ANTIGEN RECOGNITION

V. Requirement for Histocompatibility between Antigen-presenting Cell and B Cell in the Response to a Thymus-dependent Antigen, and Lack of Allogeneic Restriction between T and B Cells*

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Analysis of the cellular requirements for a humoral antibody response to thymus-dependent antigens has revealed that three cell types are necessary: T cells, B cells, and accessory or antigen-presenting cells (APC). Further studies, with a view to elucidating the regulatory role of the major histocompatibility complex (MHC) in cell-cell interactions, have yielded variable and frequently confusing results. It was established early that T cells and APC have to share MHC-encoded determinants for the in vitro proliferative response to antigen (1), and that T and B cells have to be syngeneic at the I-A subregion of the histocompatibility-2 complex (H-2) for cooperation in vivo. By contrast, homozygous T cells that had undergone differentiation in a heterozygous environment were found to cooperate efficiently with B cells of either parental haplotype in vivo (3).

These and similar studies led to the concept of adaptive differentiation, which is that the cooperating phenotype of the T cell may be altered by the environment in which differentiation takes place (4). A possible mechanism for this adaptive differentiation was suggested in the work of Zinkernagel et al. (5), whose studies indicate that the T cell recognition repertoire is controlled by both the thymic epithelium and the reticuloendothelial system of the host. Accordingly, genotypic identity alone is not necessarily sufficient to allow cooperation between immunocompetent cells, and it appears that successful interaction requires recognition of MHC determinants expressed on at least some of the cooperating cells (6, 7).

Recent studies, in which F1 T cells were selected to antigen in a parental environment, suggest that the helper T cell has to recognize determinants expressed simu-

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Abbreviations used in this paper: APC, antigen-presenting cells; BSA, bovine serum albumin; C', agarose-absorbed guinea pig complement; CFA, complete Freund's adjuvant; CRIA, cellular radioimmunoassay; CFS, fetal calf serum; FLC, fetal liver cells; H-2, histocompatibility-2 complex; KLH, keyhole limpet hemocyanin; L-15, Leibowitz L-15 medium; MHC, major histocompatibility complex; MLR, mixed lymphocyte reaction; PBS, phosphate-buffered saline; PFC, plaque-forming cell; RGG, rabbit gamma globulin; SRBC, sheep erythrocytes; TNP, trinitrophenyl derivative.
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taneously on both accessory and B cells (8), whereas work with cells from one-way
chimeras shows that the chimeric environment is able to modulate the cooperating
preferences of both T and B cells (9).

Much of the work on allogeneic restriction of cell cooperation has neglected the
role of the antigen-presenting cell in the T--B cell cooperative step. In the studies
reported here, we have examined the H-2 requirements for successful interaction
between T and B cells in the presence of APC of defined, homozygous genotype. By
so doing, we established that the interaction between APC and B cells in vivo is under
the control of the MHC, and that this restriction is not due to suppressive allogeneic
effects.

Materials and Methods

Mice. C3H.OH (H-2d), C3H.A (H-2a), and (C3H.OH × C3H.A)F1 mice were obtained
from the Small Animal Breeding Program, The University of Alberta, Edmonton, Canada.

Antigens. Keyhole limpet hemocyanin (KLH) was kindly provided by Dr. Marvin Ritten-
berg, University of Oregon Medical School, Portland, Oreg. Rabbit gamma globulin (RGG)
was purchased from the Sigma Chemical Co., St. Louis, Mo., and the trinitrophenyl (TNP)
derivatives of both antigens were prepared by haptenation with 2, 4, 6-trinitrobenzenesulfonic
acid (10). TNP-KLH had 8 TNP groups per 100,000 mol wt of KLH, and TNP-RGG had 10
TNP per molecule of RGG.

Media. Leibowitz (L-15) medium (GIBCO Canada Limited, Calgary, Alberta, Canada)
supplemented with 10% fetal calf serum (FCS; GIBCO Canada Limited) was used for the
preparation of cell suspensions, nylon wool filtration for T cell enrichment, adherence to plastic
for APC depletion, and for washing of cells. L-15 medium without FCS was used for suspending
cells for injection. RPMI-1640 medium (GIBCO Canada Limited) buffered with 25 mM Hepes
(Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.), and supplemented
with 0.3% bovine serum albumin (BSA) (Sigma Chemical Co.) was used for dilutions of
cytotoxic antisera and complement.

Cell Preparation. Single cell suspensions were prepared from primed spleen by teasing the
organs apart with syringe needles. T cell-enriched populations were obtained by the nylon wool
filtration technique of Julius et al. (11), substituting L-15 plus 10% FCS for Dulbecco's
phosphate-buffered saline (PBS). These preparations were 85–90% lysed on treatment with
monoclonal anti-Thy-1.2 antibody (New England Nuclear, Lachine, Quebec), and agarose-
absorbed guinea pig complement (C; Flow Laboratories, Inc., Rockville, Md.). B cells
were prepared from spleen by treatment with anti-Thy-1.2 antibody and absorbed complement
(twice) followed by three cycles of adherence to plastic tissue culture dishes (Corning Glass
Works, Science Products Div., Corning, N. Y.). The plastic-nonadherent cells were recovered
and washed before injection.

Irradiation. Mice were exposed to 137Cs gamma irradiation at a dose rate of 95 rad per min,
from a Gamma Cell 40 irradiator (Atomic Energy of Canada Limited, Ottawa, Ontario,
Canada).

Chimeras. (C3H.OH × C3H.A)F1 mice were lethally irradiated (950 rad) and reconstituted
within 24 h by the intravenous injection of 107 anti-Thy-1.2 plus C'-treated 13-d fetal liver cells
(FLC) from the appropriate parental strain. Survival was typically 70–85%, and survivors were
>95% reconstituted with cells of donor origin, as assessed by dye-exclusion testing, using
cytotoxic alloantisera in a two-stage procedure. Antisera were kindly provided by the Research
Resources Branch, National Institutes of Health, Bethesda, Md., and by Dr. T. L. Delovitch of
the University of Toronto.

Immunizations. Mice were immunized by the intraperitoneal injection of 0.1 ml of an
eulsion consisting of equal parts of complete Freund's adjuvant (CFA) (0.5 mg/ml heat-killed
Mycobacterium tuberculosis H37Ra) (Difco Laboratories, Detroit, Mich.) and a saline solution of
KLH or TNP-RGG to a final antigen concentration of 50 µg/0.1 ml of emulsion. Primed
spleens were harvested at 3–6 mo after priming, and purified cell suspensions were prepared as
described. Adoptive secondary recipients were challenged with 0.2 ml of a suspension of alum-precipitated TNP-KLH, containing 2 mg alum and 20 μg TNP-KLH.

Adoptive Transfers. Recipient mice were lethally irradiated (850 rad) and given 1 × 10⁷ purified T cells intravenously after 24 h. The next day, 1 × 10⁷ B cells were transferred intravenously, followed immediately by alum-precipitated antigen challenge. The secondary response was measured at day 7 by estimation of spleen plaque-forming cell (PFC) numbers and/or serum antibody levels.

PFC Assay. Direct and indirect plaques were determined by the Cunningham and Szenberg (12) slide-chamber technique. TNP-sheep erythrocytes (SRBC) were prepared according to the method of Rittenberg and Pratt (13).

Serum Antibody Determinations. Blood was collected from chloroform-anesthetized mice by cardiac puncture and was allowed to clot. Serum was recovered by centrifugation and stored at −70°C until assayed. Antibody levels were determined by the cellular radioimmunoassay (CRIA) of Longenecker et al. (14). Briefly, SRBC were haptenated and washed, and 10⁷ cells were dispensed into the wells of V-bottomed microtiter trays containing serial dilutions of the test antisera in saline. After overnight incubation at 4°C, the erythrocytes were washed extensively and further incubated with goat anti-mouse IgG antibody (N. L. Cappel Laboratories, Inc., Cochranville, Pa.), labeled with ¹²⁵I. After washing, the cells were resuspended in a small volume of saline and counted on a Beckman Bio-Gamma counter (Beckman Instruments, Inc., Fullerton, Calif.) for estimation of bound radioactivity. A quaternary hyperimmune anti-TNP serum was run as a positive control with each experiment, and counts per minute bound was expressed as a fraction of control binding.

Results

Experimental Design. The chimeric donors of T and B cells (and adoptive hosts in some instances) were prepared by reconstituting (C3H.OH × C3H.A)F₁ mice that had been lethally irradiated (950 rad) 24 h previously, with 10⁷ anti-Thy-1.2 plus C'-treated 13-d parental FLC. Analysis of these chimeras indicated that they were >95% repopulated with lymphoid cells of donor origin, and that the recovered T cells were nonreactive in mixed lymphocyte reaction (MLR) against the MHC determinants of the tolerated parent. Long-term survival of the chimeras was typically 70-85%, and the animals were free of the signs of graft vs. host disease. Chimeras were primed with antigen at least 3 mo after reconstitution and were used as cell donors 3-6 mo after priming.

To study the role of the APC in the MHC-mediated control of the T-B cell interaction, we transferred KLH-primed, chimeric T cells, and TNP-RGG-primed, chimeric B cells into lethally irradiated normal or chimeric hosts, which provided APC function. The hosts were subsequently challenged with alum-precipitated TNP-KLH, and the 7-d antibody response was determined. Carrier-primed spleen cell suspensions were passed over nylon wool columns to give a T cell-enriched helper population; typically, 90% of the nylon wool effluent cells were sensitive to lysis with anti-Thy-1.2 plus C'. B cells were purified from hapten-carrier-primed spleen by twice anti-Thy-1.2 plus C' treatment, followed by three cycles of adherence to tissue culture grade plastic surfaces. The efficacy of this method of adherent cell depletion has been previously established in our laboratory (15).

In preliminary experiments, the adoptive transfer system was calibrated by transferring graded numbers of primed syngeneic T and B cells into irradiated syngeneic hosts (data not shown). Based on these results, T and B cell numbers were chosen such that the response was linearly dependent on the number of each cell type transferred. A clear difference was seen in the helper activity of primed and unprimed T helper cells (Table III, groups X and XI).
Cooperation of Mutually Tolerant T and B Cells Across H-2 Differences In Vivo: Role of Host Haplotype. T and B cells were purified from the spleens of primed chimeras, with adherent cells removed as described, and were transferred into normal parental recipients that had been lethally irradiated 24 h previously. We were unable to exploit the radioresistance of helper cell function in situ, described by Katz et al. (2), in view of the potential for allogeneic effects arising from transferring chimeric cells into fully immunocompetent (and in some instances fully allogeneic) recipients. To further minimize the potential for allogeneic effects between host and transferred cells, the hosts were irradiated 24 h before cell transfer, a sufficient time to allow depletion of host lymphoid cells.

Histoincompatible but mutually tolerant T and B cells cooperated efficiently in generating a secondary in vivo humoral response against the TNP-hapten, as measured by CRIA, provided that the irradiated host was syngeneic with the transferred B cells (Table I). Incompatible host-B cell combinations did not show cooperation above background levels, irrespective of the T cell haplotype.

These results suggest that the T-B interaction for mutually tolerant cells is not genetically restricted, whereas that between the host APC and B cells is under H-2 control. In a repetition of part of the earlier experiments of Katz et al. (9), in which H-2 restriction of the T-B cell interaction for chimeric T and B cells was observed, we transferred primed, chimeric T and B cells into "restricting" (parental) and "nonrestricting" (F1) hosts. Our protocol differed from theirs chiefly in the greater purification of the cooperating cell types achieved by us. Based on the above results, we predicted unrestricted T-B cell cooperation in the F1 host, and this was in fact seen

| Group | T* | B‡ | Host§ | cpm ± SEMI | Percent bound¶ |
|-------|----|----|-------|------------|----------------|
| I     | OH—F1** | OH—F1 | OH | 7,673 ± 275 | 90 ± 3 |
| II    | A—F1 | A—F1 | OH | 1,556 ± 149 | 18 ± 2 |
| III   | A—F1 | OH—F1 | OH | 7,763 ± 1,613 | 91 ± 19 |
| IV    | A—F1 | OH—F1 | A | 1,144 ± 282 | 14 ± 3 |
| V     | OH—F1 | A—F1 | A | 8,133 ± 1,082 | 96 ± 13 |
| VI    | OH—F1 | A—F1 | OH | 1,850 ± 221 | 22 ± 3 |

* T cell donors were primed ~3 mo previously with 50 μg KLH/CFA intraperitoneally. 10^7 nylon wool filtered spleen cells were given intravenously to the irradiated host.
‡ B cell donors were primed ~3 mo previously with 50 μg TNP-RGG/CFA intraperitoneally. 10^7 anti-Thy-1.2 + C^-treated, plastic-nonadherent cells were transferred into the irradiated host.
§ Host animals were irradiated with 850 rad 24 h before cell transfer. Immediately after cell transfer, the host was challenged with 20 μg TNP-KLH/alum intraperitoneally. Blood for determination of serum Ab levels was taken at day 7.
¶ 7-d serum Ab levels determined by CRIA. Counts per minute represents ¹²⁵I goat antimouse IgG bound to washed TNP-SRBC pellet.
** OH, C3H.OH; A, C3H.A; F1, (C3H.OH × C3H.A)F1.
Significance levels: group I vs. group III, not significant (NS); group I vs. group IV, P < 0.001; group V vs. groups II, VI, P < 0.001.
Table II

Lack of Restriction of the T-B Cell Interaction for Purified T and B Cells in the Heterozygous Host

| Group | T* | B† | Host‡ | cpm ± SEM¶ | Percent bound‖ |
|-------|----|----|-------|------------|---------------|
| I     | A→F₁ | OH→F₁ | A     | 946 ± 178  | 22 ± 4       |
| II    | OH→F₁ | OH→F₁ | OH    | 3,571 ± 153 | 83 ± 4       |
| III   | A→F₁ | OH→F₁ | F₁    | 2,624 ± 120 | 61 ± 3       |
| IV    | OH→F₁ | OH→F₁ | F₁    | 2,918 ± 415 | 68 ± 10      |

*, ‡, §, ¶, ‖, † see legend for Table I. Significance levels: group II vs. group IV, NS; group I vs. group III, P ≤ 0.001.

(Table II, groups III and IV). Parental hosts, by contrast, showed the expected APC-B cell restriction (Table II, groups I and II).

Based on these results, it is impossible to exclude that this apparent APC-B cell restriction is not merely an artifact arising from suppression of the normal B cell response by a negative allogeneic effect exerted by the host's residual alloreactive T cells. The following experiments were set up to clarify this point.

Negative Allogeneic Effects Are Not Responsible for Apparent H-2 Restriction of the Host-B Cell Interaction. Allogeneic effects in vivo could potentially be mediated by residual alloreactive host cells recognizing and responding to MHC determinants present on either the transferred T or B cells and could result in suppression of the B cell response directed against the hapten.

A comparison was accordingly made of the ability of normal and specifically tolerant hosts to sustain a response. Parent→F₁ fetal liver chimeras were used as tolerant, irradiated adoptive recipients; these animals were >95% repopulated with lymphoid cells of donor origin and were functionally fully reconstituted with donor-derived APC (18).

To rule out allogeneic effects between host and B cells, parallel adoptive transfer experiments were carried out in which the same T and B cell combinations were transferred into previously irradiated parental and chimeric hosts (Table III).

Strictly comparable results were obtained in both instances, with allogeneic host-B cell pairings failing to cooperate. The levels of serum antibody, as determined by CRIA, closely parallel the 7-d spleen PFC response, and the two measures will therefore be used interchangeably. Both IgM and IgG responses were depressed to an equivalent extent in incompatible host-B cell combinations.

These experiments do not, however, exclude suppression of the B cell response by reaction between the host and transferred T cells. Syngeneic host-B cell combinations with either normal or chimeric hosts were set up, and the influence of helper T cell genotype was examined. Normal and chimeric hosts provided equivalent cooperating environments irrespective of the host-T cell compatibility (Table IV).

Discussion

Several levels of control of cell-cell interactions by the MHC have been described by various investigators. In this paper, we report on the role of the APC in the regulation of the T-B cell interaction in vivo, using an adoptive transfer system in which APC function is provided by the adoptive host. Our results are most compatible
Negative Allogenic Effects between Radioresistant Alloreactive Host Cells and B Cells Do Not Account for Host-B Cell Restriction: Comparison of Normal and Chimeric Hosts

| Group | T* | B§ | Host§ | PFC per spleen** | Percent Ab bound¶ |
|-------|----|----|-------|------------------|-------------------|
|       |    |    |       | Direct mean ± SEM | Indirect mean ± SEM |
| I     | A→F1 | A→F1 | A→F1 | 38,500 ± 2,010 | 170,000 ± 10,510 |
| II    | OH→F1 | OH→F1 | OH→F1 | 41,400 ± 3,130 | 138,000 ± 6,600 |
| III   | A→F1 | OH→F1 | OH→F1 | 43,500 ± 1,120 | 131,500 ± 5,140 |
| IV    | A→F1 | OH→F1 | A    | 2,700 ± 180   | 3,700 ± 540   |
| V     | A→F1 | OH→F1 | A→F1 | 4,000 ± 980   | 300 ± 450   |
| VI    | OH→F1 | A→F1 | OH   | 2,800 ± 630   | 3,400 ± 1,430 |
| VII   | OH→F1 | A→F1 | OH→F1 | 3,600 ± 630   | 4,900 ± 1,210 |
| VIII  | OH→F1 | OH→F1 | A→F1 | 1,200 ± 270   | 2,700 ± 540   |
| IX    | A→F1 | A→F1 | OH→F1 | 1,500 ± 270   | 2,300 ± 890   |
| X     | OH   | OH   | OH   | 16,571 ± 4,215 | 86,142 ± 11,385 |
| XI    | OH-unprimed | OH     | OH     | 4,132 ± 1,611 | 8,214 ± 2,307 |

*, †, §, ¶, see legend for Table I.
** 7-d splenic PFC response. Assayed on TNP-SRBC.
†† ND, not determined.

Significance levels: group I vs. group VI, PFC, P < 0.001, Ab, P < 0.001; group I vs. group VII, PFC, P < 0.001, Ab, P ≤ 0.025; group I vs. group IX, PFC, P < 0.001, Ab, P ≤ 0.025; group II vs. group III, PFC, NS; Ab NS; group II vs. group IV, PFC, P < 0.001, Ab, P ≤ 0.025; group II vs. group V, PFC, P < 0.001, Ab, P < 0.001.

Alloreactivity between the Host and Transferred Chimeric Helper T Cells Is Not Responsible for Apparent Host-B Cell Restriction: Equivalence of Normal and Chimeric Hosts

| Group | T* | B§ | Host§ | PFC per spleen** |
|-------|----|----|-------|------------------|
|       |    |    |       | Direct mean ± SEM |
| I     | OH→F1 | OH→F1 | OH→F1 | 45,000 ± 3,500 |
| II    | A→F1 | A→F1 | A→F1 | 40,500 ± 6,500 |
| III   | OH→F1 | A→F1 | A    | 35,000 ± 3,500 |
| IV    | OH→F1 | A→F1 | A→F1 | 37,000 ± 5,500 |
| V     | A→F1 | OH→F1 | OH   | 38,500 ± 2,300 |
| VI    | A→F1 | OH→F1 | OH→F1 | 46,000 ± 1,750 |

*, †, §, ¶, see legend for Tables I and III.

Significance levels: group I vs. group V, NS; group I vs. group VI, NS; group II vs. group III, NS; group II vs. group IV, P = 0.05.

with H-2 restriction expressed between APC and B cells, although the possibility of T cell-APC restriction in priming and T-B cell restriction of cooperation cannot be excluded. We were surprised to be unable to show at least H-2 restriction of the T cell-APC interaction, which has been described by Zinkernagel et al. (5) and Waldmann et al. (19) for in vitro cytotoxic T lymphocyte and in vivo T-B cell cooperative systems, respectively, and by ourselves for acutely primed T cells in the T cell proliferation assay (20). Possible reasons for this discrepancy are discussed below. The above notwithstanding, it is only under these particular conditions of H-2-unrestricted T cell activity that APC-B cell restriction can be shown. Whether, as
suggested by some workers, T cells must recognize the same determinants on APC and B cells, T cell-APC restriction will necessarily require concomitant T-B cell restriction, obscuring that between B cells and APC.

It is significant that previous demonstrations of APC-B cell restriction have relied on thymus-independent antigens. Thus, Gorczynski et al. (16) used an in vitro culture system in which suboptimal concentrations of lipopolysaccharide substitute for T cell help, and Singer et al. used the thymus-independent type 2 antigen, TNP-Ficoll, to show this with an in vitro culture system.

The alternative model of T cell-APC and consequent T-B cell restriction proposed by Sprent (8) and others (17, 18) is based on the finding that normal F1 and parent →F1 chimeric T cells contain separate subpopulations of T cells, each restricted to cooperation with one parental haplotype (18). Under ordinary conditions of in vivo priming with F1 APC or in F1 adoptive recipients, these populations give the overall impression of unrestricted cooperation, but they can be resolved by adoptive priming in irradiated parental recipients (8).

Our findings of apparent APC-B cell restriction and the lack of T-B cell restriction could reflect positive or negative allogeneic effects between the irradiated hosts and the transferred T or B cells. Initially, mutually tolerant T and B cells were transferred into supralethally irradiated parental hosts. Cooperation was observed only when the APC and B cells were syngeneic, and the response was not influenced by the T cell genotype. This result clearly excludes a negative allogeneic effect between alloreactive radioresistant T cells remaining in the host and the transferred T cells, which is a possibility that would have resulted in apparent T-B cell restriction, but it does not address the possibility of a positive allogeneic effect. The latter would be expected to give a result indistinguishable from the preceding. Additionally, it could be argued that the radioresistant T cells were able to directly suppress the response of B cells not syngeneic to the host. This possibility was ruled out in the experiments shown in Tables III and IV, in which irradiated parent →F1 chimeric animals were used as adoptive recipients. These animals were essentially fully repopulated with cells of donor parental origin, and cells recovered from the reconstituted animals were shown to be nonreactive in MLR against the H-2 determinants of the other respective parent. Comparable patterns of H-2 restriction were obtained with parental and chimeric hosts, suggesting that the role of residual alloreactive cells was insignificant.

Our results can be interpreted in terms of T-macrophage and T-B restriction only if it is postulated that our chimeric T cells are not restricted to cooperation with a given H-2 before transfer into the irradiated recipient. Two possibilities may exist for this: (a) that the T cells are effectively unprimed or (b) that they have been primed in the context of nonrestricting (F1) APC in situ and undergo selection to H-2 only in the final host. The first possibility—a lack of priming—can be excluded on the basis of the results in Table III, groups X and XI, showing a striking difference in helper activity of primed and unprimed T cells in the adoptive transfer system. Additionally, the character of the response obtained was that of a secondary immune response, with a predominant IgG component. It is impossible to exclude the second possibility definitely, although all our evidence is against it. Our earlier studies in the T cell

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2Hodes, R., J. K. Hathcock, and A. Singer. Thymus-independent type-2 responses to TNP-Ficoll involve cell interactions which are genetically restricted by products of the MHC. Manuscript submitted for publication.
proliferation assay show clearly that parent→F₁ chimeric T cells primed in situ are restricted to cooperation with syngeneic APC in the absence of detectable allogeneic effects. This argues against any substantial component of endogenous F₁-derived APC in the chimeras. Given also the time allowed for repopulation of the chimeras before priming and the supralethal doses of irradiation used to prepare them, it seems most unlikely that there should be a residuum of F₁ APC, especially in light of Sprent's results, showing disappearance of host-derived APC by 15 wk (21). Nonetheless, we must consider the possibility that a small number of F₁ APC are present, and that over the 3–6-mo priming period used by us, these host-derived APC may be sufficient to expand both parentally restricted subpopulations to detectable levels. On purely statistical grounds, however, the extent of F₁ repopulation would have to be substantial to account for our data.

Additionally, if the unrestricted behavior of T cells derived from chronic in situ priming of one-way FLC is due to significant contamination of the chimeras by F₁ APC, the equivalence of normal and chimeric secondary adoptive hosts would not be expected. These chimeras would presumably contain equivalent proportions of F₁ APC to the priming hosts, and reselection to antigen would be mediated by both parent and F₁ APC; thus, B cell-APC restriction would not be seen. Normal hosts, having only parental APC, would show the expected restriction.

Alternative (and to us preferable) explanations include the possibility that prolonged (3 mo) and vigorous (antigen in CFA) in vivo priming is sufficient to enlarge both parentally restricted T cell subpopulations in the parent→F₁ chimeras, irrespective of the APC haplotype repopulating the host. Matzinger and Mirkwood (22) have obtained equivalent findings in the induction of cytotoxic T lymphocytes restricted to minor histocompatibility antigens associated with non-self-H-2, in fully allogeneic stem cell chimeras, which they attributed to their vigorous priming conditions. Conceivably, chronic in situ priming may also induce a single unrestricted T cell population capable of cooperation with both haplotypes represented in the priming host. Alternatively, the physical form of the antigen present during either T cell induction or cooperation may be significant, as implied in our observation that soluble antigen is capable of overcoming apparent H-2 restriction for primed (and restricted) T cells in vitro (20). Finally, the possibility should be considered that the helper T cells induced by chronic in vivo priming do not require restimulation by antigen, at least in association with APC syngeneic to those present during induction.

A curious point arising from the data in Table III is that neither direct nor indirect PFC responses were observed in allogeneic host-B cell combinations. If B cells can undergo adaptive differentiation (23), histoincompatible APC and B cells from parent→F₁ chimeras would be expected to give an IgM response, the B cells in this case being essentially unprimed to hapten in the context of allogeneic APC, but still able to cooperate. It is unlikely that this result, which is compatible with the work of Sprent and Bruce (24), is due to suppression. The mouse strains used in these studies differ chiefly at the left-hand end of H-2, and although our evidence does not bear directly on this point, it seems likely that the APC-B cell interaction is under I-region control.

A key feature of our experiments, and an aspect largely neglected by previous workers with in vivo systems, is the careful depletion of extraneous cell types from the transferred cooperating populations. This is particularly important as it allowed us to
directly examine the in vivo T cell-APC-B cell interaction. Our earlier work with the T cell proliferation assay established the necessity for only a very few APC in an in vitro response, and we thus felt it necessary to rigorously deplete APC from the T and B cells before adoptive transfer to isolate the host’s role in the cooperative event. The studies of Shih et al. (6), clearly documenting the efficiency of antigen transfer to residual APC in the B cell preparations, emphasize the importance of this step.

Our failure to demonstrate T-B cell allogeneic restriction is at variance with earlier data of Katz et al. (9), who initially reported absolute restriction of the T-B cell interaction in vivo, using T and B cells derived from one-way bone marrow chimeras in an adoptive transfer system. Our attempt to repeat a part of these experiments was unsuccessful, as we were unable to show T-B cell restriction in the F1 host as opposed to the parental host. We believe that this discrepancy simply reflects the H-2 unrestricted help in our assay system, which may in turn be related to the manner of preparation of the chimeras (fetal liver vs. bone marrow). Alternatively, the degree of depletion of extraneous cell types from the transferred populations may be significant (see above). Katz has, however, recently reported that the H-2 restriction of T cells from parent→F1 chimeras is considerably less tight than was previously thought and that the “pseudorestriction” seen for cells primed in situ in the chimeric environment can be overcome by adoptive priming in the context of appropriate APC. This and other results (25-28) have suggested that the original interpretation of the data suggesting adaptive differentiation was overly stringent and that the true situation may be more one of “environmental restraint,” in which extrathymic influences during differentiation may be of critical importance.

Sprent (8), using in vivo positive selection to antigen of F1 cells in a parental environment, found that H-2 restriction was imposed during the selection process, a result not compatible with ours. The same author, using cells from stem cell chimeras, has reported unrestricted T-B cell cooperation. T and B cells derived from two-way bone marrow chimeras were used in an adoptive transfer protocol similar to ours but with an F1 host and thus F1 APC in the ultimate cooperation step. The interaction between T and B cells under these conditions was not restricted by the H-2 genotype, but did reflect the phenotype of the cells, a result that they interpreted in terms of in situ priming of the T cell subsets by both APC types (3). In a subsequent study, parental cells derived from parent plus F1→F1 chimeras were shown to comprise two functionally distinct subpopulations, with cooperative preferences dictated by the H-2 type of the APC with which priming occurred. In situ priming here is with both parent and F1 cells, the latter in sufficient proportions to give the overall picture of unrestricted cooperation (29). Both of these results can be interpreted in terms of APC-T cell and T-B cell restriction, as is done by Sprent; they are, however, equally compatible with a model of unrestricted T-B cell cooperation. APC-B cell restriction is not seen in this situation, however, as the hosts for the adoptive transfers are themselves nonrestricting F1.

Shih et al. (6) and Singer et al. (7) have both demonstrated a lack of T-B cell restriction in vivo and in vitro, and, furthermore, have claimed to find T cell-APC restriction. It is difficult to reconcile this last observation with our findings, other than on the basis of differences in experimental conditions. It could be argued that their system tends to reveal manifestations of T cell-APC restriction—for example, and H-2-restricted stimulation or restimulation of helper T cells—whereas our protocol
isolates a similarly MHC-restricted cooperative event between APC and B cells. Such a possibility is also suggested by the work of Howie and Feldmann (30) and Boswell et al. (31-33), the latter showing an APC requirement for the activation of the Lyb-5+ B cell subpopulation by both thymus-dependent and thymus-independent antigens.

We are thus left to reconcile our findings with those in the existing literature. It is unlikely that an overall synthesis can be achieved; nonetheless, certain unifying points emerge.

Our data are compatible in broad outline with either the prevailing hypothesis of sequential APC-T cell and T-B cell restriction or with our model of APC-B cell restriction. T cell-APC restriction may be present but was not revealed under our particular experimental conditions, as was discussed above. At present, it is impossible to decide between these two alternatives definitely, although our evidence is, we feel, more consistent with APC-B cell restriction and the absence of T-B cell restriction.

Our results thus support the notion that the T cell, once induced, can provide antigen-specific but H-2-unrestricted help to a B cell population, although this help requires the mediation of APC syngeneic to the B cell to be efficiently delivered; the T and B cells must be mutually tolerant for this. Others have shown (34) a requirement for APC in the in vitro B cell response to thymus dependent antigens and APC-B cell restriction has been demonstrated for the thymus independent antigen response. Although these results can be interpreted in terms of the alternative hypothesis of H-2 restriction of T cell-APC and T-B cell interactions, this analysis requires certain assumptions not supported by our data.

Summary

The restrictions imposed by the major histocompatibility complex on T-B-antigen-presenting cell (APC) interactions were studied with an in vivo adoptive transfer system, using mutually tolerant T and B cells taken from one-way fetal liver chimeras. It was found that the B cells and adoptive recipient (which provides APC function) have to share determinants encoded by the left-hand end of the H-2 complex for cooperation, whereas there is apparently no such requirement for T-B cell syngeneicity. Suppression arising from allogeneic effects between the host and the transferred T or B cells was excluded by the use of tolerant as well as normal adoptive recipients; both were functionally equivalent. We conclude that under our experimental conditions, unrestricted helper T cell function and concurrent APC-B cell genetic restriction can be demonstrated in vivo.

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