ROLE OF THE MAJOR HISTOCOMPATIBILITY COMPLEX IN T CELL ACTIVATION OF B CELL SUBPOPULATIONS

Major Histocompatibility Complex-restricted and -unrestricted B Cell Responses Are Mediated by Distinct B Cell Subpopulations

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The antigen-specific activation of B lymphocytes in T cell-dependent antibody responses has been shown to require recognition by helper T cells of products of the major histocompatibility complex (MHC)[1] expressed by cells cooperating in these responses. The specific identification of those cell interactions that are MHC restricted in T-dependent responses has, however, been the subject of controversy. The results of previous studies have been generally consistent in identifying a requirement for helper T cell recognition of MHC products expressed on antigen-presenting cells (APC) or accessory cells (1–8). In contrast, there has been considerable disagreement among those studies that have attempted to identify a requirement for T cell recognition of MHC determinants expressed on B cells in the same T cell-dependent antibody responses (3–11). Although the basis for this inconsistency has not been determined, recent findings (12) have demonstrated that the requirements for T cell recognition of B cell MHC products may in fact differ for the activation of distinct B cell subpopulations.

The existence of distinct B cell subpopulations has been established on the basis of their expression of a number of cell surface markers including Ia, IgM, IgD, F_c receptors, complement receptors, and most recently by the differentiation antigens Lyb-3, Lyb-5, and Lyb-7 (13–15). Although the possibility that these markers may define functionally distinct subsets of B cells has been suggested by a number of observations, there has until recently been little direct information concerning the activation requirements of identifiable B cell subpopulations in the generation of antigen-specific antibody responses. Recently, however, studies of in vivo adoptive transfer responses have suggested that Lyb-5^+ and Lyb-5^- B cells differ in their requirements for MHC-restricted T cell recognition (16). If a consistent association does in fact exist between the Lyb-5 phenotype of B cell subpopulations and the

[1] *Abbreviations used in this paper:* APC, antigen-presenting cells; B10, C57BL/10; $B6AF_1$, (C57BL/6 × A/J)F_1; $B6CBF_1$, (C57BL/6 × CBA/J)F_1; $B6C3F_1$, (C57BL/6 × C3H/HeJ)F_1; C, guinea pig complement; CFA, complete Freund's adjuvant; KLH, keyhole limpet hemocyanin; FCS, fetal calf serum; MEM, minimal essential medium; MHC, major histocompatibility complex; MIg, mouse immunoglobulin; PFC, plaque-forming cells; RAMB, rabbit anti-mouse brain serum; SRBC, sheep erythrocytes; TNP, trinitrophenyl.

activation requirements of those B cells, it might be predicted that responses that differ in their requirements for MHC-restricted T cell recognition will be mediated by distinct B cell subpopulations.

In a test of this prediction, the present study has characterized the requirements for MHC-restricted T cell recognition of the B cells and APC that cooperate in vitro for T cell-dependent antibody responses to the antigen trinitrophenyl (TNP)-keyhole limpet hemocyanin (KLH). First, it was shown that experimental conditions could be defined in vitro under which helper T cells uniformly recognized *H-2* products on APC, but either were or were not required to recognize *H-2* determinants expressed on B cells. Subsequently, the B cell subpopulations participating in responses under these conditions were analyzed. It was determined that under conditions in which T cell recognition of the B cell *H-2* product was required, responses were mediated by Lyb-5$^-$ B cells, the B cell subset defined by the CBA/N mutation (14). In contrast, for responses that required T cell recognition of H-2 on APC but not B cells, the participation of Lyb-5$^+$ B cells was essential for response. These findings are consistent with the concept that the Lyb-5$^+$ and Lyb-5$^-$ B cell subpopulations differ in their requirements for activation by *H-2*-restricted T cell interactions.

Materials and Methods

Animals. C57BL/10 (B10), B10.A, B10.BR, A.BY, A/J, (C57BL/6 × CBA/J)F_1 (B6CBF$_1$), (C57BL/6 × C3H/HeJ)F_1 (B6C3F$_1$), (C57BL/6 × A/J)F_1 (B6AF$_1$), (B10 × B10.A)F_1, and (B10 × B10.BR)F_1 mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. B10.A(3R), B10.A(4R), B10.A(5R), and B10.MBR mice were provided by Dr. D. H. Sachs, National Institutes of Health, Bethesda, Md. CBA/CaHN and CBA/N mice were provided by the Small Animal Section, National Institutes of Health.

Chimeras. Chimeras used in this study were prepared as previously described (6) by the transfer of 15 × 10^6 T cell-depleted bone marrow cells into lethally irradiated (950–1,000 rad) recipients, and are designated as bone marrow donor → irradiated recipient. Chimeras were immunized no earlier than 8 wk after irradiation and reconstitution. All the chimera spleen cells used in these studies were typed by indirect immunofluorescence (6) and were uniformly of donor origin without detectable (<5%) host contamination.

Antigens. KLH (Lot 730192; Calbiochem-Behring Corp., San Diego, Calif.) was conjugated with 2,4,6-trinitrobenzene sulfonate (Pierce Chemical Co., Rockford, Ill.) as previously described (17). The degree of substitution was 20 TNP residues per 100,000 mol wt KLH (TNP-KLH).

Immunization. Mice were immunized with 100 μg of KLH or TNP-KLH in complete Freund's adjuvant (CFA) (Difco Laboratories, Detroit, Mich.) intraperitoneally 3–8 wk before use.

Preparation of Cells

T CELLS. KLH-primed helper T cells were obtained either as nylon column nonadherent spleen cells or as spleen cells nonadherent to anti-mouse immunoglobulin (MIg)-coated plastic dishes. Nylon nonadherent cells were obtained by the method of Julius et al. (18). Anti-MIg plate separations were carried out by a modification of the method of Mage et al. (19). For anti-MIg separations, tissue culture dishes (25025; Corning Glass Works, Corning, N. Y.) were coated with 200 μg/ml of pig anti-MIg (the generous gift of Dr. D. H. Sachs) at 4°C overnight, then incubated with Eagle's minimal essential medium (MEM) supplemented with 5% fetal calf serum (FCS) (Lot R41003; Reheis Chemical Co., Phoenix, Ariz.) at room temperature for 1 h. 50 million spleen cells resuspended in 10 ml of MEM with 5% FCS were incubated on one plate at room temperature for 1 h and nonadherent cells were recovered. Usually 30–35% of applied cells were recovered and <5% of these cells were cell-surface Ig-positive by direct immunofluorescence.

B CELLS. B cells were prepared by depleting TNP-KLH-primed spleen cells of T cells by

treatment with a T cell-specific cytotoxic rabbit anti-mouse brain serum (RAMB) plus complement (C). The T cell specificity of this reagent has been previously characterized (17).

APC. Spleen cells from nonimmunized mice were T cell depleted by treatment with RAMB plus C and irradiated with 1,000 rad. Residual cells were incubated in RPMI 1640 medium with 10% FCS and, for antigen pulsing, in the presence of 10 μg/ml of TNP-KLH at 37°C for 4 h, and then washed five times to eliminate free antigen carryover. These antigen-pulsed cells were used as APC or accessory cells.

Culture Conditions. All cultures were performed in a volume of 2 ml per 16-mm-Diam flat-bottomed well (3524; Costar, Data Packaging, Cambridge, Mass.) and were incubated for 5 d at 37°C in a 5% CO_2 humidified air atmosphere. The medium used was RPMI 1640 supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 1 mM sodium pyruvate, 1 times nonessential amino acid, 15 mM Hepes buffer, 5×10^{-5} M 2-mercaptoethanol, and 10% FCS. Cells were harvested, resuspended in MEM, and individual cultures were assayed for hemolytic plaque-forming cells (PFC). The optimal cell density for secondary responses to TNP-KLH is 2×10^6–5×10^6 spleen cells/2 ml culture. The cell number of each individual cell population added per culture is indicated for each experiment.

PFC Assay. Sheep erythrocytes (SRBC) were conjugated with TNP (TNP-SRBC) by the method of Rittenberg and Pratt (20). Direct (IgM) PFC and total PFC (facilitated by either specific rabbit anti-MIgG or polyspecific rabbit anti-MIg) were assayed on TNP-SRBC by the slide modification of the Jerne hemolytic plaque technique (21) and IgG PFC were calculated as total PFC minus direct PFC. The TNP specificity of the PFC generated under these culture conditions has been demonstrated by inhibition with a TNP-conjugated bovine serum albumin reagent that completely and specifically inhibits anti-TNP PFC at 1×10^{-6} M TNP (Y. Asano, unpublished data). All points shown in each experiment represent the arithmetic mean responses of triplicate cultures.

Results

To study the relationship between B cell activation requirements and the identity of the B cell subpopulations activated under given experimental conditions, it was first necessary to identify response conditions that might differ in their pathways of B cell activation. To establish such conditions, the effect of antigen concentration on response was evaluated by determining the response of TNP-KLH-primed B10 spleen cells to titrated concentrations of TNP-KLH. When cultures were assayed in parallel for both IgM and IgG responses, two discrete peaks of response were seen, a predominantly IgG peak, which was consistently observed at antigen concentrations of 0.001–0.01 μg/ml (Fig. 1), and a second peak, predominantly IgM, at 0.1–20 μg/ml TNP-KLH (Fig. 1). These results defined two distinct response conditions, and thus allowed a direct evaluation of the possibility that the activation pathways functioning at low and high antigen concentrations might differ in their requirements for *H-2*-restricted T cell interaction with B cells and/or APC.

(A × B)F_1 T Cells Can Collaborate with Parent$_A$ B Cells in the Presence of Parent$_A$ but Not Parent$_B$ APC for IgG Responses to Low Concentrations of TNP-KLH. The requirements for MHC-restricted T cell recognition were compared at high or low concentrations of antigen using populations of TNP-primed B cells and KLH-primed T cells. Responses to low concentrations of TNP-KLH were evaluated either at low concentrations of soluble antigen or using TNP-KLH-pulsed APC as an alternative means of providing low effective concentrations of antigen. To establish the requirements for MHC-restricted cell recognition in T cell-dependent antibody responses to low concentrations of TNP-KLH, experiments were first carried out to determine the ability of KLH-primed (B10 × B10.A)F_1 T cells to cooperate with TNP-primed parental B10 or B10.A B cells and B10 or B10.A APC for the secondary PFC responses

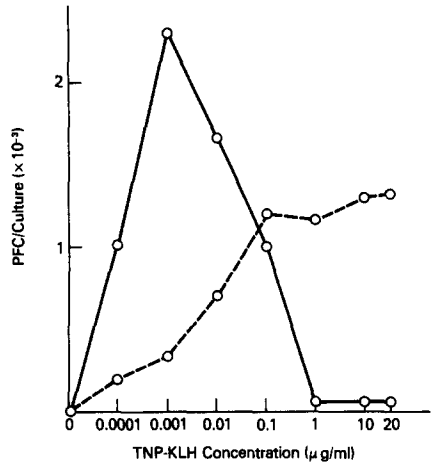

FIG. 1. Activation requirements for IgG and IgM responses are different in their stimulation antigen concentration. 3 × 10^6 TNP-KLH-primed B10 spleen cells were cultured with titrated concentrations of antigen TNP-KLH, and cultures assayed in parallel for IgG (—) and IgM (- - -) PFC responses.

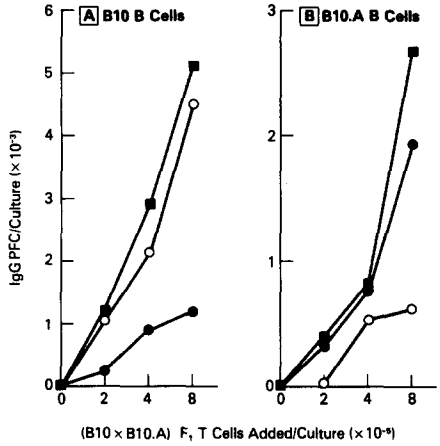

FIG. 2. (A × B)F_1 T cells can collaborate with $parent_A$ B cells in the presence of $parent_A$ but not $parent_B$ APC. Graded numbers of KLH-primed (B10 × B10.A)F_1 T cells were added to cultures containing 2 × 10^6 of either B10 (A) or B10.A (B) TNP-primed, RAMB plus C-treated spleen cells in the presence of TNP-KLH-pulsed B10 (○), B10.A (●), or (B10 × B10.A)F_1 (■) APC.

to TNP-KLH. (B10 × B10.A)F_1 T cells cultured with TNP-KLH-pulsed B10, B10.A, or (B10 × B10.A)F_1 APC were assayed for their helper activity with B10 or B10.A B cells (Fig. 2). (B10 × B10.A)F_1 T cells collaborated with B10 B cells in the presence of (B10 × B10.A)F_1 or B10 but not B10.A APC; whereas the same (B10 × B10.A)F_1 T cells collaborated with B10.A B cells in the presence of (B10 × B10.A)F_1 or B10.A, but not B10 APC (Fig. 2). Under these conditions, responses were predominantly IgG, as noted above for responses to low concentrations of soluble TNP-KLH, with direct (IgM) PFC consistently <20% of the total PFC response detected. These results demonstrated that (A × B)F_1 helper T cells are capable of cooperating with B cells

and APC of F_1 or either parental strain, but that B cells and APC must be identical in at least one parental haplotype for effective cooperation to occur.

(A × B)F_1 → Parent$_A$ Chimeric Helper T Cells Are Restricted in Their Recognition of H-2 Determinants Expressed on Both B Cells and APC for IgG Responses to Low Concentrations of TNP-KLH. The requirement that B cells and APC be syngeneic with one another could be interpreted as: (*a*) a direct *H-2* restriction in the interaction of B cells with APC; or alternatively, (*b*) that individual helper T cells are required to recognize products of the same parental *H-2* haplotype expressed on both B cells and APC. To examine the latter possibility that helper T cells were restricted in their recognition of MHC determinants expressed by both B cells and APC, experiments were performed using T helper cells from (A × B)F_1 → parent$_A$ chimeras.

It has previously been demonstrated that maturation of (A × B)F_1 T cells in a parent$_A$ chimeric environment restricts the "self" recognition of these T cells, so that they recognize the MHC determinants of parent$_A$ but not parent$_B$. The existence of such MHC restriction was therefore examined for the secondary responses of primed T and B cell populations. (B10 × B10.A)F_1 T cells or T cells from (B10 × B10.A)F_1 → A.BY or (B10 × B10.A)F_1 → A/J chimeras were co-cultured with B10 or B10.A RAMB plus C-treated spleen cells (B cells plus APC) in the presence of 0.001 μg/ml soluble TNP-KLH (Fig. 3). Although F_1 T cells cooperated equally well with B10 or B10.A (B cells plus APC), (B10 × B10.A)F_1 → A.BY helper T cells collaborated with B10 but not B10.A (B cells plus APC) and (B10 × B10.A)F_1 → A/J helper T cells collaborated with B10.A but not B10 cells. These results indicate that, as a result of their maturation in a chimeric environment, (A × B)F_1 → parent$_A$ helper T cells are also *H-2* restricted in their recognition of parent$_A$ (B cell plus APC) populations for secondary IgG responses to TNP-KLH.

A series of experiments was then carried out to distinguish among the possibilities that, under the response conditions used above, helper T cells recognize the *H-2*

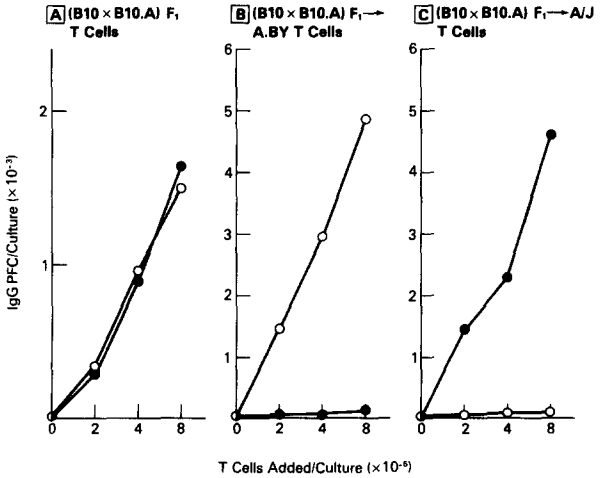

FIG. 3. (A × B)F_1 → parent$_A$ chimeric helper T cells are restricted to recognizing H-2 determinants of parent$_A$. Graded numbers of KLH-primed (B10 × B10.A)F_1 (A), (B10 × B10.A)F_1 → A.BY (B) or (B10 × B10.A)F_1 → A/J (C) T cells were co-cultured with 2 × 10^6 of B10 (○) or B10.A (●) RAMB plus C-treated TNP-primed spleen cells in the presence of soluble TNP-KLH (0.001 μg/ml).

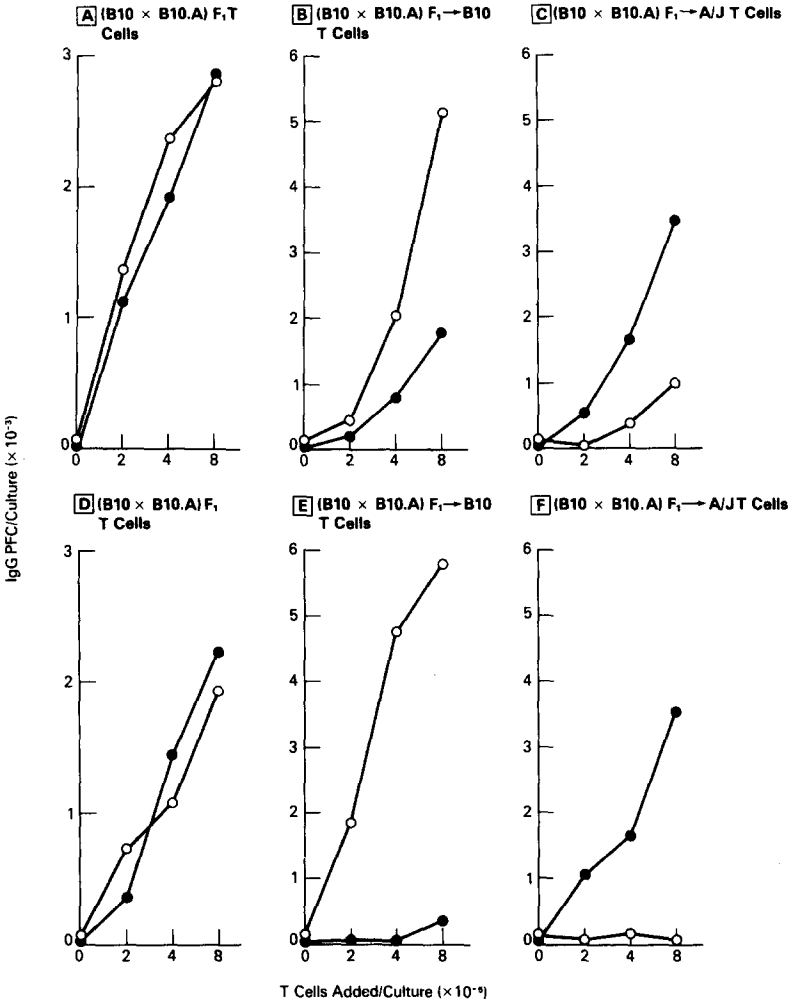

FIG. 4. (A × B)F_1 → parent$_A$ chimeric helper T cells are restricted in their recognition of parent$_A$ H-2 determinants expressed on B cells and APC. Graded numbers of KLH-primed (B10 × B10.A)F_1 (A and D), (B10 × B10.A)F_1 → B10 (B and E) or (B10 × B10.A)F_1 → A/J (C and F) T cells were co-cultured; (A–C) with 2 × 10^6 TNP-primed (B10 × B10.A)F_1 RAMB plus C-treated spleen cells in the presence of 1 × 10^5 antigen-pulsed B10 (○) or B10.A (●) APC; or (D–F) with 2 × 10^6 TNP-pulsed B10 (○) or B10.A (●) RAMB plus C-treated spleen cells in the presence of 1 × 10^5 antigen-pulsed (B10 × B10.A)F_1 APC.

determinants expressed by B cells, APC, or both. To first determine whether helper T cells recognize the *H-2* determinants expressed by APC, (B10 × B10.A)F_1, (B10 × B10.A)F_1 → B10, and (B10 × B10.A)F_1 → A/J T cells were co-cultured with (B10 × B10.A)F_1 B cells, and with antigen-pulsed B10 or B10.A APC in the absence of soluble antigen. The helper activity of (B10 × B10.A)F_1 T cells was expressed equally well in the presence of either parental APC (Fig. 4A). In contrast, (B10 × B10.A)F_1 → B10 helper T cells cooperated with (B10 × B10.A)F_1 B cells more efficiently in the presence of B10 APC than in the presence of B10.A APC (Fig. 4B), and (B10 × B10.A)F_1 → A/J helper T cells were more efficient in the presence of B10.A APC

(Fig. 4C). The ability of chimeric (A × B)F_1 → $parent_A$ T cells to cooperate preferentially with $parent_A$ APC demonstrated the ability of primed helper T cells to recognize *H-2*-encoded determinants expressed on APC for secondary IgG antibody responses in vitro. It should noted that the apparent ability of F_1 → $parent_A$ T cells to cooperate to a significant but quantitatively lesser degree with $parent_B$ APC (Fig. 4) could be accounted for by some degree of antigen transfer to F_1 APC present in the B or T cell populations.

It was then determined whether helper T cells for the secondary responses to low concentrations of TNP-KLH are required to recognize *H-2* determinants expressed on B cells as well as on APC. For these experiments, (B10 × B10.A)F_1, (B10 × B10.A)F_1 → B10, and (B10 × B10.A)F_1 → A/J T cells were cultured with antigen-pulsed (B10 × B10.A)F_1 APC and tested for their ability to help B10 or B10.A B cells. Although (B10 × B10.A)F_1 helper T cells were able to cooperate with either parental B cells in the presence of TNP-KLH-pulsed (B10 × B10.A)F_1 APC (Fig. 4D), chimeric (B10 × B10.A)F_1 → B10 helper T cells provided help to B10 but not B10.A B cells (Fig. 4E), and (B10 × B10.A)F_1 → A/J T cells cooperated with B10.A but not B10 B cells (Fig. 4F). It should be noted that the ability of (A × B)F_1 → $parent_A$ T cells to cooperate in each instance with (A × B)F_1 B cells or APC (Fig. 4) suggested that the observed *H-2* restriction of T helper activity was not the result of suppression induced by the presence of cells expressing the "inappropriate" parental haplotype. A series of experiments designed to further test this possibility also failed to identify suppression as a mechanism mediating the observed *H-2* restrictions (data not shown). Thus, it appeared that (A × B)F_1 → $parent_A$ helper T cells were restricted to recognizing $parent_A$ but not $parent_B$ H-2 determinants expressed on both B cells and APC.

(A × B)F_1 → $Parent_A$ Helper T Cells Recognize I-A Subregion Products on Both APC and B Cells for IgG Responses to Low Concentrations of TNP-KLH. Although the results presented above established conditions in which helper T cells are required to recognize *H-2*-encoded products expressed on both APC and B cells, it was not determined whether the same or different *H-2* products are recognized on these two populations. To determine which *H-2* subregion(s) encodes the products recognized by helper T cells on APC and on B cells, experiments were carried out using APC and B cell populations derived from intra-*H-2* recombinant strains. The determinants recognized on APC were analyzed by culturing T cells of (B10 × B10.A)F_1, (B10 × B10.A)F_1 → B10, and (B10 × B10.A)F_1 → A/J origin with (B10 × B10.A)F_1 B cells and antigen-pulsed APC from B10, B10.A, B10.A(3R), B10.A(4R), and B10.A(5R). (B10 × B10.A)F_1 T cells could equally well help responses stimulated by each of the antigen-pulsed APC populations used (Fig. 5A). In contrast, (B10 × B10.A)F_1 → B10 T cells showed greater helper activity when stimulated by B10, B10.A(3R), or B10.A(5R) APC than when stimulated by B10.A or B10.A(4R) APC (Fig. 5B). Reciprocal results were obtained for (B10 × B10.A)F_1 → A/J T cells that cooperated optimally with B10.A or B10.A(4R) APC (Fig. 5C). These results demonstrated that T cell recognition of *K* or *I-A* region products on APC is required for helper T cell activation.

The finding that (A × B)F_1 helper T cells could help under these conditions only when B cells and APC were of the same *H-2* haplotype (Fig. 2) suggested that helper T cells might recognize the same *H-2* subregion products on APC and on B cells.

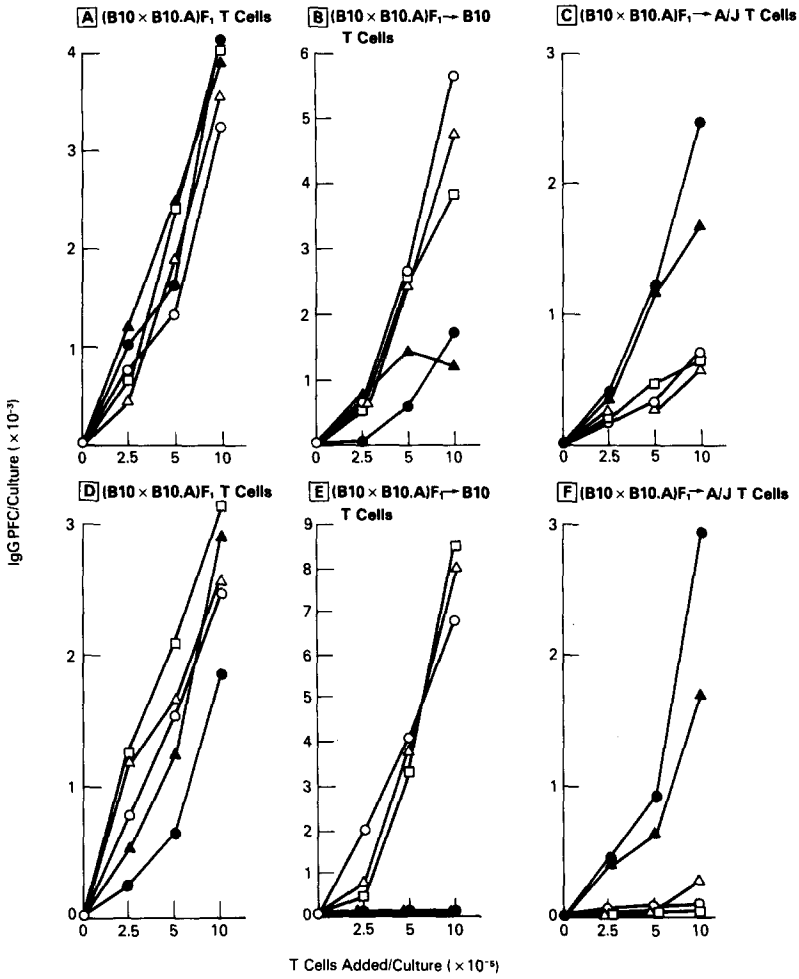

FIG. 5. Recognition of K, I-A region products is required for both helper T cell-APC and helper T cell-B cell interaction. Graded numbers of KLH-primed (B10 × B10.A)F_1 (A and D), (B10 × B10.A)F_1 → B10 (B and E) or (B10 × B10.A)F_1 → A/J (C and F) T cells were co-cultured with: (A–C) 2 × 10^6 TNP-primed (B10 × B10.A)F_1 RAMB plus C-treated spleen cells in the presence of 1 × 10^5 TNP-KLH-pulsed B10 (○), B10.A (●), B10.A(3R) (△), B10.A(4R) (▲), or B10.A(5R) (□) APC; or (D–F) 2 × 10^6 TNP-primed B10 (○), B10.A (●), B10.A(3R) (△), B10.A(4R) (▲), or B10.A(5R) (□) RAMB plus C-treated spleen cells in the presence of 1 × 10^5 TNP-KLH-pulsed (B10 × B10.A)F_1 APC.

Subregion mapping of the *H-2* products recognized on B cells by restricted T helper cells was carried out to test this prediction. In the presence of antigen-pulsed (B10 × B10.A)F_1 APC, (B10 × B10.A)F_1 → B10 T cells collaborated with B10, B10.A(3R), and B10.A(5R) B cells but not with B10.A and B10.A(4R) B cells (Fig. 5E), whereas (B10 × B10.A)F_1 → A/J T cells cooperated only with B10.A and B10.A(4R) B cells (Fig. 5F). T cells thus recognize *K*- or *I-A*-encoded products expressed on B cells as well as on APC. Additional experiments using the recombinant B10.MBR ($K^bI^kS^kD^q$) demonstrated that T cell recognition of both B cells and APC is specific for products encoded by the *I-A* subregion (data not shown). The results of these studies are summarized in Table I.

TABLE I

Summary of MHC-restricted Recognition by T Helper Cells

Strains of B cells or APC*	Origins of H-2 regions								Response in the presence of T cells from	
	K	A	B	J	E	C	S	D	(B10 × B10.A)F_1 → B10	(B10 × B10.A)F_1 → A/J
B10	b	b	b	b	b	b	b	b	+	−
B10.A(3R)	b	b	b	b	k	d	d	d	+	−
B10.A(5R)	b	b	b	k	k	d	d	d	+	−
B10.A(4R)	k	k	b	b	b	b	b	b	−	+
B10.A	k	k	k	k	k	d	d	d	−	+
B10.MBR	b	k	k	k	k	k	k	q	−	+

* T cell recognition of *H-2* products on B cells was determined in the presence of B cells of the indicated strain and TNP-KLH-pulsed (B10 × B10.A)F_1 APC. T cell recognition of *H-2* products on APC was determined in the presence of TNP-KLH-pulsed APC of the indicated strain and (B10 × B10.A)F_1 B cells.

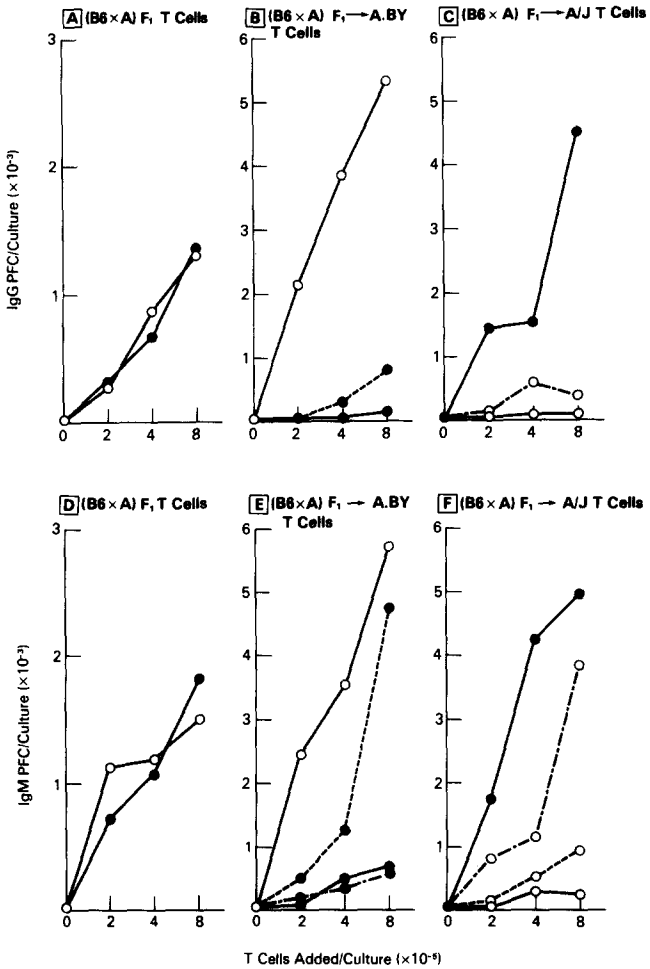

FIG. 6. Helper T cells are restricted in their *H-2* recognition of APC but not B cells at higher dose of antigenic stimulation. Graded numbers of KLH-primed B6AF_1 (A and D), B6AF_1 → A.BY (B and E), or B6AF_1 → A/J (C and F) T cells were cultured with 2 × 10^6 TNP-primed B10 (○) or B10.A (●) RAMB plus C-treated spleen cells in the presence of 0.001 μg/ml (A–C) or 10 μg/ml (D–F) of TNP-KLH. Some cultures contained 1 × 10^6 B10 (- - -) or B10.A (— - —) APC.

Helper T Cells Are Restricted in Their H-2 Recognition of APC but Not B Cells When Activated at High Concentration of TNP-KLH. The results presented above demonstrated that T cell recognition of *I-A* products expressed on B cells and on accessory cells is required for the activation of B cells mediating responses to low concentrations of TNP-KLH. The possibility remained, however, that the requirements for *H-2*-restricted recognition might differ for different pathways of B cell activation, and previous findings have in fact suggested that under different experimental conditions the in vitro PFC response to TNP-KLH requires T cell recognition of *H-2* products on accessory cells, but does not require T cell recognition of B cell *H-2* (1–8).

To directly compare the requirements for *H-2*-restricted T cell recognition under conditions of low or high antigen concentration, the same populations of TNP-primed B cells and KLH-primed T cells were stimulated with either low (0.001 μg/ml) or high (10 μg/ml) concentrations of soluble TNP-KLH (Fig. 6). B6AF$_1$ T cells helped both parental (B cells plus APC) equally well for both IgM responses to high concentration of antigen and for the predominantly IgG responses to low antigen concentration (Fig. 6 A and D). In addition, (A × B)F$_1$ → parent$_A$ T cells helped parent$_A$ but not parent$_B$ (B cells plus APC) in both conditions. The requirement for T cell recognition of *H-2* products expressed on B cells was examined by determining the ability of (A × B)F$_1$ → parent$_A$ T cells to activate parent$_B$ B cells in the presence of parent$_A$ APC and either high or low concentrations of TNP-KLH. For responses to low concentration TNP-KLH, the inability of (A × B)F$_1$ → parent$_A$ T cells to activate parent$_B$ B cells (Fig. 6 B and C) was not reversed by the addition of parent$_A$ APC, consistent with the requirement demonstrated above for T cell recognition of *H-2* product on B cells as well as APC in these responses. In contrast, for responses at high antigen concentration, the addition of parent$_A$ APC allowed (A × B)F$_1$ → parent$_A$ T cells to cooperate with either parent$_A$ or parent$_B$ B cells (Fig. 6 E and F). These results suggested that helper T cells are *H-2*-restricted in their recognition of APC under conditions of both low and high antigen concentration, but that *H-2*-restricted recognition of B cells is required only for responses at low antigen concentration.

Responses Resulting from MHC-restricted and -unrestricted T-B Cell Interaction Involve Different B Cell Subpopulations. At least two mechanisms might explain the difference in B cell activation requirements observed above under the two conditions of antigen concentration studied. First, it is possible that the same B cell populations are activated at low or high antigen concentrations, but that different activation pathways are used under these two conditions so that T cell recognition of B cell *H-2* product is required only for the activation occurring at low antigen concentration. Alternatively, it is possible that the observed differences in requirements for *H-2*-restricted T cell recognition at high and low TNP-KLH concentrations are due to the activation of different B cell subpopulations at these two conditions, and that these subpopulations differ in their requirements for *H-2*-restricted T cell recognition.

To test the possibility that different B cell subpopulations participate in the response to high and low concentrations of TNP-KLH, a comparison was made of B cells derived from the normal strain CBA/CaHN or from the defective mutant strain CBA/N. Mice of the CBA/N strain are deficient in a subpopulation of B cells that is identifiable by the expression of the Lyb-5 differentiation antigen. Thus, B cells from normal strains include both Lyb-5$^+$ and Lyb-5$^-$ subpopulations, whereas CBA/N B cells are exclusively Lyb-5$^-$. When B cells from CBA/CaHN mice (both Lyb-5$^+$ and

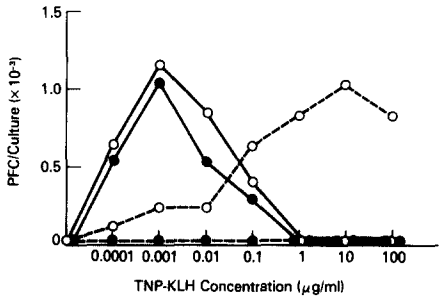

FIG. 7. The participation of Lyb-5$^+$ B cells is required for activation by higher doses of antigen, but Lyb-5$^-$ B cells can be activated at lower antigen doses. 2 × 10^6 TNP-primed CBA/CaHN (○) or CBA/N (●) RAMB plus C-treated spleen cells were co-cultured with 1 × 10^6 KLH-primed B6CBF$_1$ T cells in titrated concentrations of TNP-KLH. Cultures were assayed in parallel for IgG (—) and IgM (- - -) responses.

Lyb-5$^-$) or from CBA/N mice (Lyb-5$^-$) were cultured with B6CBF$_1$ helper T cells in the presence of graded doses of antigen, it was observed that CBA/CaHN and CBA/N B cells gave similar patterns of response at low concentrations of antigen, with a predominantly IgG peak response at 0.001 μg/ml. In contrast, whereas CBA/CaHN B cells were activated to produce IgM PFC at higher antigen concentrations, CBA/N B cells generated no significant PFC response at higher antigen concentrations (Fig. 7). These results indicate that Lyb-5$^-$ B cells could be activated at lower antigen concentrations, but that the participation of Lyb-5$^+$ B cells is required for activation by higher doses of antigen.

These findings were consistent with the possibility that under conditions in which Lyb-5$^-$ B cells are activated (e.g., at low antigen concentrations) such activation requires direct helper T cell recognition of B cell *H-2* products; but that under conditions in which Lyb-5$^+$ B cells respond (e.g., at higher antigen concentrations) there is no requirement for T cell recognition of B cell *H-2*. To test this possibility, T cells of B6CBF$_1$, B6C3F$_1$ → A.BY or B6C3F$_1$ → B10.BR origin were tested for the ability to cooperate with CBA/CaHN, CBA/N, or B10 B cells and B6CBF$_1$ APC in responses to low and high dose TNP-KLH. B cells or accessory cells present no foreign H-2 or M1s (22) determinants to T cells in any of these combinations, minimizing the possibility of complicating allogeneic effects. In response to low concentration of antigen, Lyb-5$^-$ B cells were activated in the absence of Lyb-5$^+$ B cells, because responses were generated by CBA/N as well as CBA/CaHN or B10 B cells (Fig. 8 A). Under these conditions, chimeric T cells were *H-2* restricted in their ability to help parental B cells even in the presence of F$_1$ APC (Fig. 8 B and C). In contrast, at a high dose of antigen, where responses required the participation of Lyb-5$^+$ B cells, and where CBA/N B cells were unresponsive, chimeric T cells were restricted in their recognition of APC but not B cells, as demonstrated by their ability to cooperate with either B10 or CBA/CaHN B cells in the presence of F$_1$ APC (Fig. 8 D–F). Thus, *H-2*-restricted interaction of helper T cells with responding B cells was required for the activation of Lyb-5$^-$ B cells at low antigen concentration, but not for Lyb-5$^+$ B cell-dependent responses at high antigen concentrations.

Discussion

The present study was undertaken to characterize the activation requirements of B cell populations functioning in T-dependent antibody responses to TNP-KLH. It was

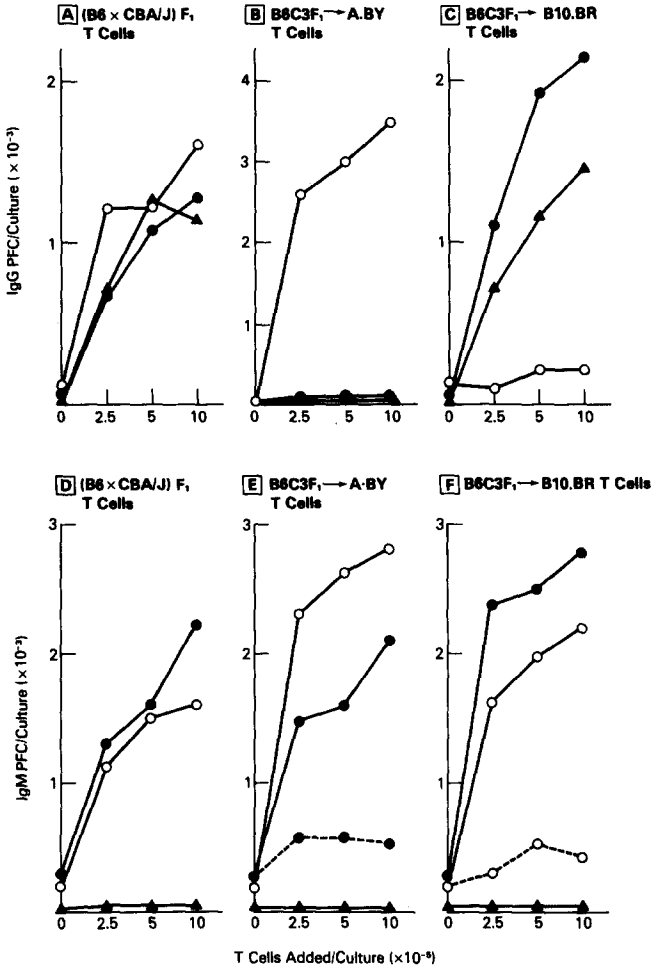

FIG. 8. Helper T cells are *H-2* restricted in their recognition of Lyb-5$^-$ B cells but not Lyb-5$^+$ B cells. Graded numbers of KLH-primed B6CBF$_1$ (A and D), B6C3F$_1$ → A.BY (B and E) or B6C3F$_1$ → B10.BR (C and F) T cells were co-cultured with 2 × 10^6 TNP-primed B10 (○), CBA/CaHN (●), or CBA/N (▲) RAMB plus C-treated spleen cells with 0.001 μg/ml (A–C) or 10 μg/ml (D–F) TNP-KLH in the presence of 1 × 10^6 B6CBF$_1$ APC. Some cultures were carried out without added B6CBF$_1$ APC (- - -).

specifically evaluated in these experiments whether responses that differ in their requirements for MHC restricted T cell recognition are mediated by the same or different B cell subpopulations. It was first demonstrated that for responses to low concentrations of TNP-KLH, (A × B)F$_1$ → parent$_A$ chimeric helper T cells were restricted in their ability to recognize parent$_A$ but not parent$_B$ *H-2* determinants expressed by both B cells and APC. In contrast, at higher antigen concentrations, helper T cells, which were *H-2* restricted in their recognition of APC, were not *H-2* restricted in their interaction with B cells. These experimental conditions were then used to determine whether the observed differences in T cell recognition reflected the activation of distinct B cell subpopulations with different activation requirements. It was determined that at low concentrations of TNP-KLH, Lyb-5$^-$ B cells were

activated, and that it was thus the activation of the Lyb-5$^-$ subpopulation which required T cell recognition of B cell *H-2* under these conditions. In contrast, responses to high antigen concentrations required the participation of Lyb-5$^+$ B cells, and activation of this B cell subpopulation required *H-2*-restricted T cell interaction with APC, but not B cells. The activation requirements, therefore, differed for responses mediated by different B cell subpopulations, and the requirement for T cell recognition of B cell *H-2* determinants appears to be specifically related to the B cell subpopulation that is activated.

A number of previous studies (1–8) have characterized the requirements for *H-2*-restricted T cell recognition in T dependent antibody responses. This work has identified a consistent requirement for helper T cell recognition of *H-2* products expressed on accessory cells or APC. There have been differences, however, among studies evaluating the requirement for T helper cell recognition of *H-2* products expressed on B cells, some studies concluding that such restricted T cell recognition of B cells is required for antibody responses (8–11), and other studies concluding that no such requirement exists (3–7). The explanation of these differences has not been apparent, and variables such as the antigen-priming status of responding populations, the predominant Ig isotype of response, and the use of in vitro or in vivo response systems have failed to correlate with the presence or absence of a requirement for *H-2*-restricted recognition of B cells. The findings presented in this report are consistent with the conclusion that the requirements for MHC-restricted recognition by T cells can differ as a reflection of the different activation requirements of distinct B cell subpopulations. Recent studies of antibody responses generated in vivo by adoptive transfer are highly relevant to this interpretation. In these studies (16), it was demonstrated that quantitatively similar IgM responses to SRBC could be generated either by Lyb-5$^-$ B cells alone or by a mixed population of Lyb-5$^+$ and Lyb-5$^-$ B cells under the same conditions. In these responses, a requirement was demonstrated for helper T cell recognition of *H-2* product expressed on B cells when responses were mediated by Lyb-5$^-$ B cells, where Lyb-5$^+$ B cells were activated without any detectable requirement for T cell recognition of B cell *H-2* product (16). These findings also demonstrated that Lyb-5$^-$ B cells are fully competent to generate IgM PFC responses, and thus that the differences in B cell activation requirements observed in the present in vitro studies are not attributable simply to differences in the Ig class of the responses elicited under different conditions. Therefore, both for in vivo IgM responses (16), and for the in vitro responses presented here, the activation of Lyb-5$^-$ B cells required *H-2*-restricted T cell recognition of B cells. Similarly, although it has not been possible to directly study the activation of isolated Lyb-5$^+$ B cells, the failure to observe T-B restriction in vivo in the presence of Lyb-5$^+$ B cells strongly suggests that Lyb-5$^+$ B cells can be activated in vitro or in vivo without *H-2*-restricted T-B interaction. Thus, it might be predicted that under conditions where *H-2* restriction requirements differ for the generation of IgM or IgG responses (23), different B cell subpopulations are in fact activated for production of each of these Ig classes.

The selective activation of different B cell subpopulations under specific experimental conditions deserves further comment. In the present studies, Lyb-5$^-$ CBA/N B cells were activated at low concentrations of TNP-KLH, but were unresponsive at higher concentrations that did activate in the presence of Lyb-5$^+$ B cells. Although the failure of Lyb-5$^-$ B cells to respond to high doses of TNP-KLH is not explained

by the present studies, these findings suggest that high concentrations of antigen may trigger active suppression that selectively inhibits the IgG response of Lyb-5$^-$ B cells. It is also unknown whether the same or different T helper cells function in the T-dependent responses of Lyb-5$^-$ and Lyb-5$^+$ B cells. Thus, the T cells that are *H-2* restricted in their activation of Lyb-5$^-$ B cells may be distinct from the T cell population that functions to activate Lyb-5$^+$ B cells, and the differential activation of these B cell subpopulations may be the result of differential T cell activation. Studies are currently in progress using monoclonal T helper cells to determine the ability of a single T cell population to activate these distinct B cell subpopulations.

A number of previous observations have also suggested that B cell subpopulations exist that differ in their requirements for activation. It has been suggested, for example, that functionally distinct subpopulations are defined by the expression or absence of complement receptors (24), or by the size and activation state of B cells (25). Recently, it has been demonstrated that Lyb-5$^+$ B cells are required for responses to antigen presented by pulsed accessory cells or macrophages, whereas Lyb-5$^-$ B cells are able to respond only to soluble antigens and not to antigen-pulsed accessory cells (26). Together with the results of the present study, these findings suggest that Lyb-5$^-$ B cells require MHC-restricted T cell recognition for their T-dependent activation, and that they are not triggered by accessory cell-presented antigen. In contrast, Lyb-5$^+$ B cells may be directly responsive to accessory cell presented antigen and may not require MHC-restricted T-B interaction for their activation.

The findings presented here have demonstrated the existence of B cell subpopulations that differ significantly in their activation requirements for T cell and accessory cell-dependent antibody responses to the antigen TNP-KLH. Lyb-5$^-$ B cells, responding to low concentrations of antigen require *I-A*-restricted T cell recognition of both B cells and APC for their activation. In contrast, responses to higher concentrations of TNP-KLH require the participation of Lyb-5$^+$ B cells, and B cell activation under these conditions requires *H-2*-restricted T cell recognition of APC, but not of B cells. Thus, as already demonstrated for T lymphocytes, the B cell population appears to consist of functionally distinct subpopulations which express critical differences in the pathways by which they are activated.

Summary

The present study has evaluated the identity of the B cell subpopulations participating in T dependent antibody responses that differ in their requirements for major histocompatibility complex-restricted T cell recognition. In vitro responses of keyhole limpet hemocyanin (KLH)-primed T cells and trinitrophenyl (TNP)-primed B cells were studied to both low and high concentrations of the antigen TNP-KLH. It was first demonstrated that for responses to low concentrations of TNP-KLH, $(A \times B)F_1 \rightarrow parent_A$ chimeric helper T cells were restricted in their ability to recognize $parent_A$ but not $parent_B$ H-2 determinants expressed by both B cells and antigen-presenting cells (APC). In contrast, at higher antigen concentrations, helper T cells were not restricted in their interaction with B cells.

It was then determined whether these observed differences in T cell recognition resulted from the activation of distinct B cell subpopulations with different activation requirements. At low concentrations of TNP-KLH it was demonstrated that Lyb-5$^-$ B cells were activated, and that it was thus the activation of the Lyb-5$^-$ subpopulation

that required T cell recognition of B cell *H-2* under these conditions. In contrast, responses to high concentration of antigen required the participation of Lyb-5$^+$ B cells, and these Lyb-5$^+$ B cells were activated by a pathway that required *H-2*-restricted T cell interaction with APC, but not with B cells.

The findings presented here have demonstrated that Lyb-5$^-$ and Lyb-5$^+$ B cells constitute B cell subpopulations that differ significantly in their activation requirements for T cell-dependent antibody responses to TNP-KLH. In so doing, these findings have established that the function of genetic restrictions in immune response regulation is critically dependent upon the activation pathways employed by functionally distinct subpopulations of B, as well as T, lymphocytes.

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