

12

NAVAL MEDICAL RESEARCH INSTITUTE BETHESDA, MARYLAND

ADA 124470



82-52

REGULATION OF T15 IDIOTYPE
DOMINANCE. MICE EXPRESSING THE
XID IMMUNE DEFECT PROVIDE NORMAL
HELP TO T15⁺ B CELL PRECURSORS

J. J. KENNY, L. S. WICKER,
G. GUELDE AND I. SCHER

DTIC
ELECTE
FEB 16 1983

J. Vorosmarti, CAPT, MC, USN

Commanding Officer

Naval Medical Research Institute

NAVAL MEDICAL RESEARCH AND DEVELOPMENT COMMAND

DTIC FILE COPY

83 02 015 051

REGULATION OF T15 IDIOTYPE DOMINANCE

I. Mice Expressing the *xid* Immune Defect Provide Normal Help to T15⁺ B Cell Precursors¹

JAMES J. KENNY,² LINDA S. WICKER,³ GRETCHEN GUELDE, AND IRWIN SCHER

From the Departments of Microbiology and Medicine, Uniformed Services University of the Health Sciences, and Naval Medical Research Institute, Bethesda, MD

The immune response to phosphocholine (PC) in many strains of mice is dominated by the T15 idiotype family of anti-PC antibodies. By introducing the CBA/N X-linked immune defect (*xid* gene) into these mice, one profoundly alters their ability to make a T15-predominant, IgM anti-PC response. This loss of T15 dominance in mice expressing the *xid* gene is not due to the presence of suppressor T cells or the lack of T15 idiotype-specific helper cells in these mice. Thus, one can reconstitute a T15 idiotype-dominant response in immune defective mice with B cells from normal mice, and in adoptive transfer assays the primed T helper cells from immune-defective mice provide qualitatively the same help to normal B cells as the T helper cells from normal mice. T15 idiotype dominance appears to be controlled by the expression and activation of Lyb-5⁺ PC-specific B cells. Thus, the majority of T15⁺ B cell precursors are restricted to this B cell subset, whereas the Lyb-5⁻ B cell subset contains predominantly T15⁻, anti-PC B cell precursors, which produce mainly IgG antibodies after activation by PC-containing antigens.

Previous studies of the immune response to phosphocholine (PC)⁴ in BALB/c mice demonstrated that this response is dominated by the T15 idiotype family of anti-PC antibodies (1-6), whereas C57BL/6 mice produce approximately equal amounts of anti-PC antibody from the T15, M511, and M603 idiotype families (7). Using the splenic focus assay to analyze the frequency of anti-PC-specific B cell precursors in these

mice, Gearhart, Cancro, and their collaborators (6, 8, 9) found that BALB/c mice have 75% T15⁺ B cell precursors, whereas C57BL/6 mice have less than 40% T15⁻ B cell precursors. These data suggested that a T15-predominant response might simply reflect the relative frequency of T15⁺ precursors in a given mouse strain. However, other studies have suggested that regulatory genes as well as regulatory T cells influence the expression of both T15⁺ and T15⁻ clones (7, 9-14). Bottomly and her co-workers (10-14) have suggested that T15 idiotype predominance in an anti-PC response is controlled by the action of a T15 idiotype-specific helper T cell. If this helper T cell is missing, BALB/c B cells no longer produce a T15-predominant anti-PC response. These investigators found that mice with low levels of T15 idiotype in their serum lacked T15-specific T helper cells. The best example of a low T15⁺ mouse is the immune-defective CBA/N strain or F₁ male progeny of CBA/N females and normal males. The F₁ male progeny from such a cross express the X-linked immune B cell defect (*xid* gene) (reviewed in References 15 and 16), whereas the heterozygous F₁ female progeny are phenotypically normal. The expression of the recessive *xid* gene causes a profound alteration in the ability to make normal anti-PC responses (17-23). Thus, when immune-defective mice are immunized with thymus-dependent (TD) PC antigens, they 1) fail to produce IgM anti-PC antibodies *in situ*, 2) produce little or no anti-PC antibody bearing the T15 idiotype, and 3) produce significant amounts of IgG anti-PC antibody only after secondary immunization (21, 22). More recent studies from our laboratory (24) indicate that this *xid*-induced alteration in the anti-PC response is due to the loss of the Lyb-5⁺ B cell subset. It appears that the T15⁺, IgM PC precursors are restricted to the Lyb-5⁺ B cell subset, and that Lyb-5⁻ B cells, in both normal and immune-defective mice, produce mainly T15⁻, IgG anti-PC antibodies. We have therefore proposed that divergent repertoires for PC exist in the Lyb-5⁺ and Lyb-5⁻ subsets of B cells. However, because immune-defective mice produce IgM antibodies to other TD antigens (15, 16) and 30 to 40% of these mice make low levels of T15⁺ antibody after secondary immunization, our findings could be explained by the lack of a T15-specific helper cell or the presence of a T15-specific suppressor cell in immune-defective mice. We have therefore attempted to repeat the experiments performed by Bottomly and her co-workers (10, 11, 14) to determine whether immune-defective mice do indeed lack the T cells necessary to preferentially activate T15⁻, IgM, PC-specific precursors. The results presented in this publication indicate that immune-defective mice provide qualitatively the same T cell help as normal mice to T15⁺ B cell precursors. Our results are consistent with the findings of Quintans *et al.* (18, 25, 26) indicating that no apparent T15-specific suppression exists in immune-defective

Received for publication April 26, 1982.

Accepted for publication June 28, 1982.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This work was supported by National Institutes of Health Grant AI-17760 and Uniformed Services University of the Health Sciences Research Nos. C07315 and C08310. Support also came from the Naval Medical Research and Development Command Task Nos. M0095-P.N.001.1030 and MR0001.01.1289 and NNMC Clinical Investigation No. 3-08-132. The opinions or assertions contained herein are the private views of the authors and should not be construed as official or as necessarily reflecting the views of the Uniformed Services University of the Health Sciences or Department of Defense. There is no objection to its presentation and/or publication. The experiments reported herein were conducted according to the principles set forth in the current edition of the *Guide for the Care and Use of Laboratory Animals*, Institutes of Laboratory Animal Resources, National Research Council.

² Reprint requests should be addressed to James J. Kenny, Department of Microbiology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814.

³ L. S. W. is a National Research Council Resident Research Associate of the Naval Medical Research and Development Command.

⁴ Abbreviations used in the paper: PC, phosphocholine; TD, thymus-dependent; CB, (CBA/N × BALB/c)F₁ progeny; DPPC, *p*-diazophenylphosphocholine; KLH, keyhole limpet hemocyanin; Hy, hemocyanin; FACS, fluorescence-activated cell sorter; RIA, radioimmunoassay; V_HPC, common idiotype associated with the V_H4 heavy chain; TRF, T cell-replacing factor; CD, (CBA/N × DBA/2)F₁ progeny; OVA, ovalbumin.

RE 63702W
Kenny, Scher

mice and that normal helper T cell activity for T15⁺ B cells is present in *xid* mice (25).

MATERIALS AND METHODS

Animals and antigens. (CBA/N × BALB/c)F₁ (CB) and (CBA/N × DBA/2)F₁ (CD) mice were obtained from Dominion Laboratories, Dublin, VA. Keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA) were obtained from Calbiochem-Behring, La Jolla, CA, and *Limulus polyphemus* hemocyanin (Hy) was obtained from Worthington Biochemical Corp., Freehold, NJ. Each of these proteins was conjugated with PC using *p*-diazophenylphosphocholine (DPPC) according to the method of Chesebro and Metzger (27). Mice were immunized i.p. with 100 μg of PC-Hy or 200 μg of KLH in complete Freund's adjuvant (CFA) and used in secondary adoptive transfer experiments 6 to 8 wk after priming. KLH and PC-Hy were used for priming T helper cells and PC-specific B cells because they are both potent immunogens in the mouse and do not cross-react at the T helper cell level as tested in the splenic fragment assay (E. S. Metcalf, unpublished observation).

Preparation of spleen cells for adoptive transfer. PC-Hy-primed spleen cells were treated with monoclonal anti-Thy-1.2 (generously provided by Dr. Phil Lake) and rabbit complement (C). B cell preparations contained less than 5% Thy-1.2⁺ cells as determined by analysis on the fluorescence-activated cell sorter (FACS) using biotin-conjugated anti-Thy-1.2 and fluorescein-labeled avidin (generously provided by Dr. Steven Kessler). KLH-primed T helper cells were obtained either by depletion of Ig⁺ cells on rabbit anti-mouse F(ab')₂-coated petri dishes as described by Mage *et al.* (28) or by passage over nylon columns as described by Julius *et al.* (29). T cell preparations contained less than 5% Ig⁺ and/or Ia⁺ cells.

Adoptive transfers. PC-Hy-primed B cells and KLH-primed T cells from either male or female mice were injected i.v. into irradiated (900 R) CB or CD female recipients. Mice were immunized i.p. with 50 μg of PC-KLH in saline or i.v. with 25 μg PC-KLH, which was mixed with the T and B cell preparations before injection. Seven or 8 days after cell transfer, mice were bled and/or their spleen cells were assayed for IgM anti-PC PFC.

Analysis of serum anti-PC antibodies. The inhibition radioimmunoassay (RIA) used for detection of the T15 and V_HPC^a idiotypes and the direct binding RIA used to detect IgM and IgG anti-PC antibodies have been previously described (21, 30).

PFC analysis. IgM anti-PC PFC were determined by the Cunningham and Szenberg slide method (31) using sheep red cells coupled with DPPC according to Claflin *et al.* (5). The percentage of T15⁺ PFC was determined by incorporating affinity-purified rabbit anti-T15 into the plaquing mixture at a final concentration of 1:1000. The preparation and testing of this anti-T15 idiotypic-specific reagent was previously described (21).

RESULTS

Reconstitution of the T15-idiotype response in immune-defective male mice. Immune defective male mice make little or no anti-PC antibody until 14 to 21 days after antigenic challenge, and the antibody produced at this time is primarily IgG and T15⁻ (21; and Kenny *et al.*, manuscript submitted for publication). This response pattern could be due to the existence of a suppressor T cell that is specific for T15 in these mice, or may simply reflect the normal response kinetics of Lyb-5⁻ PC-precursor B cells. To test for *in situ* suppressor T cells, anti-Thy-1.2 + C-treated spleen cells obtained from unprimed normal female or defective male mice were transferred into nonirradiated immune-defective CD male mice. These B cell recipients were immunized i.p. with 200 μg of PC-KLH in CFA, and their anti-PC responses were compared with the responses in both normal females and immune-defective males receiving only PC-KLH (Tables I and II). The results in Table I show that male mice reconstituted with various numbers of female B cells produced predominantly T15⁺ IgM PFC at every cell dose tested. Linear regression analysis of the PFC response showed that this response was directly proportional to the number of B cells transferred ($r^2 = 0.999$) and that the response plateaued in mice that received 20×10^6 female B cells.

To ascertain that the splenic PFC response reflected the profile of the total anti-PC response, we obtained serum from another group of recipients reconstituted with 25×10^6 female or male B cells (Table II). These sera were analyzed for the

TABLE I
Reconstitution of the IgM anti-PC PFC response in nonirradiated immune-defective male mice

Group Responding	Number of B Cells Transferred ($\times 10^6$) ^a	PFC/Spleen ^b	% T15 ⁺ ^c
1 ♂	40	15,105 (1.25)	94 ± 2
2 ♂	20	15,215 (1.29)	96 ± 1
3 ♂	5	4,913 (1.21)	94 ± 2
4 ♂	1	1,025 (1.12)	86 ± 5
5 ♂	0.5	651 (1.21)	90 ± 4
6 ♂	—	<500 ^d	N.T. ^e
7 ♀	—	34,949 (1.23)	97 ± 1

^a Spleen cells from normal CD female mice were treated with two rounds of anti-Thy-1.2 plus rabbit C to obtain a B cell-enriched cell preparation.

^b After reconstitution, mice were immunized with 200 μg of PC-KLH in CFA. Spleen cells were assayed 7 days after cell transfer. Data are presented as the geometric mean (standard error of the mean) of five individual mice. A linear regression analysis was performed on the data from groups 2 through 5. The coefficient of determination (r^2), which indicates the quality of fit achieved by the regression, was 0.913 in a linear-log plot and 0.999 in a log-log plot.

^c The percentage of T15⁺ PFC was determined by incorporating affinity-purified rabbit anti-T15 antibodies into the plaquing mixture at a final concentration of 1:1000. Data are presented as the arithmetic mean (± standard error of the mean) of five individual mice.

^d Three of the five control immune defective males gave no PFC, and two animals produced 267 and 623 PFC, respectively. These PFC were not tested (N.T.) to determine the percentage of T15⁺.

TABLE II
Reconstitution of the T15 idiotype response in nonirradiated immune-defective male mice

Group Responding ^a	B Cells Transferred ^b	μg Anti-PC Antibody/ml of Serum ^c			
		T15 idiotype	V _H PC idiotype	IgM	IgG
1 ♂	♀	150	232	90	97
2 ♂	♂	5	39	<5	36
3 ♂	—	12	30	<5	53
4 ♀	—	647	702	723	>1500

^a CD male mice (groups 1 and 2) were injected with 25×10^6 B cells from either normal CD females or immune-defective CD males. Groups 3 and 4 represent control groups that did not receive additional B cells.

^b B cell preparation and immunization were described in Table I. Mice were bled 14 days after immunization.

^c T15 and V_HPC idiotype-positive antibodies were evaluated as described in Reference 21. IgM and IgG anti-PC antibodies were assayed as described in Reference 30.

presence of IgM and IgG anti-PC antibodies and for antibodies bearing the T15 and V_HPC idiotypes. The results in Table II show that B cells derived from normal female donors reconstituted a T15 dominant response in the defective male recipients, whereas additional male B cells had no effect on the quality or quantity of the anti-PC antibody produced. Together, the results in Tables I and II indicate that immune-defective mice do not possess a natural T15-specific suppressor, and that they possess the T helper cells necessary to give a T15-dominant IgM response. The fact that reconstituted males gave a twofold to fourfold lower PFC response and a sevenfold to 15-fold lower serum antibody response than normal females was probably due to the efficiency with which the donor B cells home to the lymphoid tissue; however, one cannot rule out the possibility that the male T cell is deficient in some helper function. Although the data in Tables I and II indicate that adequate primary T help is developed in immune-defective mice, Bottomly and her collaborators (10, 11) have suggested that male mice fail to develop primed T15-specific helper cells. We therefore performed adoptive transfer experiments using KLH-primed T-helper cells and PC-Hy-primed B cells from normal female and immune-defective male mice to determine whether a qualitative or quantitative difference in the secondary helper function of male and female T cells could be observed.

T helper cells from immune-defective and normal mice provide equivalent help. CB female mice were irradiated (900 R) and reconstituted with KLH-primed T helper cells and/or PC-primed B cells as shown in Table III. Reconstituted mice were

immunized i.p. with 50 μ g of PC-KLH in saline and bled 7 days later. Serum from these mice was then analyzed for the presence of IgM and IgG anti-PC antibodies and for antibodies bearing the T15 and V_HPC idiotypes. As seen in Table III, immune-defective male and normal female T helper cells provide equivalent amounts of help to both male and female B cells. Male T cells did not alter either the quality or the quantity of the serum anti-PC response produced by female B cells. These data show that the source of the B cell controlled both the idio type and the isotype of the anti-PC antibodies produced. Thus, when mice were reconstituted with PC-primed B cells from normal female donors, the V_HPC⁺ anti-PC response was comprised of approximately 50% T15⁺ and 50% T15⁻ antibodies, and both IgM and IgG antibodies were produced (groups 1 and 2). On the other hand, when mice were reconstituted with male-derived, PC-primed B cells, T15⁻, IgG antibodies were produced (groups 3 and 4).

The data in Table III also indicate that immune-defective mice provide T cell help that is equivalent to normal mice at the cell numbers used in this experiment. However, because we transferred four times as many B cells and five times as many T cells as Bottomly and Mosier (11), we performed several experiments to see if more limiting numbers of T and B cells would alter the idio type profile of the responding B cells. Irradiated CB female mice were reconstituted with 15 \times 10⁶ PC-primed female B cells and four different concentrations of KLH-primed T cells from either male or female donors. Seven days after immunization with PC-KLH, serum from these mice was obtained and analyzed for the T15 and V_HPC idiotypes. Male and female T cells provided equivalent help for female B cells at all doses tested (Table IV).

The data in Tables III and IV indicate there is no difference in the serum anti-PC response obtained with T-helper cells from normal or immune-defective mice. However, the serum response is composed of both IgM and IgG anti-PC antibodies, and these antibodies may be produced in lymphoid tissues other than the spleen. We therefore carried out a series of experiments in which the idio type profile of the direct PFC from the spleens of reconstituted recipients was analyzed. These experiments allowed us to compare our results directly to those of other investigators.

CB female mice were reconstituted with either 5 \times 10⁶ or 20 \times 10⁶ PC-primed female B cells and 2 \times 10⁵ or 10 \times 10⁶ T helper cells from KLH-primed immune-defective males or normal females. The lower dose of T and B cells was equivalent to the cell numbers transferred by Bottomly and Mosier (11),

TABLE III
Serum anti-PC response in irradiated CB female recipients reconstituted with CB male and female lymphocytes

Group	B Cell Source ^a	T Cell Source ^a	μ g Anti-PC Antibody/ml Serum ^b			
			T15 Idiotypic ^c	V _H PC Idiotypic ^c	IgM ^d	IgG ^d
1	♀	♀	271	491	155	480
2	♀	♂	486	641	280	730
3	♂	♀	<10	84	<10	210
4	♂	♂	<10	69	<10	330
5	—	♀	<10	36	N.T. ^e	N.T.
6	—	♂	<10	43	N.T.	N.T.
7	♀	—	<10	35	N.T.	N.T.
8	♂	—	<10	24	N.T.	N.T.
9	—	—	<10	11	N.T.	N.T.

^a CB female mice were irradiated with 900 rad and reconstituted with 20 \times 10⁶ PC-Hy-primed B cells and/or 10 \times 10⁶ KLH-primed T cells prepared as described in *Materials and Methods*. After reconstitution, recipients were immunized i.p. with 50 μ g of PC-KLH in saline.

^b Serum was obtained 7 days after reconstitution and immunization.

^c See legend of Table II.

^d N.T. = not tested.

TABLE IV
Serum anti-PC response in irradiated CD female recipients reconstituted with limited numbers of T helper cells

Group	T-Helper Cells ^a		μ g Anti-PC Antibody/ml Serum ^b	
	Number (\times 10 ⁵)	Source	T15 Idiotypic	V _H PC Idiotypic
1	14.5	♀	197	382
2	4.8	♀	102	205
3	1.2	♀	18	65
4	0.4	♀	11	43
5	14.5	♂	214	380
6	4.8	♂	73	153
7	1.2	♂	15	35
8	0.4	♂	11	28

^a KLH-primed T helper cells and PC-Hy-primed B cells were prepared as described in *Materials and Methods*. Irradiated (900 R) CD female mice were reconstituted with 15 \times 10⁶ PC-primed B cells and T cells as indicated above. After reconstitution, mice were immunized i.p. with 50 μ g of PC-KLH in saline.

^b Serum was obtained 7 days after immunization, and the PFA were performed as indicated in Table III.

whereas the higher cell numbers were equivalent to the experiment shown in Table III above. The data in Table V show that at the high cell dose, the idio type profile of the anti-PC response was identical with male and female T helper cells. However, at the lower cell dose, male T cells seemed to activate more T15⁻ precursors than the female T cells, even though both responses were still predominantly T15⁺ (groups 3 and 4). The fact that male T cells provided quantitatively less help to the IgM-PFC precursors at both cell doses and activated significantly more T15⁻ precursors at the lower T cell dose suggested that by further limiting the T cell help we might shift the predominantly T15⁺ response to a predominantly T15⁻ response. Even at the low cell dose used in Table V we obtained a fourfold higher PFC response than that obtained by Bottomly and co-workers (10, 11, 14); this suggested that our cell preparations were more active on a per cell basis. Therefore, in an attempt to duplicate Bottomly and Mosier's findings (11), we 1) prepared the KLH-primed T cells on nylon wool columns rather than by the usual panning procedure, 2) transferred very limiting numbers of T helper cells, and 3) immunized i.v. with 25 μ g of PC-KLH rather than i.p. with 50 μ g. Eight days after cell transfer and PC-KLH immunization, idio type analysis of the splenic PFC was performed. The results in Table VI show that with this very limiting T cell help, we obtained total IgM PFC responses equivalent to those obtained by Bottomly and Mosier (11); however, a non-T15-predominant response was observed only when no T cell help was provided (groups 6 and 7). T helper cells from immune-defective and normal mice produced equivalent T15-dominant responses with 1 \times 10⁶ and 0.5 \times 10⁶ T cells, but the male T cells failed to provide significant help at the 0.1 \times 10⁶ cell dose.

DISCUSSION

The data presented in this publication show that 1) mice that express the *xid* immune defect can make a T15 idio type-predominant, anti-PC antibody response when provided with normal female B cells; 2) the T helper cells from normal female mice do not alter the idio type response of defective male B cells; thus, the adoptive transfer anti-PC responses of male B cells remain IgG and T15⁻ even when activated by primed female helper T cells; and 3) the primed helper T cells from immune-defective male mice provide qualitatively the same help to female B cells as primed female T helper cells. These data confirm the findings of Quintans *et al.* (18, 25, 26) that immune-defective mice do not possess a T15-specific suppressor cell and that they provide normal help to T15⁺ B cells. These data indicate that T15-idio type predominance in an anti-

PC response is controlled by the phenotype of the B cell. Our data fail to support the findings of Bottomly and her collaborators (10, 11) that mice expressing the *xid* gene lack T15-specific helper cells. Even under conditions of limiting T cell help, we did not observe low levels of T15⁺ antibody production when female B cells were activated by KLH-primed male T cells. In our hands, normal female B cells produce predominantly T15⁺, IgM PFC (Table V) when activated with optimum numbers of either male or female T helper cells. On the other hand, both male and female B cells produce IgG anti-PC antibodies that are predominantly T15⁻. These IgG, T15⁻ antibodies appear to be produced mainly by Lyb-5⁻ B cells, and they exhibit the same idiotypic profile after activation of the Lyb-5⁻ B cells by either male or female T cells (24).

How might one explain the difference between our finding that both male and female T helper cells give T15-predominant help to IgM PC-precursor B cells and Bottomly and Mosier's (11) finding that only normal female T cells can provide T15-predominant help? Assuming that technical problems and differences in reagents are not the basis of the observed differences, we would like to propose a hypothesis which might explain the results obtained by Bottomly and her collaborators (10-14). This hypothesis is based on alternate activation pathways for the two B cell subsets expressed in mice rather than preferential idiotypic selection by different helper T cell subsets.

Recent studies in our laboratory (21, 24) indicate that T15⁻ IgM antibodies are produced by Lyb-5⁺ B cells, whereas Lyb-5⁻ B cells from immune-defective mice produce mainly IgG-T15⁻ anti-PC antibodies. Nevertheless, Lyb-5⁻ B cells can produce IgM T15⁻ PFC in secondary adoptive transfer assays when optimum T cell help is provided. For example, greater than 60% of the PC-specific, Lyb-5⁻ precursors from PC-KLH

immune CD females produce T15⁻ IgM anti-PC antibodies (Metcalfe and Kenny, unpublished data).

It is possible that Bottomly and Mosier (11) stimulated a substantial proportion of female Lyb-5⁻ PC-specific B cell precursors with their primed male helper cells and thus observed a predominantly T15⁻ response. However, one must then explain how this T15⁻ response was converted to a predominantly T15⁺ response by the addition of ovalbumin (OVA) plus OVA-primed female T cells (11). Bottomly and Mosier (11) suggested that the female OVA-primed cells provided the T15-idiotypic specific helper cells that were supposedly absent in the immune-defective male mice. To provide this T15-specific help, the OVA-primed T cells do not require PC and OVA to be physically linked, because they presumably recognize both OVA and T15 idiotypic determinants. Alternatively, the activation of female OVA-specific T cells by OVA could simply stimulate the production of additional soluble T helper factors such as T cell-replacing factor (TRF). Antigen plus TRF is known to activate Lyb-5⁺ B cells (32, 33), whereas the Lyb-5⁻ B cells are generally refractory to activation by TRF-like factors. Thus, when T cell help is limiting, the additional soluble factors provided by the OVA-specific T cells could preferentially activate and expand the T15⁺, Lyb-5⁺ precursors and thereby shift the response from predominantly T15⁻ to predominantly T15⁺. This alternate hypothesis is supported by the recent findings of Singer *et al.* (34), who have shown that cloned T helper cells will activate TNP-specific, MHC-incompatible Lyb-5⁺ B cells even when carrier and hapten are unlinked. These same T cell clones can activate TNP-specific Lyb-5⁻ B cells but this latter activation requires both the physical linkage of the hapten and carrier molecules and MHC compatibility between the T cell clones and Lyb-5⁻ B cells (34).

In a similar system, Bottomly and Jones (14) found that cooperation between cloned, carrier-specific helpers and normal PC-specific B cells (Lyb-5⁺ and Lyb-5⁻) resulted in a predominantly T15⁻ IgM response. In these experiments, the phenotype of the responding B cell was not determined; however, both physical linkage of hapten and carrier and MHC compatibility between the T helper clones and B cells were required (35). These findings suggest that Lyb-5⁻, PC-specific precursors may be playing a major role in this response. However, if Bottomly and Mosier (11) are correct and T15 idiotypic-specific T helper cells play a major role in regulation of the PC repertoire, then the addition of OVA and OVA-primed T cells to KLH-specific T cell clones and PC-primed B cells should convert a predominantly T15⁻ response to a predominantly T15⁺ response. This idiotypic conversion should not occur when the OVA-primed T cells come from mice expressing the *xid* gene or from mice with low levels of circulating T15⁺ antibodies.

The data in Tables IV-VI show that T cells from immune-defective male mice provide qualitatively the same help as T helpers from normal female mice. However, we consistently observed a lower PFC response with male T cells (Tables V and VI, and Reference 24) and male T cells titrated out faster than female T cells (Table VI). This quantitative difference in PFC responses has been confirmed in the anti-PC serum antibody levels when these two assays were performed in the same experiment (24). PFC were not analyzed in the experiment shown in Table III. We believe that the lower quantitative responses observed with male T helper cells reflects a lower absolute number of male T cells in these adoptive transfer experiments. When male T cell preparations were analyzed on

TABLE V

IgM anti-PC PFC response in irradiated CB female mice reconstituted with CB male and female lymphocytes

Group	Cell Source & Number Transferred ($\times 10^{-6}$) ^a		PFC/Spleen ^b ($\times 10^{-3}$)	% T15 ⁺ ^{c,d}
	B Cells	T Cells		
1	♀ 20	♀ 10	720 (1.14)	90 ± 2.7
2	♀ 20	♂ 10	456 (1.04)	90 ± 2.1
3	♀ 5	♀ 2	66 (1.03)	90 ± 1.2
4	♀ 5	♂ 2	47 (1.20)	82 ± 3.7

^a PC-Hy-primed CB female B cells and KLH-primed T cells from CB male and female mice were prepared as described in *Materials and Methods*.

^b Data are presented as the geometric mean (standard error of the mean) of five individual mice. Assays were performed 7 days after reconstitution and i.p. immunization with 50 µg of PC-KLH in saline. B and T cells transferred individually (data not shown) gave less than 5% of the experimental values.

^c As indicated in Table I.

^d Student's t-test was performed by comparing the arithmetic means of the anti-T15 inhibition data. Group 4 was significantly different from the other three groups ($P < 0.02$).

TABLE VI

IgM anti-PC PFC response in irradiated CB female mice reconstituted with limiting numbers of T helper cells

Group	T Cell Source ^a ($\times 10^{-6}$)	PFC/Spleen ^b	% T15 ⁺ ^c
1	1.0 ♀	15,201 (1.20)	69 ± 6
2	1.0 ♂	13,237 (1.20)	73 ± 2
3	0.5 ♀	8,021 (1.33)	76 ± 4
4	0.5 ♂	7,216 (1.27)	80 ± 5
5	0.1 ♀	6,202 (1.30)	72 ± 7
6	0.1 ♂	1,767 (1.39)	47 ± 12
7	—	1,425 (1.35)	54 ± 12

^a The KLH-primed T cells for this experiment were purified on nylon wool columns as described by Julius *et al.* (29). These KLH-primed, nylon wool-purified T cells were transferred along with 5×10^6 PC-primed B cells into irradiated (650 rad) CB female recipients. Twenty-five micrograms of PC-KLH were mixed with the cells before i.v. injection.

^b As indicated in Table I.



DTIC COPY REQUESTED A 21

the FACS, twice as many null cells were present as in the female T cell preparations (Kenny, unpublished observation). We made no attempt to normalize the male and female cell preparations to equivalent numbers of Ig⁺ or Thy-1.2⁺ cells before cell transfer.

In summary, these data show that spleen cells from immune-defective mice provide qualitatively the same type of T cell help to normal B cells as T cells from normal mice. If a T15-specific helper T cell is indeed necessary to obtain a T15-idiotype predominant PFC response, then mice expressing the *xid* gene must possess this T cell in their helper cell repertoire. Our data clearly indicate that the B cell responsible for T15-predominance is contained within the Lyb-5⁺ B cell subset and that this B cell subset produces virtually all the T15⁺, IgM anti-PC antibodies during an immune response to PC. These data and other data from our laboratory (24) support the hypothesis of Slack *et al.* (36) that idiotype and isotype expression may be dependent on activation of different B cell subsets.

Acknowledgments. The authors would like to acknowledge the excellent technical assistance of Ms. A. K. Berning, and we thank Drs. E. S. Metcalf and A. Singer for their helpful suggestions after reviewing this manuscript. We also thank Ms. Rita Guimond for her patient preparation of this manuscript.

Note Added in Proof. After submission of this manuscript, similar data were published by Quintáns, J., Z. S. Quan, and Miguel A. Arias. 1982. Mice with the *xid* defect have helper cells for T15 idiotype dominant anti-phosphorylcholine primary and secondary plaque-forming cell responses. *J. Exp. Med.* 155:1245.

REFERENCES

- Köhler, H. 1975. The response to phosphorylcholine: dissecting an immune response. *Transplant. Rev.* 27:24.
- Cosenza, H., M. H. Julius, and A. A. Augustin. 1977. Idiotypes as variable region markers: analogies between receptors on phosphorylcholine-specific T and B lymphocytes. *Immunol. Rev.* 34:3.
- Scher, A., and M. Cohn. 1972. Inheritance of an idiotype associated with the immune response of inbred mice to phosphorylcholine. *Eur. J. Immunol.* 2:319.
- Cosenza, H., and H. Köhler. 1972. Specific inhibition of plaque formation to phosphorylcholine by antibody against antibody. *Science* 176:1027.
- Clafin, J. L., R. Lieberman, and J. M. Davie. 1974. Clonal nature of the immune response to phosphorylcholine. I. Specificity, class and idiotype of phosphorylcholine-binding receptors on lymphoid cells. *J. Exp. Med.* 139:58.
- Gearhart, P. J., N. H. Sigal, and N. R. Klinman. 1975. Heterogeneity of the BALB/c antiphosphocholine antibody response at the precursor cell level. *J. Exp. Med.* 141:56.
- Ruppert, V. J., K. Williams, and J. L. Clafin. 1980. Specific clonal regulation in the response to phosphorylcholine. I. Genetic factors controlling the expression of a distinct idiotype. *J. Immunol.* 124:1068.
- Gearhart, P. J., and J. J. Cebra. 1978. Idiotype sharing by murine strains differing in immunoglobulin allotype. *Nature* 272:264.
- Cancro, M. P., N. H. Sigal, and N. R. Klinman. 1978. Differential expression of an equivalent clonotype among BALB/c and C57BL/6 mice. *J. Exp. Med.* 147:1.
- Bottomly, K., B. J. Mathieson, H. Cosenza, and D. E. Mosier. 1979. Idiotype-specific regulation of the response to phosphorylcholine by T cells from mice with high and low levels of circulating idiotype. *In B Lymphocytes in the Immune Response. Proceedings of the 2nd International Symposium.* Edited by M. Cooper, D. E. Mosier, I. Scher, and E. S. Vitetta. Elsevier/North Holland, New York. P. 323.
- Bottomly, K., and D. E. Mosier. 1979. Mice whose B cells cannot produce the T15 idiotype also lack an antigen-specific helper T cell required for T15 expression. *J. Exp. Med.* 150:1399.
- Bottomly, K., C. A. Janeway, Jr., B. J. Mathieson, and D. E. Mosier. 1980. Absence of an antigen-specific helper T cell required for the expression of the T15 idiotype in mice treated with anti- μ antibody. *Eur. J. Immunol.* 10:159.
- Bottomly, K., and C. A. Janeway, Jr. 1981. Selected populations of a T_H reactive T cells contain helper T cells but lack Th1d, and antigen-specific helper T cell required for dominant production of the T15 idiotype. *Eur. J. Immunol.* 11:270.
- Bottomly, K., and F. Jones III. 1981. Idiotype dominance manifested during a T-dependent anti-phosphocholine response requires a distinct helper T cell. *In B Lymphocytes in the Immune Response. Proceedings of the 2nd International Symposium.* Edited by N. R. Klinman, D. E. Mosier, I. Scher, and E. S. Vitetta. Elsevier/North-Holland, New York. P. 415.
- Scher, I. 1931. B lymphocyte development and heterogeneity: analysis with the immune-defective CBA/N mouse strain. *In Immune Defects in Laboratory Animals.* Edited by E. Gershwin and B. Merchant. Plenum Press, New York. P. 163.
- Scher, I. 1932. The CBA/N mouse strain: an experimental model illustrating the influence of the X chromosome on immunity. *In Advances in Immunology.* In press.
- Quintáns, J. 1977. The "patchy" immunodeficiency of CBA/N mice. *Eur. J. Immunol.* 7:749.
- Quintáns, J., and R. B. Kaplan. 1978. Failure of CBA/N mice to respond to thymus-dependent and thymus-independent phosphorylcholine antigens. *Cell. Immunol.* 38:294.
- Mond, J. J., R. Lieberman, J. K. Inman, D. E. Mosier, and W. E. Paul. 1977. Inability of mice with a defect in B lymphocyte maturation to respond to phosphorylcholine on immunogenic carriers. *J. Exp. Med.* 146:1138.
- Kishimoto, T., S. Shigemoto, T. Watanabe, and Y. Yamamura. 1979. Demonstration of phosphorylcholine-specific IgE B cells in CBA/N mice. *J. Immunol.* 123:1039.
- Kenny, J. J., G. Gualde, J. L. Clafin, and I. Scher. 1931. Altered idiotype response to phosphorylcholine in mice bearing an X-linked immune defect. *J. Immunol.* 127:1629.
- Clough, E. R., D. A. Levy, and J. J. Cebra. 1981. CBA/N x BALB/c F₁ male mice can be primed to express quantitatively equivalent secondary anti-phosphocholine responses. *J. Immunol.* 126:387.
- Köhler, H., S. Stryk, and J. Fung. 1931. Immune response to phosphorylcholine. VII. The response of CBA/N mice to PC-LPS. *J. Immunol.* 126:1749.
- Wicker, L. S., G. Gualde, I. Scher, and J. J. Kenny. 1932. Antibodies from the Lyb-5⁺ B cell subset predominate in the secondary IgG response to phosphorylcholine. *J. Immunol.* 123:950.
- Quintáns, J., J. P. McKearn, and D. Kaplan. 1979. A study of B cell subpopulations involved in the reconstitution of an X-linked immune defect of B cell differentiation. *J. Immunol.* 122:1750.
- Quintáns, J., M. R. Loken, Z. S. Quan, R. F. Dick, and B. Regueiro. 1931. Alteration of clonal profile. II. Studies on the capacity of BALB/c splenic B cells to perpetuate responsiveness to phosphorylcholine and T15 idiotype dominance. *Eur. J. Immunol.* 11:236.
- Chesebro, B., and H. Metzger. 1972. Affinity labeling of a phosphorylcholine binding mouse myeloma protein. *Biochemistry* 11:766.
- Mage, M. G., L. L. McHugh, and T. L. Rothstein. 1977. Mouse lymphocytes with and without surface immunoglobulin: preparative scale separation on polystyrene tissue culture dishes coated with specifically purified anti-immunoglobulin. *J. Immunol. Methods* 15:47.
- Julius, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* 3:645.
- Banerji, B., J. J. Kenny, I. Scher, and C. R. Alving. 1932. Antibodies against liposomes in normal and immune-defective mice. *J. Immunol.* 123:1603.
- Cunningham, A. J., and A. Szanberg. 1968. Further improvements in the plaque technique for detecting single antibody-forming cells. *Immunology* 14:599.
- Ahmed, A., and I. Scher. 1979. Murine B cell heterogeneity defined by anti-Lyb 5, an allotypic antigen specific for a late-appearing B lymphocyte subpopulation. *In B Lymphocytes in the Immune Response. Proceedings of the 1st International Symposium.* Edited by M. Cooper, D. E. Mosier, I. Scher, and E. S. Vitetta. Elsevier/North-Holland, Amsterdam. P. 115.
- Yaffe, L., and I. Scher. 1981. Analysis of receptiveness of B cells to the T cell factors TRF and AEF in CBA/N and DBA/2Ha immune defective mice. *Fed. Proc.* 40:3. (Abstr. 5007).
- Singer, A., Y. Asano, M. Shigeta, K. S. Hathcock, A. Ahmed, C. G. Fathman, and R. J. Hodes. 1982. Distinct B cell subpopulations differ in their genetic requirements for activation by T helper cells. *Immunol. Rev.* 64:137.
- Jones, B., and C. A. Janeway, Jr. 1931. Cooperative interaction of B lymphocytes with antigen-specific helper T lymphocytes is MHC restricted. *Nature* 292:547.
- Slack, J., G. P. Der-Balian, M. Nahm, and J. M. Davie. 1980. Subclass restriction of murine antibodies. II. The IgG plaque-forming cell response thymus-independent type 1 and type 2 antigens in normal mice and mice expressing an X-linked immunodeficiency. *J. Exp. Med.* 151:853.