Brief Definitive Report

MICE WITH THE *xid* DEFECT HAVE HELPER CELLS FOR T15 IDIOTYPE-DOMINANT ANTI-PHOSPHORYLCHOLINE PRIMARY AND SECONDARY PLAQUE-FORMING CELL RESPONSES*

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Several laboratories have reported that immunodefective *xid* mice are unable to give anti-phosphorylcholine (PC) plaque-forming cell (PFC) responses (1–3), although they are known to possess PC-specific antigen-binding B cells (4), functional helper T cells (5, 6), and are capable of producing anti-PC immunoglobulin (IgE) antibodies (7). More recently it has been reported that *xid* mice can produce serologically detected anti-PC antibodies that lack the T15 idiotype marker (8, 9), one of the dominant idiotypes of murine anti-PC antibodies (10). If B cell idiotypes are instrumental in inducing regulatory T cells with specificity for allotype or idiotype, *xid* mice might be expected to be deficient in T15-specific regulatory T cells because of their inability to produce this B cell idiotype. Evidence to support this prediction has been presented by Bottomly and Mosier (11). Their results show that carrier-primed helper T cells from (CBA/N × BALB/c) F_1 (NBF_1) male mice cannot provide help for $T15^+$ secondary anti-PC PFC responses. These observations contrast sharply with findings from our laboratory on unirradiated *xid* NBF_1 mice whose anti-PC responses have been restored by a transplant of normal immunocompetent cells. The B cells engrafted in NBF_1 recipients generate thymus-dependent anti-PC PFC responses that express the T15 idiotype. Furthermore, the level of T15 expression in NBF_1 recipients is comparable to the level detected *in situ* in the mice used as donors of B cells (6). The results of our experiments therefore negate the claim that *xid* mice cannot provide adequate help for $T15^+$ responses. Because of the obvious contradictions between our conclusions and those of Bottomly and Mosier (11), we have reinvestigated this problem using a variety of thymus-dependent (TD) PC antigens and different experimental protocols. Our new results confirm that commercially available *xid* mice provide adequate help for primary and secondary anti-PC PFC responses.

Materials and Methods

Previously Described Materials and Methods. The following reagents and procedures have been described elsewhere: irradiation (12), preparation of PC-containing antigens (6, 12), anti-T cell reagents (12), PFC assay (6, 12), and the hybridoma anti-HOPC 8 antibody AB1-2 (13).

Mice. (CBA/N × BALB/c)F_1 (NBF_1) male and female mice were purchased from Laboratory Supply Co., Indianapolis, IN. BALB/c mice were obtained from Cumberland View Farms, Clinton, TN. (BALB/c × CBA/N)F_1 (BNF_1) mice were bred in our animal facilities. BALB/c congenitally athymic mice, originally obtained from Oak Ridge Laboratories, Oak

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Ridge, TN, were also bred in our facilities. In the transfer experiments involving athymic mice, F_1 T cells were obtained from (CBA/N × BALB/cOak)F_1 male and female donors.

B Cell Preparations. Donors of splenic B cells were primed intraperitoneally with either 200 μg PC-KLH or 200 μg PC-FGG in complete Freund's adjuvant (CFA) 6–12 wk before killing. Donor spleen cells were treated twice with anti-Thy-1.2 and anti-Lyt-2 plus rabbit complement (C′) (Accurate Chemical & Scientific Corp., Westbury, NY), washed extensively in Hanks' balanced salt solution, and then injected intravenously into the recipients, as described in the text. In some cases, the donor mice additionally received 0.3–0.5 ml of rabbit anti-mouse thymocyte serum (ATS) (M. A. Bioproducts, Walkersville, MD) i.v. 3 and 5 d beforehand; responses generated by cells treated thus were identical to those from cells not exposed to ATS.

T Helper Cell Populations. BNF_1 and NBF_1 male and female mice were primed with carrier in CFA in the tail and hind footpads; 4–8 d later, inguinal, popliteal, and periaortic lymph node cells were washed extensively in Hanks' balanced salt solution and used as sources of helper T cells. In some cases, as noted in the text, animals were carrier-primed intraperitoneally and subsequently used as lethally irradiated hosts.

Results and Discussion

We have compared the ability of carrier-primed lymph node cells from *xid* and normal mice to restore TD responses to PC-keyhole limpet hemocyanin (KLH) and PC-fowl gamma globulin (FGG) in athymic BALB/c nude mice. In responses to both antigens, cells from *xid* and normal mice provided similar help for the T15-dominant, TD response elicited in athymic mice. Table I illustrates the results of one experiment using FGG-primed lymph node cells and PC-FGG as antigen. The carrier specificity of the reconstitution is demonstrated by the relative lack of effect in mice reconstituted with KLH-primed cells and immunized with PC-FGG. These experiments establish two points: PC-specific B cells in congenitally athymic BALB/c mice are predominantly $T15^+$; and these B cells can be helped to generate TD anti-PC PFC responses by both normal and *xid* carrier-primed cells.

Our experiments have examined primary anti-PC responses, whereas, in the reports of other laboratories, secondary responses were analyzed. To extend our observations to immune B cells, BALB/c mice were immunized intraperitoneally with PC-KLH in CFA and, 6–8 wk later, their splenic cells were rigorously depleted of T cells by double treatment with anti-Thy-1 + anti-Lyt-2 + C′. 10^7 primed B cells were transferred to unirradiated, FGG-primed NBF_1 male mice and to unprimed, irradiated B/C recipients, which served as a negative control for T cell helper activity. One carrier-primed, nonreconstituted *xid* mouse was also immunized with PC-FGG to confirm the complete lack of host contribution to PFC responses. Table II presents the results of this experiment. It can be seen that the *xid* recipient provides adequate T cell help for T15 dominant, secondary IgM anti-PC responses. It is important to point out that these results cannot be attributed to PFC responses bearing a minor T15 idiotope because our monoclonal anti-idiotypic reagent can cause complete, long-term unresponsiveness to PC in BALB/c mice (13); if the expression of the T15 idiotopes that we detect is not selectively regulated by idiotype-specific help, then the role of a regulatory idiotype-specific T helper cell must be reconsidered. Aliquots of the B cells used in this experiment were also transferred to irradiated, carrier-primed NBF_1 and BNF_1 male mice to determine if irradiation would expose the defective helper activity of the *xid* recipient (14). For this part of the experiment, T cell priming was performed with an intraperitoneal injection of 200 μg FGG in CFA 5 d before exposure to 600 rad and transfer of immune B cells and 20 μg PC-FGG. The day 7 secondary responses were 707,072 PFC/NBF_1 spleen and 264,845 PFC/BNF_1 spleen (geometric means of

TABLE I

Reconstitution of TD Anti-PC Responses in Athymic BALB/c Nude Mice with Carrier-primed Lymph Node Cells from xid and Normal Mice

Cells	Antigen	Anti-PC PFC/spleen			
		Day 5	Percent $T15^+$	Day 8	Percent $T15^+$
—	PC-FGG	871 (2.94 ± .17)	ND*	1,000 (3. ± .02)	ND
xid (FGG primed)	PC-FGG	69,183 (4.84 ± .07)	95	21,380 (4.33 ± .09)	95
Control (FGG primed)	PC-FGG	64,565 (4.81 ± .10)	95	30,199 (4.48 ± .06)	95
Control (KLH primed)	PC-FGG	5,370 (3.73 ± .04)	ND	2,884 (3.46 ± .23)	95

NBF_1 males and females were primed in the tail with 100 μg FGG in CFA. 6 d later, the inguinal, popliteal, and periaortic lymph nodes of the male vs. female mice were suspended in Hanks' balanced salt solution, washed extensively, and transferred in aliquots of 5×10^6 lymph node cells per mouse into groups of eight BALB/c *nu/nu* mice. 20 μg PC-FGG were injected i.v. immediately after cell transfer. As specificity control, eight *nu/nu* mice received KLH-primed lymph node cells and PC-FGG, and four *nu/nu* mice received antigen without cell reconstitution. At 5 and 8 d after antigenic challenge, one-half of each group was assayed for anti-PC PFC responses. Presented are the geometric means of the PC-specific and percent $T15^+$ responses at each time point, with the logarithm of the mean and standard error in parentheses. Percent $T15^+$ was determined by incorporating a hybridoma anti-idiotypic antibody into the plaquing mixture.

* Not done.

TABLE II

Secondary Anti-PC Responses in NBF_1 Male Mice Reconstituted with PC-immunized BALB/c Splenic B Cells

	Anti-PC PFC/spleen			
	Day 5	Percent $T15^+$	Day 8	Percent $T15^+$
NBF_1 Control	<1,000		ND*	
B cell Control	<1,000		1,905 (3.28 ± .28)	ND
NBF_1 Reconstituted	181,970 (5.26 ± .03)	97	45,709 (4.66 ± .02)	97

Spleen cells from BALB/c mice primed with 200 μg PC-KLH in CFA i.p. 10 wk earlier were treated twice with anti-Thy-1.2 + anti-Lyt-2 + rabbit C′ and transferred intravenously to unprimed, 600 rad-irradiated BALB/c recipients (four per group) and to unirradiated NBF_1 male hosts that had been primed with 100 μg FGG in CFA i.p. 5 d previously (six per group); each animal received 10^7 PC-primed splenic B cells. All recipients were immunized with 20 μg PC-FGG i.v. immediately after cell transfer. As a negative specificity control, one primed NBF_1 male that had not been reconstituted was also given antigen. One-half of each group was assayed for anti-PC PFC responses 5 and 8 d after challenge. Presented are the geometric means of the anti-PC PFC and percent $T15^+$ responses for each time point, with the logarithm of the mean and standard error given in parentheses. Percent $T15^+$ was determined by the extent of PFC inhibition after incorporation of an anti-idiotypic antibody into the plaquing mixture.

* Not done.

five individual responses). Anti-idiotypic serum and a PC-containing bacterial polysaccharide inhibited plaque formation >95%. Thus, under conditions of optimal carrier priming, irradiated *xid* recipients can provide help for T15 dominant anti-PC responses. We interpret Phillips and Campbell's (14) finding of deficient radioresistant helper cell activity in low-dose sheep erythrocyte (SRBC)-primed mice as secondary to ineffectual priming of *xid* helper cells (perhaps due to low levels of circulating IgM that may normally focus limiting concentrations of SRBC in the lymphoid tissues).

In these experiments, we elicited anti-PC responses of considerably greater magni-

TABLE III

Secondary Anti-PC Responses by BNF_1 B Cells: Comparison of Help from Normal vs. xid Lymph Node Cells with Help from Normal vs. xid carrier-primed Irradiated Hosts

	T cells		Antigenic challenge	Anti-PC PFC/spleen		
	Source	Priming		Total PFC	Non-T15 PFC	Percent $T15^+$
I.	NBF_1 LN	BSA	PC-BSA	2,495 (3.40 ± 0.10)	773 (2.89 ± 0.10)	69 (38–86)
	BNF_1 LN	BSA	PC-BSA	3,103 (3.49 ± 0.05)	676 (2.83 ± 0.11)	78 (55–86)
	—	—	PC-BSA	700 (2.85 ± 0.24)	373	46 (6–69)
II.	NBF_1 host 600-rad-irradiated	KLH	PC-KLH	65,455 (4.82 ± 0.04)	49,251 (4.69 ± 0.04)	25 (18–39)
	BNF_1 host 600-rad-irradiated	KLH	PC-KLH	36,269 (4.56 ± 0.13)	21,395 (4.33 ± 0.13)	41 (26–52)

Spleen cells from BNF_1 mice primed with 200 μg PC-FGG in CFA i.p. 6 wk previously were treated twice with monoclonal anti-Thy-1.2 and anti-Lyt-2 reagents plus rabbit C′ and then transplanted in aliquots of 5×10^6 cells per mouse into: (I) lethally irradiated BNF_1 hosts, with carrier-primed lymph node cells from either NBF_1 or BNF_1 males as the source of T cell help; and (II) lethally irradiated *xid* vs. normal hosts that had been carrier primed beforehand. In part I, the splenic B cells were co-transferred with 2.4×10^6 lymph node (LN) cells from either BNF_1 or NBF_1 male donors, which had been primed with 100 μg BSA in CFA in the tail and hind footpads 7 d earlier, into 600-rad-irradiated BNF_1 recipients (four per group). An additional two animals received B cells only, as a negative control. 20 μg PC-BSA were injected intravenously immediately after cell transfer. The same pool of B cells was used in part II as well, where 5×10^6 splenic B cells were transferred to 600-rad-irradiated BNF_1 or NBF_1 male mice (four per group) that had been primed 3 d previously with 2 μg KLH in CFA i.p. These hosts were challenged with 20 μg PC-KLH i.v. Both parts were assayed for anti-PC responses 7 d after immunization. Presented are the geometric means of the total and non-T15 anti-PC PFC responses for each group, with the logarithm of each mean and standard error in parentheses; non-T15 responses consisted of those PFC not inhibited by the anti-idiotypic serum incorporated into the plaquing mixture. Included also are the calculated percent $T15^+$ of each group, with the range of individual animals in the group given in parentheses.

tude than those analyzed by Bottomly and Mosier (11). To exclude the possibility that the putative deficit in helper T cells from *xid* mice could only be observed in suboptimal conditions, we performed an experiment using a less immunogenic TD PC antigen, PC-bovine serum albumin (BSA). As a source of B cells we used PC-FGG-primed BNF_1 mice which, unlike BALB/c mice, normally generate significant numbers of $T15^-$ anti-PC PFC responses. Carrier-primed lymph node cells were taken from NBF_1 male and BNF_1 mice injected with BSA in CFA in the base of the tail and footpads 4 d before use. The experiment also included a negative B cell control (unprimed, lethally irradiated NBF_1 recipients of B cells and PC-BSA) and a positive control (KLH-primed, lethally irradiated BNF_1 and NBF_1 recipients of B cells and PC-KLH). The results presented in Table III clearly show that in both optimal and suboptimal PFC responses, *xid* and control mice do not differ in their ability to provide help for $T15^+$ responses. In an experiment of similar design, the helper activity present in carrier-primed cells from *xid* mice was found to be sensitive to treatment with monoclonal anti-Thy-1 and C′ and anti-Lyt-1 + C′ but insensitive to anti-Lyt2 + C′ (results not shown). We conclude that *xid* helper cells are Thy-1^+, Lyt-1^+2^- lymphocytes.

We have also determined whether or not the activity of the helper cells present in *xid* mice is influenced by circulating idiotype. We investigated this in the course of experiments aimed at shifting idiotypic profiles of PFC induced in NBF_1 male mice injected repeatedly with BALB/c ($T15^+$) serum or immunized in vivo with PC-KLH under conditions known to generate $T15^-$ antibodies in *xid* mice ([8, 9], and Z. S.

Quan, unpublished results). Neither the passive transfer of T15 idiotype nor the active production of $T15^-$ antibody had a detectable effect on the idiotypic profiles of PFC responses generated by NBF_1 female B cells transplanted to treated NBF_1 male mice (results not shown). In view of the positive demonstrations both here and elsewhere (6, 9) that helper T cells for dominant $T15^+$ responses are present in *xid* mice, and given the ineffectiveness of circulating idiotype to alter idiotype expression, two explanations could account for our findings. The first attributes the induction of helper T cells for $T15^+$ responses to the presence of $T15^+$ nonantigen-reactive B cells in *xid* mice (4) or to the presence of nonclonally distributed idiotypelike surface markers on B cell progenitors (15). Because the population of sIg^+ progenitors of antigen-reactive B cells can be expanded by nonspecific antigenic stimulation (16), it is likely that conflicting findings on B cell-dependent T cell regulation will be generated unless such effects are controlled for. The conflicts may well be compounded by the possibility that the pool of idiotopes recognized by T cells may not be identical to the pool of idiotopes defined by the anti-idiotypic reagents used by different investigators. In all our studies we have used mice raised and maintained under standard conditions; the degree to which immunization with environmental antigens contributes to our findings remains to be determined. The second assumes that either only one type of helper T cell is necessary for IgM PFC responses (17) or that idiotype-restricted T helper cells are not responsible for idiotype dominance (18). These possibilities are currently under investigation.

Summary

We have examined the abilities of helper T cells from commercially available (CBA/N × BALB/c)F_1 (NBF_1) *xid* male and phenotypically normal female mice to help $T15^+$ and $T15^-$ B cells to produce thymus-dependent phosphorylcholine (PC)-specific direct plaque-forming cell responses. Carrier-primed T cells from both male and female mice were found (*a*) to restore $T15^+$ TD responses in congenitally athymic BALB/c mice, (*b*) to help PC-primed BALB/c splenic B cells produce predominantly $T15^+$ responses, and (*c*) to provide help for $T15^+$ and $T15^-$ PFC responses generated by PC-primed normal F_1 splenic B cells. Furthermore, carrier-primed irradiated *xid* and normal recipients contributed adequate helper activity for T15 dominant responses. We therefore conclude that male and female NBF_1 mice are equally capable of helping $T15^+$ responses.

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