleen cells depleted of adherent accessory cells are fully reconstituted by either parent$_A$ or parent$_B$ accessory cells. Responses to 10 μg/ml TNP-KLH of 5 × 10⁵ spleen cells were significantly diminished ($P < 0.01$) by Sephadex G-10 passage as indicated. 4 × 10⁴ SAC were added to cultures containing Sephadex G-10-passed spleen cells and TNP-KLH. Less than 5 PFC per culture were observed in the absence of antigen.

both PFC per culture and as unprimed culture equivalent responses, i.e. corrected for the lower cell number used in secondary versus primary cultures. It should be noted that in cultures containing only unprimed cells, essentially no IgG anti-TNP PFC are detected at any cell number (data not shown).

In the absence of added accessory cells, KLH-primed F₁ T cells did not cooperate with TNP-primed B10 or B10.A B cells (Fig. 5 A, B); upon the addition of B10 SAC, effective cooperation was observed between KLH-primed F₁ T cells and either TNP-primed B10 or B10.A B cells for the generation of both secondary IgM and IgG anti-TNP PFC (Fig. 5 A, B). Indeed, the IgM and IgG PFC responses observed in the presence of B10 SAC were essentially identical regardless of whether the B cells were B10 or B10.A. Thus, antigen priming did not induce a requirement for helper T-cell recognition of the identical H-2 determinants on both accessory cells and B cells.

(A × B)F₁ → Parent$_A$ Chimeric Helper T Cells can Cooperate with Either Parent$_A$ or Parent$_B$ B Cells. So far, it has been demonstrated that helper T cells are required to recognize the K or I-A region-encoded H-2 determinants expressed on accessory cells and that successful collaboration does not require helper T-cell recognition of identical determinants on both accessory cells and B cells. These two results taken together strongly suggested that no requirement existed for helper T-cell recognition of H-2 determinants expressed by B cells. The experimental approach used to directly examine this possibility again utilized the fact that (A × B)F₁ → parent$_A$ chimeric helper T cells were restricted to recognizing parent$_A$ but not parent$_B$ H-2 determinants expressed on accessory cells.

Unprimed spleen cells were again separated into subpopulations of T cells, B cells, and accessory cells. T cells from three different chimeric combinations were examined:
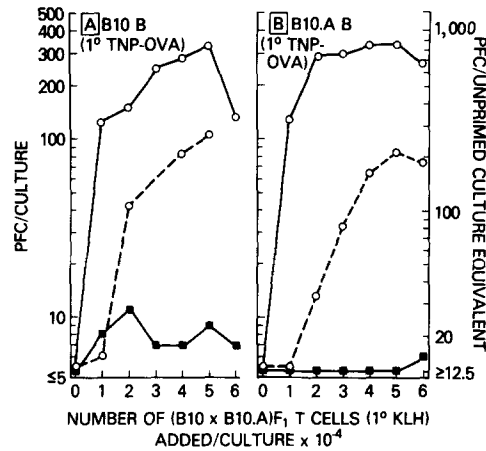
Fig. 5. No requirement for carrier primed helper T-cell recognition of identical H-2 determinants expressed on both accessory cells and hapten-primed B cells. Graded numbers of KLH-primed F_1 T cells were added to cultures containing 10 µg/ml TNP-KLH, either 1.6 × 10^5 B10 or B10.A TNP-OVA-primed B cells, and either no SAC (■) or 2 × 10^4 B10 SAC (○). Cultures were assayed for the number of IgM (———) and IgG (– – –) anti-TNP PFC generated. Results are expressed as both PFC per culture and as PFC per unprimed culture equivalent, the latter corrected for the lower numbers of cells used in secondary versus primary cultures. Less than 5 PFC per culture were observed either in the absence of antigen or in cultures devoid of B cells, containing only TNP-KLH, T cells, and/or SAC.

(a) (B10 × B10.A)F_1 → B10 (Fig. 6A, B), (b) (B10 × B10.A)F_1 → B10.A (Fig. 6C, D), and (c) BDF$_1$ → B6 (Fig. 6E, F). In each case, chimeric helper T cells were mixed with B cells and accessory cells from each parent. The results in each case were identical: (A × B)F_1 → parent$_A$ chimera helper T cells only cooperated with parent$_A$ accessory cells but, in the presence of parent$_A$ accessory cells, cooperated equally well with B cells from either parent$_A$ or parent$_B$. For example, (B10 × B10.A)F_1 → B10 chimera T cells did not cooperate with either B10 or B10.A B cells for the generation of anti-TNP PFC responses in the absence of adherent accessory cells or in the presence of B10.A adherent accessory cells (Fig. 6A, B). Responses were obtained, however, in the presence of B10 adherent accessory cells and were obtained regardless of whether the B cells were B10 or B10.A (Fig. 6A, B).

Three conclusions can be drawn from these experiments. First, these experiments confirm that no requirement exists for helper T-cell recognition of identical H-2 determinants on both accessory cells and B cells. The absence of this requirement in these experiments is unlikely to be due to helper T-cell recognition of H-2 specificities shared between parent$_A$ and parent$_B$ because (A × B)F_1 → parent$_A$ T cells cannot be shown to recognize any H-2 determinants at all expressed on parent$_B$ accessory cells. Second and more importantly, these experiments demonstrate that a requirement exists for helper T-cell recognition of H-2 determinants expressed on accessory cells but that no such requirement was observed for helper T-cell recognition of H-2 determinants expressed on B cells. Finally, these experiments demonstrate that if the lack of cooperation between (A × B)F_1 → parent$_A$ chimera T cells and parent$_B$ accessory cells is a result of active haplotype specific suppression, such suppression must be specific only for accessory-cell-T-cell interactions and not for T-cell-B-cell interactions.
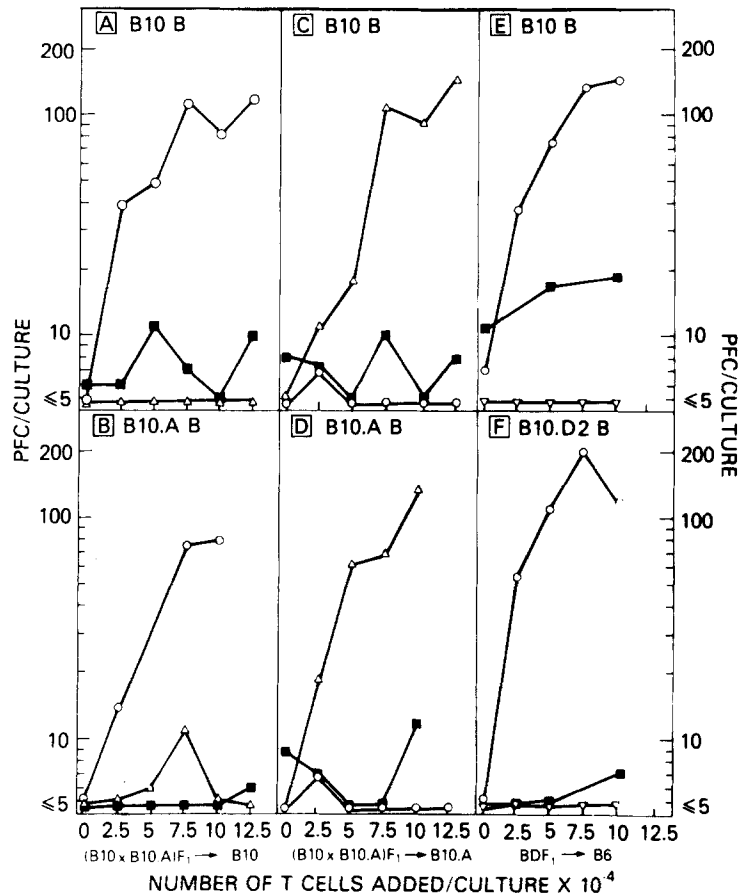
FIG. 6. (A × B)F₁ → parentₐ chimeric T cells cooperate with either parentₐ or parentB B cells in the presence of parentₐ accessory cells. Graded numbers of chimeric T cells were added to cultures containing 10 μg/ml TNP-KLH; 4 × 10⁶ B cells from either B10, B10.A, or B10.D2; and either no SAC (■) or 4 × 10⁴ SAC from B10 (O), B10.A (△), or B10.D2 (▽). Less than 5 PFC per culture were observed either in the absence of antigen or in cultures devoid of B cells.

Restrictions on Cooperation between (A × B)F₁ → Parentₐ Helper T Cells and ParentB (B + Accessory) Cells Can Be Reversed by the Addition of Antigen-Pulsed Parentₐ Accessory Cells. The previous experiments have shown that restrictions exist on (A × B)F₁ → parentₐ chimeric helper T-cell recognition of H-2 determinants expressed only by accessory cells. If this is correct, it would be expected that the lack of cooperation between (A × B)F₁ → parentₐ T cells and parentB (B + accessory) cells (Fig. 1) could be reversed by the addition of parentₐ SAC. This prediction was tested using (B10 × B10.A)F₁ → B10.A chimeric helper T cells, parental (B + accessory) cells, and TNP-KLH-pulsed SAC as antigen-presenting cells (Fig. 7). Indeed, F₁ → B10.A chimeric T cells did cooperate with B10 (B + accessory) cells upon the addition of TNP-KLH-pulsed B10.A SAC (Fig. 7 A). It should be noted that the ability to observe such reversal of restriction was dependent upon both the haplotype and the source of accessory cells used, in that B10.A peritoneal cells did not mediate such reversal of restriction. This is consistent with the observation made in this and other experimental
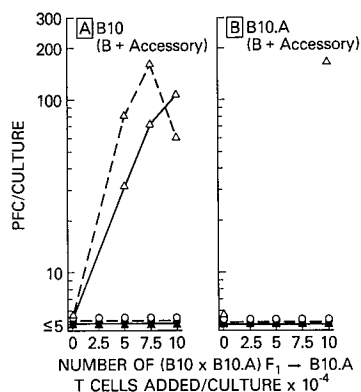
Fig. 7.   TNP-KLH-pulsed parent_A SAC can reverse the lack of cooperation between $(A \times B)F_1$ → parent_A chimeric T cells and parent_B (B + accessory) cells. Graded numbers of chimeric T cells were added to cultures containing 4×10^5 (B + accessory) cells from either B10 or B10.A; and either 4×10^4 (——) or 8×10^4 (- - -) unpulsed (closed figures) or TNP-KLH-pulsed (open figures) SAC from B10 (O) or B10.A (△). Less than 5 PFC/culture were observed for cultures devoid of B cells.

systems that spleen is a more potent source of accessory cells than the peritoneum. The ability of SAC to reverse apparent restrictions on T-cell-B-cell collaboration demonstrates that the inability of $(A \times B)F_1$ → parent_A chimeric T cells to cooperate with parent_B (B + accessory) cells is only a result of the inability of these T cells to collaborate wth parent_B accessory cells.

It was also demonstrated, in the same experiment, that helper T cells are only stimulated by antigen that is presented on the appropriate accessory cell. F_1 → B10.A chimeric T cells were added to cultures with B10.A (B + accessory) cells (Fig. 7 B). No restrictions to cellular cooperation exist in these cultures, so all that should be required to elicit a response is the introduction of antigen. Indeed, the addition of TNP-KLH-pulsed B10.A SAC did elicit a response (Fig. 7 B); however, the addition of TNP-KLH-pulsed B10 SAC did not (Fig. 7 B), even though both antigen and B10.A accessory cells were present in the same cultures. Hence, it can be concluded that responses are elicited only if antigen and the appropriate H-2 determinants are presented to helper T cells by the same accessory cell.

No Restrictions on Helper T-Cell Recognition of B-Cell H-2 Determinants is Observed In Vivo.   To examine whether the conclusions drawn from the preceding in vitro experiments could be extended to in vivo conditions, short-term adoptive transfer experiments were performed using unprimed spleen cell subpopulations of T cells (NNA spleen cells), B cells (RαMB + C' treated spleen cells), and added accessory cells (RαMB + C'-treated and 1,000 rads irradiated spleen cells). In this experiment (Fig. 8) both the B cells and the irradiated hosts were B10; the T cells were from (B10 × B10.A)F_1 → B10.A chimeric spleens which do not cooperate with B10 accessory cells in vitro and so would be expected not to cooperate with any radiation-resistant accessory cells resident in the irradiated B10 hosts. The strains of the irradiated host and the cell populations were intentionally selected so that any contribution the host might make to the observed in vivo responses would be negated by using F_1 → parent chimeric T cells that were unable to recognize the H-2 determinants expressed by cells in that host. Consequently, it should be possible to ascertain whether the addition
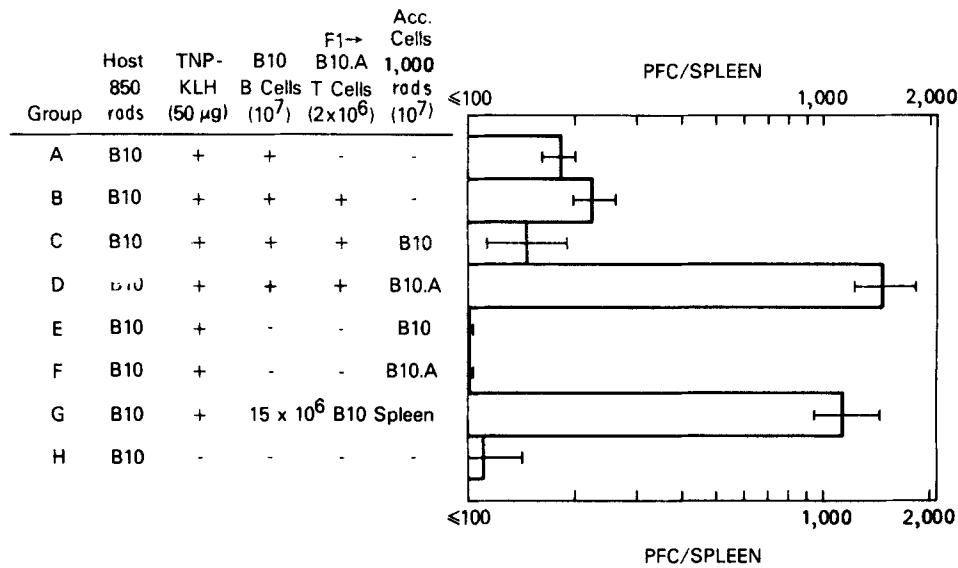
Group	Host 850 rads	TNP-KLH (50 μg)	B10 B Cells (10^7)	F1→ B10.A T Cells (2×10^6)	Acc. Cells 1,000 rods (10^7)
A	B10	+	+	-	-
B	B10	+	+	+	-
C	B10	+	+	+	B10
D	B10	+	+	+	B10.A
E	B10	+	-	-	B10
F	B10	+	-	-	B10.A
G	B10	+	15 × 10^6 B10 Spleen		
H	B10	-	-	-	-

Fig. 8. Parent$_A$ accessory cells reverse the lack of cooperation between (A × B)F$_1$ → parent$_A$ chimeric T cells and parent$_B$ (B + accessory) cells in vivo. B10-recipient mice were lethally irradiated with 850 rads and reconstituted with unprimed B cells (RαMB + C′-treated spleen cells), unprimed (B10 × B10.A)F$_1$ → B10.A chimeric T cells (NNA spleen cells) and/or B10- orB10.A-added accessory cells (RαMB + C′-treated spleen cells irradiated with 1,000 rads). The irradiated recipient mice were simultaneously reconstituted and immunized with 50 μg TNP-KLH intravenously. 6 d after reconstitution the spleens from these adoptively transferred mice were assayed for the number of anti-TNP PFC/spleen.

of B10.A accessory cells can reverse the unresponsiveness between F$_1$ → B10.A chimeric T cells and B10 B cells in vivo for the generation of responses to TNP-KLH. As assayed on day 6, only minimal responses were obtained in the absence of added T cells or added accessory cells (Fig. 8 A). In addition, collaboration was not observed in B10 hosts reconstituted with F$_1$ → B10.A chimeric T cells and B10 B cells alone, in that responses were no different with chimeric T cells than without (Fig. 8 A, B). In contrast, collaboration was observed between the F$_1$ → B10.A chimeric T cells and the B10 B cells upon the addition of irradiated B10.A but not B10-added accessory cells (Fig. 8 C, D). It should be noted that the magnitude of these responses reflect the fact that all the cell populations were unprimed; nevertheless, the responses observed with unprimed separated cell populations were comparable to those obtained by reconstitution of irradiated B10 hosts with intact, unprimed B10 spleen cells (Fig. 8 G).

Thus, it can be concluded that in vivo, as well as in vitro, (A × B)F$_1$ → parent$_A$ chimeric helper T cells are restricted in their recognition of H-2 determinants expressed on accessory cells, but that they are not similarly restricted in their recognition of H-2 determinants expressed on B cells, if recognition of B-cell H-2 determinants occurs at all.

Discussion

The present study on the role of H-2-region gene products in regulating interactions among helper T cells, B cells, and accessory cells differs importantly from most

previous studies in that the interacting cell types were separated into three functionally distinct subpopulations of cells, all of which were required in culture for generating responses to the antigen TNP-KLH. These experiments have demonstrated that: (a) helper T cells are not required to recognize the identical MHC determinants on both accessory cells and B cells; (b) a strict requirement exists for helper T-cell recognition of MHC determinants on accessory cells, but that a similar requirement was not observed for recognition of MHC determinants on B cells; (c) helper T cells are triggered by antigen only if both antigen and the appropriate H-2 determinants are presented by the same accessory cell; and (d) no requirement for MHC homology was observed for the potential interactions between accessory cells and B cells.

Helper T Cells That Recognize Accessory Cell H-2 Determinants Do Not Also Recognize B-Cell H-2 Determinants. The observation that $(A \times B)F_1 \rightarrow$ parent$_A$ chimeric helper T cells collaborate with accessory cells from parent$_A$ but not from parent$_B$ demonstrates that these chimeric helper T cells are able to distinguish the accessory cells of parent$_A$ from those of parent$_B$. Because parent$_A$ and parent$_B$ were H-2 congenic-resistant strains, these experiments demonstrate that helper T cells not only recognize the H-2 determinants expressed on accessory cells, but are required to do so to mediate help. A different interpretation of these data is that the interaction of $(A \times B)F_1 \rightarrow$ parent$_A$ chimeric T cells with parent$_B$ accessory cells is specifically suppressed. Because cultures containing accessory cells from both parent$_A$ and parent$_B$ are not suppressed (Table I, Fig. 7), then the suppresson, if it exists, must be both specifically induced by parent$_B$ accessory cells and only effect recognition of parent$_B$ accessory cells. However, experiments to be reported elsewhere appear to exclude such haplotype-specific suppression as the mechanism for the failure of $(A \times B)F_1 \rightarrow$ parent$_A$ chimeric T cells to cooperate with parent$_B$ accessory cells.[3]

Although $(A \times B)F_1 \rightarrow$ parent$_A$ chimeric helper T cells do not recognize or cooperate with parent$_B$ accessory cells, these same chimeric T-cell populations cooperate equally well with B cells from either parent. Consequently, these data are consistent with the notion that helper T cells do not recognize B-cell H-2 determinants at all. However, these data cannot exclude the possibility that a second helper T-cell subpopulation exists that is specific for recognizing only B-cell-expressed H-2 determinants if it is hypothesized that such a second helper T-cell population is not restricted in its H-2-specific receptor repertoire by the chimeric host environment. However, if such a second subpopulation of helper T cells exists, these helper T cells alone cannot be sufficient to provide help, because responses were not observed under conditions in which only this subpopulation of helper T cells would have been triggered, i.e. in cultures containing $(A \times B)F_1 \rightarrow$ parent$_A$ chimeric T cells and parent$_B$ accessory cells. (Table I, Fig. 6). Whether or not such a second subpopulation of helper T cells exists, it can be concluded from these experiments that helper T cells are required to recognize H-2 determinants expressed on accessory cells, but that these same helper T cells do not also recognize the H-2 determinants on B cells.

This conclusion is supported by an additional set of observations. It has been demonstrated that normal $(A \times B)F_1$ helper T cells are not required to recognize the identical H-2 determinants expressed on both accessory cells and B cells. A substantial amount of data has been accumulated demonstrating that normal $(A \times B)F_1$ T cells

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[3] Hodes, R. J., K. S. Hathcock, and A. Singer. Manuscript in preparation.

actually constitute two independent populations of T cells, each specific for recognizing one parental haplotype (4, 19–21). Because individual F_1 T cells express receptors specific for only one parental's haplotype, the ability of both naive and antigen-primed (A × B)F_1 T cells to simultaneously cooperate with parent$_A$ accessory cells and parent$_B$ B cells implies that individual helper T cells either recognize the H-2 determinants expressed on accessory cells or recognize the H-2 determinants expressed on B cells, but not both. Thus, individual F_1 T cells that recognize the H-2 determinants expressed on parent$_A$ accessory cells could not also recognize the different H-2 determinants expressed on parent$_B$ B cells.

Similarly, studies demonstrating that accessory cells can present antigen to allogeneic helper T cells and B cells for the generation of antigen-specific antibody responses (22) also demonstrate that the helper T cells in those experiments could not have recognized identical H-2 determinants on both the allogeneic accessory cells and the syngeneic B cells.

Why helper T cells preferentially recognize accessory-cell and not B-cell H-2 determinants is not clear. Nevertheless, it appears that alloreactive T cells mediating mixed lymphocyte reactions (MLR) also preferentially recognize accessory-cell H-2 determinants rather than B-cell H-2 determinants. This point has been clearly demonstrated in the guinea pig (23), although it is still controversial in the mouse. Nevertheless, evidence that murine dendritic cells are more potent stimulators of MLR than lymphocytes has recently been reported (24); in addition, recent data demonstrate quite clearly that SAC are far more potent stimulators of murine MLR than B cells, if B cells are stimulatory at all.[4]

The absence of helper T-cell recognition of B-cell H-2 determinants in the experiments reported here is consistent with results previously obtained by some investigators (21, 25) but clearly differs from conclusions reached by others (4, 5, 7, 19). However, it should be emphasized that in the presence of a strict requirement for helper T-cell recognition of H-2 determinants expressed on accessory cells, studies demonstrating H-2 restriction on interactions between helper T cells and B cells must be carefully evaluated for the possibility that: (a) the observed restrictions to cooperation were actually a result of restrictions on accessory-cell-T-cell interactions, or (b) if H-2 congenic-resistant strains were not used, that the observed restrictions were actually a result of the unsuspected role of non-H-2-gene products either in T-cell-B-cell interactions or accessory-cell-B-cell interactions.

A Unique Hole in the Receptor Repertoire of (A × B)F_1 → Parent$_A$ Chimeras. Because the present data demonstrate that the host environment in which T cells mature importantly effects which H-2 determinants helper T cells are able to recognize, these data are relevant to our understanding of the repertoire of H-2-specific receptors expressed by T cells. The present data could be interpreted as demonstrating that (A × B)F_1 → parent$_A$ chimeric T cells only express receptors specific for recognizing parent$_A$ accessory-cell H-2 determinants. However, an alternative interpretation of these data is that (A × B)F_1 → parent$_A$ chimeric T cells express receptors specific for recognizing all H-2 determinants except those specific for recognizing the H-2 determinants of parent$_B$. In other words a hole has been created in the T-cell repertoire of (A × B)F_1 → parent$_A$ chimeras resulting in the specific absence of T cells able to

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recognize parent$_B$ H-2 determinants. Such a hole in the T-cell repertoire would be unique to (A × B)F$_1$ → parent$_A$ chimeras and would be specific for recognizing parent$_B$ MHC determinants because the only H-2 determinants expressed on the precursor T cells and not also expressed in the thymus of the chimeric host are those of parent$_B$. This asymmetry would be peculiar to (A × B)F$_1$ → parent$_A$ chimeras in that it would not occur in normal mice. That such a unique hole might indeed exist in the T-cell repertoire of (A × B)F$_1$ → parent$_A$ chimeras is suggested by some of the data presented in this report: (a) (A × B)F$_1$ → parent$_A$ chimera T cells contain T cells which recognize third-party allogeneic accessory cells, although under the conditions used in the present experiments, the subsequent alloreaction (itself evidence for recognition) resulted in suppression of PFC responses (Table I); (b) these chimeric T-cell populations contain T cells which recognize parent$_A$ accessory cells as evidenced by the generation of specific PFC responses; and (c) these chimeric T-cell populations do not possess T cells capable of recognizing parent$_B$ accessory cells as suggested by the absence of both T-cell-help and demonstrable T-cell-mediated suppression. The mechanism by which holes might be created in the H-2-specific T-cell repertoire and its implications will be detailed elsewhere.

If viewed as a specific hole in their T-cell repertoire, the inability of (A × B)F$_1$ → parent$_A$ chimeric T cells to cooperate with parent$_B$ accessory cells does not necessarily conflict with reports that allogeneic T cells and accessory cells can collaborate with each other (22, 26). These two seemingly contradictory results can be reconciled if: (a) (A × B)F$_1$ → parent$_A$ chimeric T cells have a unique hole in their T-cell repertoire so that they are only unable to recognize parent$_B$ H-2 determinants, and (b) collaboration between helper T cells and accessory cells does not require genetic identity, but rather, requires helper T-cell recognition of both antigen and H-2 determinants presented by accessory cells, recognition which can be masked by interfering alloreactions.

Whether these speculations are correct or not, this study supports the general concept that regulation of cell interactions by H-2-region genes is currently best understood not as a requirement for H-2 identity, but rather as a requirement for specific recognition of cell-surface H-2 determinants expressed on the interacting cell populations; and specifically supports the viewpoint that at least one required population of helper T cells is only required to recognize the H-2 determinants expressed by accessory cells.

Summary

Requirements for helper T-cell recognition of H-2 determinants expressed on adherent accessory cells and on B cells was individually assessed in the anti-hapten PFC responses to TNP-KLH. Complicating allogeneic effects were minimized or avoided by the use of helper T cells from normal F$_1$ hybrids, parent → F$_1$ chimeras, and F$_1$ → parent chimeras. The results of both in vitro and in vivo experiments demonstrated that: (a) helper T cells are not required to recognize the identical H-2 determinants on both accessory cells and B cells; (b) helper T cells are required to recognize K or I-A region-encoded determinants expressed on accessory cells; (c) no requirement was observed in vitro or in vivo for helper T-cell recognition of B-cell-expressed H-2 determinants; and (d) no requirement was observed for H-2 homology between accessory cells and B cells.

The absence of required helper T-cell recognition of the identical H-2 determinants on both accessory cells and B cells was demonstrated in two ways: (a) naive or KLH-primed (A × B)F_1 hybrid helper T cells collaborated equally well with B cells from either $parent_A$ or $parent_B$ in the presence of accessory cells from either parent; (b) A → (A × B)F_1 chimeric spleen cells depleted of accessory cells collaborated equally well with accessory cells from either $parent_A$ or $parent_B$, even though the B cells only expressed the H-2 determinants of $parent_A$.

A requirement for helper T-cell recognition of K or I-A region-encoded H-2 determinants on accessory cells was also demonstrated in two ways: (a) (A × B)F_1 → $parent_A$ chimeric spleen cells depleted of accessory cells collaborated with accessory cells from $parent_A$ but not $parent_B$; and (b) (A × B)F_1 → $parent_A$ chimeric helper T cells collaborated with normal F_1 B cells only in the presence of parental or recombinant accessory cells that expressed the K or I-A region-encoded determinants of $parent_A$.

Although restricted in their ability to recognize H-2 determinants on accessory cells, it was demonstrated both in vitro and in vivo that (A × B)F_1 → $parent_A$ chimeric helper T cells were able to collaborate with B cells from either $parent_A$ or $parent_B$. In vitro in the presence of accessory cells from $parent_A$, (A × B)F_1 → $parent_A$ chimeric helper T cells collaborated equally well with B cells from either parent. In addition, the inability of (A × B)F_1 → $parent_A$ chimeric helper T cells to collaborate with (B + accessory) cells from $parent_B$ was successfully reversed by the additon of $parent_A$ SAC as added accessory cells. In vivo, upon the addition of $parent_A$ accessory cells, (A × B)F_1 → $parent_A$ chimeric helper T cells collaborated with $parent_B$ B cells in short-term adoptive transfer experiments.

References

1. Katz, D. H., T. Homoaka, and B. Benacerraf. 1973. Cell interactions between histoincompatible 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.

2. Rosenthal, A. S., and E. M. Shevach. 1973. Function of macrophages in antigen recognition by guinea pig T lymphocytes. *J. Exp. Med.* 138:1194.

3. von Boehmer, H., L. Hudson, and J. Sprent. 1975. Collaboration of histoincompatible T and B lymphocytes using cells from tetraparental bone marrow chimeras. *J. Exp. Med.* 142:989.

4. Sprent, J. 1978. Restricted helper function of F_1 hybrid T cells positively selected to heterologous erythrocytes in irradiated parental strain mice. I. Failure to collaborate with B cells of the opposite strain not associated with active suppression. *J. Exp. Med.* 147:1142.

5. Sprent, J. 1978. Restricted helper function of F_1 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. *J. Exp. Med.* 147:1159.

6. Kappler, J. W., and P. Marrack. 1977. The role of *H-2* linked genes in helper T-cell function. I. In vitro expression in B cells of immune response genes controlling helper T cell activity. *J. Exp. Med.* **146:**1977.

7. Yamashita, U., and E. M. Shevach. 1978. The histocompatibility restrictions on macrophage T-helper cell interaction determine the histocompatibility restrictions on T-helper cell B-cell interaction. *J. Exp. Med.* **148:**1171.

8. Benacerraf, B. 1978. A hypothesis to relate the specificity of T lymphocytes and the activity of *I* region-specific Ir genes in macrophages and B lymphocytes. *J. Immunol.* **120:**1809.

9. 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.

10. Zinkernagel, R. M., G. N. Callahan, A. Althage, S. Cooper, J. W. Streilen, 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.

11. Sprent, J. 1978. Restricted helper function of $F_1 \rightarrow$ Parent bone marrow chimeras controlled by K-end of *H-2* complex. *J. Exp. Med.* **147:**1838.

12. Hodes, R. J., and A. Singer. 1977. Cellular and genetic control of antibody responses in vitro. I. Cellular requirements for the generation of genetically controlled primary IgM responses to soluble antigens. *Eur. J. Immunol.* **7:**892.

13. Cudkowicz, G., and M. Bennett. 1971. Peculiar immunobiology of bone marrow allografts. II. Rejection of parental grafts by resistant F_1 hybrid mice. *J. Exp. Med.* **134:**1513.

14. Hodes, R. J., G. B. Ahmann, K. S. Hathcock, H. B. Dickler, and A. Singer. 1978. Cellular and genetic control of antibody responses in vitro. IV. Expression of *Ia* antigens on accessory cells required for responses to soluble antigens including a response under *Ir* gene control. *J. Immunol.* **121:**1501.

15. Singer, A., C. Cowing, K. S. Hathcock, H. B. Dickler, and R. J. Hodes. 1978. Cellular and genetic control of antibody responses in vitro. III. Immune response gene regulation of accessory cell function. *J. Exp. Med.* **147:**1611.

16. Cowing, C., B. D. Schwartz, and H. B. Dickler. 1978. Macrophage Ia antigens. I. Macrophage populations differ in their expression of Ia antigens. *J. Immunol.* **120:**378.

17. Rittenberg, M. B., and K. L. Pratt. 1969. Antitrinitrophenyl (TNP) plaque assay. Primary response of Balb/c mice to soluble and particulate immunogen. *Proc. Soc. Exp. Med.* **132:**575.

18. Jerne, N. K., and A. A. Nordin. 1963. Plaque formation in agar by single antibody-producing cells. *Science (Wash. D. C.).* **140:**405.

19. Swierkosz, K. Roch, P. Marrack, and J. W. Kappler. 1978. The role of H-2 linked genes in helper T-cell function. II. Isolation on antigen-pulsed macrophages of two separate populations of F_1 helper T cells each specific for antigen and one set of parental H-2 products. *J. Exp. Med.* **147:**554.

20. Thomas, D. W., and E. M. Shevach. 1976. Nature of the antigenic complex recognized by T lymphocytes. I. Analysis with an in vitro primary response to soluble protein antigens. *J. Exp. Med.* **144:**1263.

21. McDougal, J. S., and S. P. Cort. 1978. Generation of T helper cells in vitro. IV. F_1 T helper cells primed with antigen-pulsed parental macrophages are genetically restricted in their antigen-specific activity. *J. Immunol.* **120:**445.

22. Pierce, C. W., J. A. Kapp, and B. Benacerraf. 1976. Regulation by the H-2 gene complex of macrophage-lymphoid cell interactions in secondary antibody responses in vitro. *J. Exp. Med.* **144:**371.

23. Greineder, D. K., and A. S. Rosenthal. 1975. Macrophage activation of allogeneic lymphocyte proliferation in the guinea pig mixed leukocyte culture. *J. Immunol.* **114:**1541.

24. Steinman, R. M., and M. G. Witmer. 1978. Lymphoid dendritic cells are potent stimulators of the primary mixed lymphocyte reaction in mice. *Proc. Natl. Acad. Sci. U. S. A.* **75:**5132.

25. Erb, P., and M. Feldmann. 1975. The role of macrophages in the generation of T helper cells. II. The genetic control of macrophage-T-cell interaction for helper cell induction with soluble antigens. *J. Exp. Med.* **142:**460.
26. Thomas, D. W., and E. M. Shevach. 1977. Nature of the antigenic complex recognized by T lymphocytes: specific sensitization by antigens associated with allogeneic macrophages. *Proc. Natl. Acad. Sci. U. S. A.* **74:**2104.