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 F1 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 F1 hybrid mice with the CBA/N defect. The results indicate that the X-linked defect of CBA/N does not affect the ability of F1 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/N9 × BALB/cδ)F1 (NBFI) mice, including F1 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/N9 × BALB/cδ)F1 (NBFI) 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 10⁵ daltons of protein): PC₃-MGG, TNP₃-MGG, PC₅-MGG/TNP₇, TNP₁₉-KLH.

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

* Supported by graduate training grant 1-T32 HD-07009.
‡ Supported by grant AI-14530, Research Career Development Award AI-00268, and a Basil O'Connor Starter Research grant from the National Foundation March of Dimes.
### Table I

Demonstration of PC-Specific Helper T Cells in NBF₁ and BALB/c Mice

| Cells transferred* | Helper cells | TNP-primed B cells | Antigen | Indirect anti-TNP PFCs per spleen§ |
|--------------------|--------------|-------------------|---------|-----------------------------------|
| Exp. 1             |             |                   |         |                                   |
| NBF₁              | 2 × 10⁷ PC-primed spleen | 5 × 10⁶ | PC₃-MGG-TNP; | 54,954 (4.74 ± 0.10) |
|                   | 2 × 10⁷ PC-primed spleen | 5 × 10⁶ | TNP₃-MGG; | 1,047 (3.02 ± 0.17) |
|                   | 2 × 10⁷ unprimed spleen | 5 × 10⁶ | PC₃-MGG-TNP; | <500 |
|                   | 2 × 10⁷ unprimed spleen | 5 × 10⁶ | TNP₃-MGG; | <500 |
| Exp. 2             |             |                   |         |                                   |
| BALB/c            | 2 × 10⁷ PC-primed spleen | 5 × 10⁶ | PC₃-MGG-TNP; | 9,120 (3.96 ± 0.10) |
|                   | 1 × 10⁷ PC-primed T cells | 5 × 10⁶ | PC₃-MGG-TNP; | <500 |
|                   | 1 × 10⁷ PC-primed T cells | 5 × 10⁶ | TNP₃-MGG; | <100 |

* 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 F₂)Fl (NBF₁ female mice in exp. 1 and BALB/c mice in exp. 2 were used as cell donors.

Cell Transfers. Hapten-specific helper activity was assayed in an adoptive transfer system. Syngeneic recipients were given 600 rads total body irradiation from a 1⁴C 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-idiotypic 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 HOPEC-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 NBF₁ female. The combination of 2 × 10⁷ PC-MGG primed cells and 5 × 10⁶ purified, TNP-primed B cells responded with 54,954 indirect
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 × 10⁷ PC-MGG primed spleen cells and 5 × 10⁶ 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, NBF₁ 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 NBF₁ 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 × 10⁷ PC-primed NBF₁ female spleen cells combined with 5 × 10⁶ B cells gave 26,915 anti-TNP PFC's, while 2 × 10⁷ NBF₁ male spleen cells tested with same indicator B cells yielded 33,133 PFCs. Since NBF₁ 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 NBF₁ male mice were greater than or equal to responses in the groups with cells from NBF₁ female mice. A representative experiment using 1 × 10⁷ T cells and 5 × 10⁶ 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-MGG-TNP7 | –        | 2,323 (3.37 ± 0.08)      | 97,724 (4.99 ± 0.12) |
| 2‡     | PC             | PC-MGG-TNP7 | +        | 162 (2.21 ± 0.33)        | 79 (1.90 ± 0.26) |
| 3**    | KLH            | TNP<sub>7</sub>-KLH | – | 46,174 (6.17 ± 0.05) | 1,318,257 (6.12 ± 0.05) |
| 4**    | KLH            | TNP<sub>7</sub>-KLH | + | 66,069 (4.67 ± 0.04) | 1,412,438 (6.15 ± 0.05) |

* BALB/c mice were used as donors and recipients in all groups. Recipients were given 600 rad 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<sup>7</sup> PC-MGG primed spleen cells, 5 × 10<sup>9</sup> TNP-KLH primed B cells (anti-Thy 1:2 plus guinea pig complement treated), plus 100 μg PC<sub>7</sub>-MGG-TNP<sub>7</sub>.
** Groups 3 and 4 received 3 × 10<sup>7</sup> TNP-KLH primed spleen cells and 100 μg TNP<sub>7</sub>-MGG-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<sub>1</sub> male mice are unable to produce H8-positive humoral responses to PC (4–6), whereas NBF<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> females to anti-idiotype treatment, but we may conclude that both NBF<sub>1</sub> 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<sub>1</sub> 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

| Group | Cells transferred* | PC-primed spleen cells | TNP-primed B cells | Antigen | Anti-H8 | Indirect anti-TNP PFCs per spleen§ | Control |
|-------|---------------------|------------------------|-------------------|---------|---------|------------------------------------|---------|
| Exp. 1 | 1 2 × 10⁷ NBF₁, γ | 5 × 10⁷ NBF₁, γ | PC₆-MGG-TNP₇ | -- | 20,606 (4.31 ± 0.07) | 100 |
| 2 | 2 × 10⁷ NBF₁, γ | 5 × 10⁷ NBF₁, γ | TNP₇-MGG | -- | 582 (2.83 ± 0.17) | -- |
| 3 | 2 × 10⁷ NBF₁, γ | 5 × 10⁷ NBF₁, γ | PC₆-MGG-TNP₇ | + | 6,730 (3.83 ± 0.07) | 30 |
| Exp. 2 | 1 2 × 10⁷ NBF₁, δ | 5 × 10⁷ NBF₁, δ | PC₆-MGG-TNP₇ | -- | 20,323 (4.31 ± 0.07) | 100 |
| 2 | 2 × 10⁷ NBF₁, δ | 5 × 10⁷ NBF₁, δ | TNP₇-MGG | -- | 847 (2.93 ± 0.13) | -- |
| 3 | 2 × 10⁷ NBF₁, δ | 5 × 10⁷ NBF₁, δ | PC₆-MGG-TNP₇ | + | 3,698 (3.37 ± 0.10) | 15 |

* As in Table I.
§ Percent control was calculated as follows: (group 3—group 2)/(group 1—group 2).

### Summary

F₁ 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 F₁ female littermates. Inhibition of helper activity with anti-idiotypic antiserum indicates that PC-specific T cells from both NBF₁ 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 NBF₁ male mice, which occurs in the absence of anti-PC antibody synthesis, also demonstrates the endogenous origin of the T-cell receptor.

We are grateful to Paul Griffith for skillful technical assistance and to Miguel Arias for care of the NBF₁ mouse colony.

*Received for publication 2 October 1978.*

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