

## **Brief Definitive Report**

### **INABILITY OF MICE WITH A DEFECT IN B-LYMPHOCYTE MATURATION TO RESPOND TO PHOSPHORYLCHOLINE ON IMMUNOGENIC CARRIERS**

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CBA/N mice bear an X-linked genetic defect in the function of thymus-independent (B) lymphocytes (1). Several lines of evidence suggest that this defect results from the failure of development of a subpopulation of B lymphocytes which is found in mature mice of normal strains (2). The most prominent defects of CBA/N mice are their failure to respond to several thymus-independent antigens including the 2,4,6-trinitrophenyl (TNP) and 2,4-dinitrophenyl (DNP) derivatives of Ficoll (3), type III pneumococcal polysaccharide (4), polyinosinic-polycytidilic acid (5), levan and dextran (J. J. Mond, D. E. Mosier, and W. E. Paul, unpublished observations). CBA/N mice display less profound but clear defects in certain thymus-dependent antibody responses (6). Recently, we have shown that mice with the CBA/N-immune defect can respond to certain T-independent antigens, namely TNP-lipopolysaccharide (TNP-LPS [7]), the TNP derivative of *Brucella abortus* (TNP-BA)<sup>1</sup> and TNP conjugates of polyacrylamide beads.<sup>2</sup> These results suggest that T-independent antigens can be separated into two groups based on the matrix in which the TNP group is presented.

Because of these findings, it was particularly striking to find that male progeny of a cross between CBA/N female and either BALB/c or DBA/2 male mice, which are hemizygous for the CBA/N defect, failed to respond to phosphorylcholine (PC) derivatives of all carriers tested including several carriers which, if derivatized with TNP, would stimulate an anti-TNP response on the part of these defective mice. This is a provocative finding because the response to this PC antigen is dominated by antibody of one major idiotype (TEPC-15 idiotype), the expression of which has been demonstrated to occur substantially later in life than the expression of many antibodies to other antigens, most notably to the TNP group (8).

#### **Materials and Methods**

(CBA/N × DBA/2)F<sub>1</sub> and (CBA/N × BALB/c)F<sub>1</sub> male and female mice were obtained from Division of Research Services, National Institutes of Health. Since the defect of the CBA/N mice is X-linked, all F<sub>1</sub> males resulting from crosses of normal males with CBA/N females are defective

<sup>1</sup> J. J. Mond, I. Scher, M. Blaese, D. E. Mosier, and W. E. Paul. Manuscript submitted for publication.

<sup>2</sup> J. J. Mond, D. E. Mosier, and W. E. Paul. Manuscript in preparation.

and similar to the CBA/N mouse in their immunological characteristics while female progeny of such crosses are phenotypically normal.

**Antigens.** Antigens used in these studies included PC-ovalbumin (PC-OVA), PC-mycobacterium H37 Ra (PC-myco), PC-*B. abortus* (PC-BA) and rough strain pneumococcus (R36A) which bears PC as an immunodominant determinant. Antigens were prepared by the method of Chesebro and Metzger (9).

**Enumeration of Plaque-Forming Cells (PFC) and Anti-PC Antibodies.** PFC specific for phosphorylcholine were enumerated by using cII polysaccharide conjugated to sheep erythrocytes (10). cII contains PC as a predominant antigen. All PFC were inhibitable with concentrations of PC <  $10^{-4}$  M. PC antibodies bearing the TEPC 15 idiotype (T15) were determined by hemagglutination inhibition (HI) as previously described (11).

**Cell Culture.** Spleen cells were cultured in modified Mishell-Dutton medium containing 10% endotoxin free fetal calf serum and  $5 \times 10^{-5}$  M 2-mercaptoethanol in flat bottom microtiter trays (Falcon no. 3040 Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) at a density of  $10^6$  cells in 0.2 ml medium per well. Plates were incubated for 3-4 days in a humidified 5% CO<sub>2</sub>, 95% air atmosphere. Cells were collected and IgM PFC were enumerated by a modification of Jerne and Nordin hemolysis in gel assay (12).

### Results and Discussion

Results in Table I show T15 titers in CBA/N × DBA/2N defective F<sub>1</sub> males and their normal female littermates after immunization with 100 µg of PC-OVA, PC-Myco, or PC-BA or with  $10^6$  R36A organisms in complete Freund's adjuvant. F<sub>1</sub> male mice failed to mount T15<sup>+</sup> responses to PC on T-dependent (OVA) or on T-independent (BA, R36A) carriers although these mice can respond to TNP on OVA (13) and on BA.<sup>1</sup> F<sub>1</sub> females developed significant T15 responses as early as 2 wk after immunization with each of these antigens.

As noted above, it has been shown that CBA/N mice and (CBA/N × DBA/2)F<sub>1</sub> males and females can respond to certain T-independent antigens including TNP-BA. To directly compare the immunogenicity of PC and of TNP conjugates we studied in vitro primary responses to PC and TNP derivatives of *B. abortus* as well as to R36A.

In vitro responses of (CBA/N × BALB/c)F<sub>1</sub> male and female spleen cells to PC-BA, TNP-BA, and R36A are presented in Table II. F<sub>1</sub> female cells respond to a wide dose range of dilutions of TNP-BA and of PC-BA. In fact, the antibody responses of F<sub>1</sub> female cells to TNP-BA and PC-BA are very comparable at high dilutions of organisms, suggesting that in F<sub>1</sub> female mice the two antigens have a substantial similarity in immunogenicity. F<sub>1</sub> male cells are able to mount significant anti-TNP responses to TNP-*B. abortus*, although over a more narrow range of antigen concentrations than was true of F<sub>1</sub> female cells. However, F<sub>1</sub> male cells failed entirely to respond to PC-BA, at all antigen concentrations tested. Similarly, they did not mount an anti-PC response to R36A despite the fact that F<sub>1</sub> female cells made a significant in vitro response to R36A.

F<sub>1</sub> male and female mice were also immunized with PC-BA intravenously and PFC to PC-sheep erythrocytes (SRBC) were determined 4 and 6 days later (Table III). F<sub>1</sub> female mice developed large numbers of direct PFC's which were shown to be PC specific by the capacity of PC to inhibit plaque formation. Preliminary experiments had established that the peak response of normal mice to PC-BA occurred 4-6 days after immunization, and was comparable in magnitude in BALB/c and (CBA/N × DBA/2N)F<sub>1</sub> female mice. In sharp contrast to these results, F<sub>1</sub> male mice formed no measurable anti-PC responses after such

TABLE I  
*T15 Responses of CBA/N × DBA/2 F<sub>1</sub> Males and Females to Phosphorylcholine Conjugates of T-Dependent and T-Independent Carriers\**

	HI titer ( $\log_2$ ) ± SE	
	F <sub>1</sub> ♂	F <sub>1</sub> ♀
No Ag (5)‡	0	0.6 ± 0.6
PC-myco (13)	0	5.5 ± 0.8
PC-BA (4)	0	6.3 ± 0.6
PC-OVA (7)	0	6.9 ± 1.2
R36A (4)	0	4.9 ± 0.4

\* (CBA/N × DBA/2N)F<sub>1</sub> male and female mice were immunized with 100 µg of antigen or 10<sup>6</sup> R36A organisms emulsified in complete Freund's adjuvant. PC-myco was emulsified in incomplete Freund's adjuvant. 2-6 wk later sera were assayed for T15 idiotype by HI.

‡ Number of animals per group.

TABLE II  
*In Vitro Response of (CBA/N × BALB/c) F<sub>1</sub> Males and Females to PC-BA, TNP-BA and R36A\**

Antigen	Dilution	PFC/culture	
		F <sub>1</sub> ♂	F <sub>1</sub> ♀
PC-BA	10 <sup>-1</sup>	0	12
	10 <sup>-3</sup>	0	76
	10 <sup>-5</sup>	0	158
	10 <sup>-7</sup>	0	75
TNP-BA	10 <sup>-1</sup>	101	285
	10 <sup>-3</sup>	90	227
	10 <sup>-5</sup>	13	166
	10 <sup>-7</sup>	9	74
R36A	10 <sup>-4</sup>	0	40
	10 <sup>-5</sup>	0	35
	10 <sup>-6</sup>	0	0

\* 10<sup>6</sup>(CBA/N × DBA/2N)F<sub>1</sub> male and female spleen cells were cultured in flat bottom Falcon microtiter Mishell, Dutton medium. Antigens were PC-BA, TNP-BA, and R36A. PFC to PC-SRBC or to TNP-SRBC were determined 4 days later. Results represent means of duplicate wells.

TABLE III  
*In Vivo Response of (CBA/N × BALB/c) F<sub>1</sub> Males and Females to PC-BA\**

Antigen	PC-PFC/spleen			
	F <sub>1</sub> ♂		F <sub>1</sub> ♀	
	Day 4	Day 6	Day 4	Day 6
PC-BA	0	0	83,750 ± 21,250	23,167 ± 10,925

\* (CBA/N × BALB/c) F<sub>1</sub> male and female mice (three per group) were injected with a 10<sup>-2</sup> dilution of PC-BA intravenously. On day 4 and 6 after immunization spleens were enumerated for PFC to PC-SRBC. Results represent the mean ± SEM of three individual animals.

immunization. We have recently reported that such immunization with TNP-BA induces TNP-specific PFC in both male and female F<sub>1</sub> mice.<sup>1</sup>

The inability of these genetically defective mice to mount anti-PC responses in the face of relatively normal ability to develop anti-TNP (13) antibodies as well as antibodies to other specificities including azobenzeneearsonate and 3-nitro, 4-hydroxy, 5-bromophenyl acetyl (J. J. Mond, O. Mäkelä, and W. E.

Paul, unpublished observations) when immunized with these determinants conjugated to comparable carriers might be explained by the absence of precursors of anti-PC antibody-forming cells. Indeed, absence of such precursors and of PC-binding cells has been reported to occur in neonatal mice (14). It is possible that defective mice whose B-cell maturation is arrested or abnormal might never develop lymphocytes expressing this late-appearing receptor. Alternatively, mice with CBA/N X-linked-immune defect may lack an entire lineage of B lymphocytes. This lineage may be responsible for all of the PC response in normal strains either (a) because the expression of V-region genes controlling the T15 idiotype and other PC specificities may be limited to cells of this lineage or (b) because cells of the alternative lineage are tolerized in the neonatal period by contact with PC-bearing environmental antigens. In either case, the CBA/N would lack the line of B cells responsive to PC-antigens in normal mice and thus would fail to respond to PC on any carrier. A final possibility is that CBA/N mice do have PC-binding B cells but that these cells are defective in that they have a high threshold for activation or that they retain the high susceptibility to tolerance induction normally associated with very immature B cells. In any case, it seems most unlikely that the X-linked defect leading to the failure of CBA/N mice to respond to PC can represent the absence of a structural gene for anti-PC antibody since neither H-chain (15) or L-chain (16) genes have been reported to be X-linked.

### Summary

Mice with the CBA/N defect are unresponsive to the hapten phosphorylcholine (PC) even when presented on a variety of immunogenic carriers. Since these mice have the variable region gene for PC, their inability to respond may reflect deletion or suppression of the line of B lymphocytes which is responsible for the anti-PC response.

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### References

1. Scher, I., A. Ahmed, D. M. Strong, A. D. Steinberg, and W. E. Paul. 1975. X-linked B-lymphocyte immune defect in CBA/HN mice. I. Studies of the function and composition of spleen cells. *J. Exp. Med.* 141:788.
2. Ahmed, A., I. Scher, S. O. Sharow, A. H. Smith, W. E. Paul, D. H. Sachs, and K. W. Sell. 1977. B-lymphocyte heterogeneity: development and characterization of an alloantisera which distinguishes B-lymphocyte differentiation alloantigens. *J. Exp. Med.* 145:101.
3. Cohen, P. L., I. Scher, and D. E. Mosier. 1976. *In vitro* studies of the genetically determined unresponsiveness to thymus-independent antigens in CBA/N mice. *J. Immunol.* 116:300.
4. Amsbaugh, D. F., C. T. Hansen, B. Prescott, P. W. Stashak, D. R. Barthold, and P. J. Baker. 1972. Genetic control of the antibody response to type III pneumococcal polysaccharide in mice. *J. Exp. Med.* 136:931.
5. Scher, I., M. Frank, and A. D. Steinberg. 1973. The genetics of the immune response to a synthetic double-stranded RNA in a mutant CBA mouse strain. *J. Immunol.* 110:1396.

6. Gershon, R. K., and K. Kondo. 1976. Deficient production of a thymus-dependent high affinity antibody subset in mice (CBA/N) with an X-linked B lymphocyte defect. *J. Immunol.* 117:701.
7. Mosier, D. E., I. Scher, and W. E. Paul. 1976. *In vitro* responses of CBA/N mice: spleen cells of mice with an X-linked defect that precludes immune responses to several thymus-independent antigens can respond to TNP-lipopolysaccharide. *J. Immunol.* 117:1363.
8. Klinman, N. R., and J. L. Press. 1975. The characterization of the B-cell repertoire specific for the 2,4-dinitrophenyl and 2,4,6-trinitrophenyl determinants in neonatal BALB/c mice. *J. Exp. Med.* 141:1133.
9. Chesebro, B., and H. Metzger. 1972. Affinity labeling of a phosphorylcholine binding mouse myeloma protein. *Biochemistry*. 11:760.
10. Baker, P. J., P. W. Stashak, and B. Prescott. 1969. Use of erythrocytes sensitized with purified pneumococcal polysaccharides for the assay of antibody and antibody producing cells. *Appl. Microbiol.* 17:422.
11. Lieberman, R., M. Potter, W. Humphrey, Jr., E. B. Mushinski, and M. Vrana. 1975. Multiple individual and cross-specific idiotypes on 13 levan-binding myeloma proteins of BALB/c mice. *J. Exp. Med.* 142:106.
12. Jerne, N. K., and A. A. Nordin. 1963. Plaque-formation in agar by single antibody producing cells. *Science (Wash. D. C.)*. 140:405.
13. Janeway, C. A., Jr., and D. R. Barthold. 1975. An analysis of the defective response of CBA/N mice to T-dependent antigens. *J. Immunol.* 115:898.
14. Klinman, N. R., A. R. Pickard, N. H. Sigal, P. T. Gearhart, E. S. Metcalf, and S. K. Pierce. 1976. Assessing B cell diversification by antigen receptor and precursor cell analysis. *Ann. Immunol. (Paris)*. 127C:489.
15. Lieberman, R., M. Potter, E. B. Mushinski, W. Humphrey, Jr., and S. Rudikoff. 1977. Genetics of a new IgV<sub>H</sub> (T15 idiotype) marker in the mouse regulating natural antibody to phosphorylcholine. *J. Exp. Med.* 139:983.
16. Claflin, J. L. 1976. Genetic marker in the variable region of kappa chains of mouse anti-phosphorylcholine antibodies. *Eur. J. Immunol.* 6:666.