Brief Definitive Report

PHOSPHORYLCHOLINE-SPECIFIC HELPER T CELLS IN MICE WITH AN X-LINKED DEFECT OF ANTIBODY PRODUCTION TO THE SAME HAPTEN

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CBA/N mice have a recessive X-linked defect of B-lymphocyte differentiation characterized by abnormal expression of B-cell surface markers and an inability to mount antibody responses to certain antigens (1–3). Previous studies have shown that CBA/N mice and their F_1 male offspring with the defective gene cannot produce antibody in response to the hapten phosphorylcholine (PC) (4–6). The precise nature of the defect has not been resolved, although it was shown not to be the result of suppressive mechanisms or abnormal antigen handling (5). Failure to respond to PC may be caused by an arrest of B-lymphocyte differentiation or an absence of a group of V-region genes coding for certain immunoglobulin specificities. A germ-line loss of V-region genes for PC receptors would be expected to affect T cells as well, because it has been shown that PC-specific T- and B-cell receptors show parallel expression of idiotypes (7). In the following experiments, an adoptive transfer system was used to test the PC-specific helper activity of F_1 hybrid mice with the CBA/N defect. The results indicate that the X-linked defect of CBA/N does not affect the ability of F_1 offspring to produce PC-specific helper T cells. Furthermore, inhibition of helper activity with anti-idiotypic antiserum demonstrates that genes coding for anti-PC specificities of the HOPC-8 (H8) idiotype are present in (CBA/N♀ × BALB/c♂)F_1 (NBF_1) mice, including F_1 males expressing the gene defect in their B-cell responses.

Materials and Methods

Animals. 6–8 wk old BALB/c mice were purchased from Cumberland View Farms, Clinton, Tenn.; A/He mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. CBA/N and (CBA/N♀ × BALB/c♂)F_1 (NBF_1) mice are being bred in our laboratories; the original CBA/N breeding pairs were supplied by Dr. C. Hansen, NIH, Bethesda, Md

Antigens. BALB/c IgG mouse gamma globulin (MGG) was prepared from normal serum by ammonium sulfate precipitation and purification on an Ultrogel ACA-34 column (LKB Produkter AB, Bromma, Sweden). The MGG was then absorbed on a PC-Sepharose column (8). Keyhole limpet hemocyanin (KLH) was obtained from Calbiochem, San Diego, Calif. PC was conjugated to MGG with *p*-diazonium phenylphosphorylcholine (8). Trinitrobenzene sulfonic acid (TNP) was reacted with KLH, MGG, or PC-MGG according to standard procedures (9). Conjugation ratios were as follows (hapten groups per 10^5 daltons of protein): PC_3-MGG, TNP_5-MGG, PC_3-MGG/TNP_7, TNP_{19}-KLH.

Immunizations. T-cell priming was accomplished by intraperitoneal and footpad immuni-

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TABLE I

Demonstration of PC-Specific Helper T Cells in NBF_1 and BALB/c Mice

	Cells transferred*		Antigen	Indirect anti-TNP PFCs per spleen§
	Helper cells	TNP-primed B cells‡		
Exp. 1‖ NBF_1 ♀	2×10^7 PC-primed spleen	5×10^6	PC_3-MGG-TNP_7	54,954 (4.74 ± 0.10)
	2×10^7 PC-primed spleen	5×10^6	TNP_5-MGG	1,047 (3.02 ± 0.17)
	2×10^7 unprimed spleen	5×10^6	PC_3-MGG-TNP_7	<500
	2×10^7 unprimed spleen	5×10^6	TNP_5-MGG	<500
Exp. 2 BALB/c	2×10^7 PC-primed spleen	5×10^6	PC_3-MGG-TNP_7	9,120 (3.96 ± 0.10)
	2×10^7 PC-primed spleen	5×10^6	TNP_5-MGG	<100
	—	5×10^6	PC_3-MGG-TNP_7	<100
	2×10^7 PC-primed spleen α-Thy 1.2 + C′ treated	5×10^6	PC_3-MGG-TNP_7	<100
	1×10^7 PC-primed T¶	5×10^6	PC_3-MGG-TNP_7	10,333 (4.01 ± 0.10)
	1×10^7 PC-primed T	5×10^6	TNP_9-MGG	<500
	1×10^7 PC-primed T	—	PC_3-MGG-TNP_7	<500

* Syngeneic recipients were given 600 rads and injected i.v. with various cell mixtures and 100 μg soluble antigen.

‡ B cells were purified by treatment with anti-Thy 1.2 serum and guinea pig complement.

§ Anti-TNP responses were assayed on day 7 after cell transfer. Direct PFC responses were negligible in all groups. Indirect PFCs were developed by using a sheep anti-mouse IgG antiserum. PFC responses represent the geometric mean of at least five animals per group. The log of the geometric mean and standard error are reported in parentheses.

‖ (CBA/N ♀ × BALB/c ♂)F_1 (NBF_1) female mice in exp. 1 and BALB/c mice in exp. 2 were used as cell donors.

¶ Enrichment for T cells was accomplished by passage of spleen cells through nylon wool columns (10).

zation with 200 μg PC-MGG in complete Freund's adjuvant (CFA); cells were assayed not earlier than 4 wk after priming. Animals immunized with 200 μg TNP-KLH in CFA intraperitoneally were used as donors of B cells at least 6 wk postimmunization.

Cell Transfers. Hapten-specific helper activity was assayed in an adoptive transfer system. Syngeneic recipients were given 600 rads total body irradiation from a ^{137}Cs source 6 h before cell transfer and maintained in sterile cages with water containing terramycin. PC-MGG primed cells were mixed with TNP primed B cells and 100 μg antigen, either TNP-MGG, or PC-MGG-TNP, and the inoculum was injected intravenously. B cells were prepared by treating spleen cells with anti-Thy 1.2 serum and guinea pig complement. In certain experiments, PC-primed T cells were purified by spleen cell filtration through nylon wool columns (10) and contained 95% Ig(−) cells as determined by immunofluorescence. Experimental groups receiving anti-idiotypic serum were treated as follows: recipients were injected intraperitoneally with 0.2 ml of a 1:2 dilution of anti-H8 immediately before irradiation and were given an additional 0.1 ml of anti-H8 intravenously along with the cell inoculum. In vivo treatment of BALB/c mice with anti-idiotype according to this procedure inhibited >95% of their anti-PC PFC responses. (Control responses were >150,000 PFC/spleen.)

Antisera. Anti-idiotypic antiserum was prepared in A/He mice by immunization with the BALB/c myeloma protein HOPC-8 (11). AKR anti-CBA ascites (anti-Thy 1.2) was purchased from Litton Bionetics, Inc., Kensington, Md.

Hemolytic Plaque Technique. A modification of the hemolytic plaque technique was used to detect antibody secreting cells (12, 13), using TNP-conjugated sheep erythrocytes as indicator cells (14). Secondary IgG anti-TNP plaque-forming cell (PFC) responses were assayed 7 days after cell transfer. IgG PFC were developed with a sheep anti-mouse IgG antiserum. In all cases, direct (IgM) PFC responses were negligible and are, therefore, not reported.

Results and Discussion

PC-specific helper activity was determined by comparing the response of PC primed spleen cells and TNP primed B cells to the antigens PC-MGG-TNP and TNP-MGG in an adoptive transfer system. Exp. 1 of Table I demonstrates the presence of PC-specific helper cells in the NBF_1 female. The combination of 2×10^7 PC-MGG primed cells and 5×10^6 purified, TNP-primed B cells responded with 54,954 indirect

TABLE II

Comparison of PC-Specific Helper T Cells in Female vs. Male NBF_1 Mice

Cells transferred*		Antigen	Indirect anti-TNP PFCs per spleen§
PC-primed helper cells	TNP-primed B cells‡		
2×10^7 NBF_1 ♀ spleen	5×10^6 NBF_1 ♀	PC_8-MGG-TNP_7	26,915 (4.43 ± 0.05)
2×10^7 NBF_1 ♂ spleen	5×10^6 NBF_1 ♀	PC_2-MGG-TNP_7	33,133 (4.52 ± 0.07)
2×10^7 NBF_1 ♀ spleen	5×10^6 NBF_1 ♀	TNP_5-MGG	<500
2×10^7 NBF_1 ♂ spleen	5×10^6 NBF_1 ♀	TNP_5-MGG	<500

* Spleen cells from NBF_1 ♀ or ♂ mice primed with PC-MGG were mixed with TNP-primed NBF_1 ♀ B cells and antigen. The mixture was injected i.v. into irradiated (600 rads) NBF_1 recipients.

‡, § as in Table I.

anti-TNP PFCs when challenged with PC-MGG-TNP, but only produced 1,047 PFCs in response to the antigen TNP-MGG. No helper activity was detected if unprimed spleen cells were used as a source of T-cell help. We also looked for PC-specific helper activity in both BALB/c and CBA/N parental strains. In agreement with previous reports (7), PC-specific help was found in BALB/c mice; in exp. 2, the mixture of 2×10^7 PC-MGG primed spleen cells and 5×10^6 TNP-primed B cells gave 9,120 PFCs to PC-MGG-TNP and less than 100 PFCs to TNP-MGG. The response in this system is a measurement of PC-specific helper T cells, because TNP-primed B cells alone would not respond, and anti-Thy 1.2 plus complement treatment of the PC-primed cell population eliminated the response to PC-MGG-TNP. Additionally, nylon-wool purified, PC-primed T cells were effective in helping TNP-primed B cells. We were unable, however, to induce PC-specific help in the CBA/N parental strain. Since normal CBA/CaJ mice also failed to provide PC-specific T help in this assay, we conclude that the failure of CBA/N is not due to their X-linked defect.

To assess the ability of immune defective mice to mount T-cell responses to PC, NBF_1 hybrid mice were used, because the males show defective anti-PC B-cell responses, and the females can be used as control mice. A comparison between the helper activities of male and female NBF_1 mice is presented in Table II. Using spleen cells as a source of helper T cells, it is evident that males have at least as much PC-specific helper activity as their female littermates. 2×10^7 PC-primed NBF_1 female spleen cells combined with 5×10^6 B cells gave 26,915 anti-TNP PFC's, while 2×10^7 NBF_1 male spleen cells tested with same indicator B cells yielded 33,133 PFCs. Since NBF_1 male mice have a higher proportion of T cells in their spleens than do female mice, further experiments were performed by using equal numbers of nylon-wool purified T cells to compare the relative numbers of PC-specific cells in the splenic T-cell populations of males and females. Various numbers of purified T cells were assayed, and in all cases anti-TNP responses in the groups that had received T cells from NBF_1 male mice were greater than or equal to responses in the groups with cells from NBF_1 female mice. A representative experiment using 1×10^7 T cells and 5×10^6 B cells gave 44,668 PFCs (4.65 ± 0.04) with male helper T cells and 13,490 PFC's (4.13 ± 0.07) using female helper T cells. The X-linked immune defect does not impair the potency of helper activity as seen in this assay system.

To further characterize the specificity of anti-PC helper T cells, anti-idiotypic antiserum was used to inhibit helper activity. As shown in Table III, the injection of anti-H8 serum with the cell inoculum suppressed 90% of the BALB/c response to PC-MGG-TNP, confirming the previously reported dominance of H8 idiotype expression of PC-specific T-cell help in this strain (7). Since exp. 2, Table III demonstrates that

TABLE III

Specificity Control for Anti-H8 Antiserum in Adoptive Transfer

Group*	T-cell priming	Antigen	Anti-H8‡	Anti-TNP PFCs per spleen§		Control‖
				Direct	Indirect	
						%
1¶	PC	PC_3-MGG-TNP_7	−	2,323 (3.37 ± 0.1β)	97,724 (4.99 ± 0.12)	100
2¶	PC	PC_3-MGG-TNP_7	+	162 (2.21 ± 0.33)	79 (1.90 ± 0.26)	10
3**	KLH	TNP_{19}-KLH	−	46,774 (4.67 ± 0.05)	1,318,257 (6.12 ± 0.05)	100
4**	KLH	TNP_{19}-KLH	+	66,069 (4.82 ± 0.04)	1,412,538 (6.15 ± 0.05)	107

* BALB/c mice were used as donors and recipients in all groups. Recipients were given 600 rads and injected i.v. as described below.

‡ Recipients were given 0.2 ml of a 1:2 dilution of anti-H8 serum i.p. 6 h before cell transfer and 0.1 ml anti-H8 i.v. with the cell inoculum.

§ As in Table I legend (§).

‖ % control of indirect anti-TNP PFCs.

¶ Groups 1 and 2 received 2×10^7 PC-MGG primed spleen cells, 5×10^6 TNP-KLH primed B cells (anti-Thy 1.2 plus guinea pig complement treated), plus 100 μg PC_3-MGG-TNP_7.

** Groups 3 and 4 received 3×10^7 TNP-KLH primed spleen cells and 100 μg TNP_{19}-KLH.

both direct (IgM) and indirect (IgG) PFC responses to TNP-KLH in adoptive transfer are unaffected by anti-idiotype treatment, the anti-H8 does not suppress TNP-specific B cells or non-PC-specific T-cell help.

Earlier studies have shown that CBA/N and NBF_1 male mice are unable to produce H8-positive humoral responses to PC (4–6), whereas NBF_1 females and BALB/c mice have detectable levels of H8-positive antibodies in their circulation even before exposure to exogenous PC antigens (5). According to the results in Table IV, both NBF_1 male and female anti-PC helper T cells possess H8-positive idiotype determinants, although in both cases suppression by anti-H8 serum was not complete; anti-TNP responses in animals that received NBF_1 female helper cells were only 70% suppressed, while responses by recipients of male helper cells were 85% suppressed. We are uncertain as to the significance of the slightly lower susceptibility of the T cells from NBF_1 females to anti-idiotype treatment, but we may conclude that both NBF_1 males and females largely share idiotype determinants among PC-specific T cells.

These studies demonstrate the ability of mice with an X-linked immune defect to produce normal T-cell responses to the hapten phosphorylcholine, an antigen against which they are unable to synthesize antibodies. Since the specificity for PC exists in the immune repertoire of mice with the X-linked defect, failure to produce antibodies to PC is probably not a result of a loss of genes coding for V-region specificities. Rather, the B-lymphocyte deficiency should be attributed to a maturational defect, as previously suggested (2, 5).

The results presented here strongly suggest the endogenous origin of PC-specific T-cell receptors, thus reinforcing the conclusions of Julius et al. in an independent system (15). Furthermore, adequate T-cell priming for a hapten does not require the presence of circulating antibody; this result argues against the existence of an Ig-dependent, antigen-specific helper T-cell population (16). Passively acquired antibody cannot function as the T-cell receptor in this system, because male F_1 hybrids with the immune defect are unable to mount antibody responses to PC and lack detectable anti-PC antibodies in their serum (5).

TABLE IV

Idiotype Expression of PC-Specific Helper Activity in NBF_1 Mice

	Group	Cells transferred*		Antigen	Anti-H8	Indirect anti-TNP PFCs per spleen§	Control
		PC-primed spleen cells	TNP-primed B cells‡				
							%
Exp. 1	1	2×10^7 NBF_1 ♀	5×10^6 NBF_1 ♀	PC_3-MGG-TNP_7	−	20,606 (4.31 ± 0.07)	100
	2	2×10^7 NBF_1 ♀	5×10^6 NBF_1 ♀	TNP_5-MGG	−	682 (2.83 ± 0.17)	—
	3	2×10^7 NBF_1 ♀	5×10^6 NBF_1 ♀	PC_3-MGG-TNP_7	+	6,730 (3.83 ± 0.07)	30‖
Exp. 2	1	2×10^7 NBF_1 ♂	5×10^6 NBF_1 ♀	PC_3-MGG-TNP_7	−	20,323 (4.31 ± 0.07)	100
	2	2×10^7 NBF_1 ♂	5×10^6 NBF_1 ♀	TNP_5-MGG	−	847 (2.93 ± 0.13)	—
	3	2×10^7 NBF_1 ♂	5×10^6 NBF_1 ♀	PC_3-MGG-TNP_7	+	3,698 (3.57 ± 0.10)	15

*, ‡, § As in Table I.

‖ Percent control was calculated as follows: ([group 3—group 2)/(group 1—group 2]).

Summary

F_1 male mice with the CBA/N X-linked defect that are unable to produce plaque-forming cell responses to phosphorylcholine (PC) provide normal PC-specific helper T-cell activity when compared to F_1 female littermates. Inhibition of helper activity with anti-idiotypic antiserum indicates that PC-specific T cells from both NBF_1 female and male mice possess predominantly BALB/c myeloma protein HOPC-8 idiotypic determinants. Therefore, the CBA/N defect cannot be explained as a deletion of genes coding for V-region anti-PC specificities. The demonstration of helper activity in NBF_1 male mice, which occurs in the absence of anti-PC antibody synthesis, also demonstrates the endogenous origin of the T-cell receptor.

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