

INTERACTION OF HELPER T CELLS AND Lyb5⁺ B CELLS
RESPONDING TO PHOSPHORYLCHOLINE IS MHC-
RESTRICTED*

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There is continuing controversy about the rules governing the interaction of helper T lymphocytes with B lymphocytes in the induction of antibody responses *in vitro*. While the activation of T helper cells depends upon antigen recognition in the context of self major histocompatibility complex (MHC) determinants, the interaction of helper T cells with B cells is restricted by MHC products under some conditions (1, 2) but not under others (3, 4). There are two competing explanations for these observations. One hypothesis that has been advanced by Andersson et al. (5) proposes that resting B cells, but not activated B cell blasts, require MHC-restricted T cell help. In contrast, Singer et al. (6) have argued that only Lyb5⁻ B cells require MHC-restricted help and that activation of the Lyb5⁺ B cell subpopulation is unrestricted. This paper addresses the issue of whether or not the Lyb5⁺ B cell subpopulation is MHC-restricted in its interaction with helper T cells.

We have studied the T cell interaction requirements of normal B cells responding to phosphorylcholine (PC) coupled to keyhole limpet hemocyanin (KLH). The IgM anti-PC response is dominated by one idio type, T15. Since *xid* mice cannot produce the T15 idio type (7), and lack the Lyb5⁺ subset by definition, it has been inferred that T15 production is uniquely associated with the Lyb5⁺ B cell subset in normal mice (8). We have therefore asked whether the T15-dominant IgM anti-PC response supported by H-2-restricted, KLH-specific T helper cell lines required an H-2-restricted interaction of T and B cells. We find that the *in vitro* PC-KLH response is T15-dominant and the interaction of T and B cells is H-2 restricted, so we conclude that the interaction of T cells and Lyb5⁺ B cells is subject to the same MHC-restriction demonstrated for Lyb5⁻ cells and resting B cells. We also find that Lyb5⁻ *xid* B cells can be activated for polyclonal IgM synthesis by an apparently MHC-unrestricted T-B interaction.

Materials and Methods

Mice. CBA/N × BALB/c F₁ (NC F₁) males and females, BALB/c Icr, and BALB.B mice were bred in the Laboratory Animal Facility of the Institute for Cancer Research and used at 8–16 wk of age.

Antigens. Phosphorylcholine was coupled to keyhole limpet hemocyanin (Calbiochem,

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329

Volume 160 July 1984 329–334

San Diego, CA) or *Brucella abortus* (USDA, Ames, IA) as previously described (9). Trinitrophenyl-KLH was also prepared as usual (6).

Cell Cultures. Long-term T cell lines were produced and maintained by a protocol similar to that first described by Augustin et al. (10). Briefly, NC F₁ mice were immunized subcutaneously at the base of the tail with 50 µg of KLH in complete Freund's adjuvant. 7–9 d later, nylon wool-passed T cells were prepared from draining lymph nodes. The T cell lines were cultured at 5×10^6 cells per 3 ml RPMI 1640 plus 5% human serum in upright tissue culture flasks (Falcon 3012, Cockeysville, MD), and stimulated with antigen weekly by adding 1×10^6 syngeneic irradiated (1,500 R) spleen cells that had been pulsed for 1 h with 100 µg/ml KLH. Helper cell activity was determined by adding graded numbers of T cells from long-term (6 wk or greater) cultures prepared as above to T-depleted spleen cells. Depletion of splenic T cells was accomplished by incubating spleen cells in a mixture of monoclonal anti-Thy-1.2 and anti-Lyt-2 antibodies (clones HO 13-4, 30H-12, and 138.68, from Drs. Marshak-Rothstein, Ledbetter, and Fitch, respectively) followed by addition of pre-tested rabbit complement (low-tox M, Accurate Sci. Corp., Westbury, NY). The recovered cells contained 85–90% surface immunoglobulin-positive cells and fewer than 2% Thy-1-positive cells by FACS analysis. Of the recovered B cells, 15–20% were judged to be pre-activated on the basis of increased size by light scatter analysis and commitment to DNA synthesis as assessed by acridine orange or chromamycin A3 staining of DNA content. This population of T cell-depleted spleen cells will be referred to as “B cells plus macrophages” (B + Mφ) although small numbers of other cell types may be present. Such preparations were shown in preliminary experiments to be unresponsive to the T cell mitogens PHA and Con A. 5×10^5 B + Mφ were cultured with 1×10^3 – 5×10^4 KLH T helper (T_H) cells in 0.2-ml microcultures (Costar 3596, Data Packaging Corp., Cambridge, MA) in RPMI 1640 supplemented with 5% human serum, 15 mM Hepes, 50 µg/ml Gentamicin, 2 mM glutamine, and 5×10^{-2} mM 2-mercaptoethanol.

Assays and Data Analysis. The numbers of antigen-specific PFC were measured by the hemolytic plaque assay as described previously (11). PC-specific PFC were determined to bear the T15 idiotype by plaque inhibition with heterologous anti-T15 serum at a dilution that would completely inhibit PFC formation by a T15⁺ hybridoma but would not inhibit PFC formation by PC-specific hybridoma cells bearing the closely related 511 or 603 idiotypes, by >20%. Polyclonal activation of B cells was determined by the protein A PFC assay (12) using rabbit anti-µ serum to detect all IgM-secreting cells. The PFC data presented is thus all for IgM responses. Each PFC assay was performed on 3–5 replicate cultures per experimental group, and data are expressed as the geometric mean \times/\pm the relative standard error factor per 10^6 B + Mφ added to culture.

Results and Discussion

Long-term KLH-specific T Cell Lines Are H-2 Restricted for T-B Interaction in the Primary In Vitro IgM Response to TNP-KLH, but Unrestricted in Their Ability to Support a Polyclonal IgM Response. Long-term T cell lines from NC F₁ females (H-2^{k/d}) were shown in preliminary experiments to support a primary antibody response to TNP-KLH in vitro when syngeneic B + Mφ preparations were used. The addition of T cells from the long-term cultures also generated a substantial polyclonal IgM response. The specific but not the polyclonal IgM response was antigen-dependent and the magnitude of both responses was linearly related to the number of T cells added to culture. Proliferation experiments showed that the KLH-T_H lines had lost alloreactivity and were H-2 restricted for antigen recognition at the time they were used in helper cell assays.

The experiment shown in Table I was designed to test the H-2 restriction of T-B cell cooperation between the KLH-T_H lines and unprimed B + Mφ. NC F₁ KLH-T_H line cells were added to either syngeneic BALB/c (H-2^d) or allogeneic

TABLE I
T and B Cell Interaction Is MHC-Restricted for the In Vitro TNP-KLH Response but Not for Polyclonal IgM Synthesis

2 × 10 ⁴ CBA/N × BALB/c F ₁ female KLH-T _H	5 × 10 ⁴ F ₁ Female irr. spleen	Antigen	PFC/10 ⁶ cells*			
			5 × 10 ⁵ BALB/c B + Mφ		5 × 10 ⁵ BALB.B B + Mφ	
			TNP	IgM	TNP	IgM
-	+	-	0	92 (1.14)	0	0
-	+	TNP-KLH (10 μg/ml)	0	142 (1.18)	18 (1.43)	105 (1.68)
-	+	TNP-KLH (10 ng/ml)	0	40 (1.00)	0	58 (1.44)
-	+	TNP-BA	38 (1.23)	63 (1.59)	48 (1.18)	83 (1.44)
+	+	-	72 (1.73)	377 (1.17)	0	424 (1.09)
+	+	TNP-KLH (10 μg/ml)	565 (1.08)	1,102 (1.29)	8 (1.00)	827 (1.14)
+	+	TNP-KLH (10 ng/ml)	286 (1.36)	452 (1.20)	10 (1.26)	592 (1.12)
+	+	TNP-BA	201 (1.20)	759 (1.21)	133 (1.23)	667 (1.15)

* TNP-specific direct PFC were assayed using TNP-coupled SRBC. Total IgM PFC were measured using protein A-coupled SRBC and rabbit anti-μ chain serum as described in Materials and Methods. Numbers are geometric means (×/+ relative standard error factors) of triplicate cultures. Similar results were obtained in two additional replicate experiments.

BALB.B (H-2^b) B + Mφ in the presence of irradiated F₁ spleen cells (H-2^{k/d}) to provide syngeneic antigen-presenting function. The cultures were stimulated with TNP-KLH at high and low antigen concentrations and TNP-specific and total IgM PFC were measured after 5 d. The results show that the TNP-specific response depended upon an H-2-restricted interaction between T_H cells and B cells, whereas the polyclonal IgM response showed no such dependence. Higher antigen concentrations did not circumvent the H-2-restricted interaction for the TNP-KLH response. Further note that the presence of T_H cells augmented the nominally T-independent response to TNP-*B. abortus* and that such augmentation does not seem to depend upon H-2-restricted T-B interaction.

Since TNP-KLH has been shown to elicit TNP-specific responses in vitro (13) and in vivo (14) in CBA/N mice, it seems likely that at least part of the in vitro TNP-KLH response supported by KLH-T_H lines is derived from the Lyb5⁻ B cell subpopulation. We wanted to confirm directly this inference, since it has been reported that *xid* B cells are deficient in their ability to generate primary in vitro IgM anti-TNP responses to TNP-KLH (6). Accordingly, NC F₁ female KLH-T_H line cells were added to either NC F₁ male (*xid*) or F₁ female B + Mφ and stimulated with TNP-KLH. As can be seen in Table II, both F₁ male and F₁ female B cells clearly responded to TNP-KLH under these conditions, although the PFC responses of F₁ female B cells were somewhat larger. We also observed a substantial polyclonal IgM response with *xid* B cells that we would infer from the results in Table I to be due to an MHC-unrestricted T-B interaction. It is thus clear that not all Lyb5⁻ B cells depend upon MHC-restricted T-B interactions to be activated to antibody synthesis.

Interaction of Helper T Cells and B Cells Is MHC-restricted for the Antibody Response to PC-KLH. The following experiments were performed with PC-KLH, an antigen that preferentially addresses the Lyb5⁺ subpopulation (7, 8),

TABLE II
Lyb5⁻-Like B Cells of CBA/N × BALB/c F₁ Male Mice Are Activated by an MHC-Restricted T Cell Line for Both Specific and Polyclonal Antibody Responses

CBA/N × BALB/c F ₁ female KLH-T _H	Antigen [†]	PFC/10 ⁶ Cells*			
		CBA/N × BALB/c F ₁ male B + Mφ		CBA/N × BALB/c F ₁ female B + Mφ	
		TNP	IgM	TNP	IgM
—	—	0	40 (1.00)	10 (1.55)	40 (1.00)
—	TNP-KLH	0	40 (1.00)	10 (1.27)	40 (1.00)
—	TNP-BA	91 (1.13)	128 (1.26)	298 (1.16)	211 (1.32)
10 ⁴	—	37 (1.20)	1,200 (1.41)	343 (1.28)	2,420 (1.11)
10 ⁴	TNP-KLH	234 (1.35)	2,270 (1.19)	1,008 (1.10)	3,528 (1.17)
10 ⁴	TNP-BA	176 (1.08)	1,390 (1.40)	1,495 (1.10)	3,851 (1.04)

* Data presented as in Table I. Since the CBA/N defect is X-linked, F₁ male mice are phenotypically defective and F₁ female mice are phenotypically normal.

[†] Antigen concentrations were 10 μg/ml TNP-KLH and 1–2 × 10⁷ TNP-*B. abortus* organisms/ml.

TABLE III
T and B Interaction for the Lyb5⁺ Response to PC-KLH Is MHC-Restricted

1 × 10 ⁴ CBA/N × BALB/c F ₁ KLH- T _H	1 × 10 ⁵ CBA/N × BALB/c F ₁ 1,500 irr. spleen	Antigen [†]	PFC/10 ⁶ Cells*			
			5 × 10 ⁵ BALB/c B + Mφ		5 × 10 ⁵ BALB.B B + Mφ	
			PC	IgM	PC	IgM
—	+	—	0	74 (1.26)	0	56 (1.22)
—	+	PC-KLH	0	53 (1.32)	0	56 (1.22)
—	+	PC-BA	15 (1.68)	888 (1.22)	20 (1.44)	563 (1.15)
+	+	—	0	1,268 (1.08)	0	1,996 (1.32)
+	+	PC-KLH	135 (1.24)	1,477 (1.22)	12 (1.57)	2,595 (1.06)
+	+	PC-BA	103 (1.23)	3,321 (1.44)	113 (1.08)	4,123 (1.08)

* Direct PC-PFC were assayed against PC-coupled SRBC. >95% of PC-specific PFC were inhibited by rabbit anti-T15 antibodies. Data are expressed as in Table I. Two additional replicate experiments gave similar results.

[†] Antigen concentrations were 10 μg/ml PC-KLH and 1–2 × 10⁷ PC-*B. abortus* organisms/ml.

to address whether MHC-unrestricted T help is always sufficient to activate Lyb5⁺ B cells. In experiments not shown, we confirmed that F₁ male B cells would not respond to PC-KLH in the presence of T_H lines.

Table III presents the results of an experiment in which H-2^{k/d} F₁ T cell lines, F₁ antigen-presenting cells, and B + Mφ from H-2^d or H-2^b mice were stimulated with PC-KLH. The results show that T-B interaction for the PC-KLH response is H-2 restricted just as it is for the TNP-KLH response. Note that the polyclonal IgM response does not show such H-2 restriction (as would be expected from the results above) nor does the T_H-dependent augmentation of the PC-*B. abortus* response. To substantiate that the PC-KLH response arose from the Lyb5⁺ subset, we determined that 95–100% of PFC were inhibited with anti-T15 antibodies (data not shown). Further note that PC-*B. abortus* stimulated an equivalent anti-PC response with B cells derived from either BALB/c or BALB.B mice, indicating that BALB.B mice do not have an H-2-linked defect in their ability to respond to PC, and that both B + Mφ preparations were capable of equivalent responses.

These results show that our long-term T cell lines are MHC-restricted when activating two antigen-specific antibody responses, the responses to TNP-KLH

that seems to be derived from both the Lyb5⁻ and Lyb5⁺ B cell subpopulations, and the response to PC-KLH that appears to be derived exclusively from the Lyb5⁺ B cell subset. At the same time, interaction of T helper cell lines and B cells for the induction of polyclonal IgM synthesis is apparently MHC-unrestricted, as previously reported (15). However, the Lyb5 phenotype of B cells seems to have little or no impact on whether or not their activation by T cells is MHC-restricted. The polyclonal activation of NC F₁ male B cells, which we would argue are at least a first approximation of the normal Lyb5⁻ subset, appears not to be MHC-restricted. The IgM, T15⁺ response to PC-KLH, which would appear from the CBA/N studies to be confined to the Lyb5⁺ subset, is MHC-restricted. The hypothesis advanced by Singer et al. (6), that Lyb5⁻ B cells require MHC-restricted T-B interaction and Lyb5⁺ B cells do not, would therefore appear to be rejected by our data.

One likely explanation for the dichotomy between MHC-restricted antigen-specific responses and MHC-unrestricted polyclonal responses is that the polyclonal response is derived from the 15–20% of B cells in cell cycle (see Materials and Methods) when the cultures were initiated. Removal of these pre-activated B cells by density gradient separation substantially diminishes the T_H-dependent polyclonal IgM response (D. E. M., unpublished observations). The augmentation of antigen-specific responses to TNP-*B. abortus* and PC-*B. abortus* T_H cell lines also is not MHC-restricted (Tables I and II). We would therefore argue that both Lyb5⁻ and Lyb5⁺ B cells that have been stimulated to enter cell cycle by antigenic stimuli in vivo or “T-independent” antigens in vitro become susceptible to MHC-unrestricted T help. We thus propose that it is the stage of B cell activation that primarily determines the requirement for MHC-restricted T-B interaction. The Lyb5⁻ B cell subset might appear to be more dependent on MHC-restricted T help under conditions where it fails to achieve the activation status necessary to respond to MHC-unrestricted T helper cells or their factors.

Summary

The requirements for T cell/B cell interaction for the induction of primary in vitro antibody responses to phosphorylcholine (PC)-keyhole limpet hemocyanin (KLH) were examined. Long-term helper T cell lines derived from KLH-primed (CBA/N × BALB/c) F₁ female mice (H-2^{k/d}) were able to support a T15-idiotype dominant, IgM anti-PC response of BALB/c (H-2^d) B cells and macrophages, but could not activate PC-specific responses by BALB.B (H-2^b) B cells, even in the presence of irradiated H-2^{k/d} antigen-presenting cells. Polyclonal IgM secretion in the same cultures did not appear to depend upon a major histocompatibility complex (MHC)-restricted T-B interaction. Since IgM anti-PC responses seem to be entirely derived from the Lyb5⁺ B cell subpopulation, we conclude that at least some Lyb5⁺ B cells can only be activated by MHC-restricted T-B interactions. We also found that *xid* B cells from (CBA/N × BALB/c) F₁ male mice could be polyclonally activated by helper T cell lines by an apparently MHC-unrestricted interaction. Our data thus suggests that residence in the Lyb5⁻ or Lyb5⁺ B cell subset does not determine the T:B interaction requirements for antibody synthesis.

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