SYNERGISTIC GENETIC DEFECT IN B-LYMPHOCYTE FUNCTION

I. Defective Responses to B-Cell Stimulants and Their Genetic Basis

By CONSTANTIN BONA, JAMES J. MOND, AND WILLIAM E. PAUL

From the Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205; and the Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20014

Animals with genetically determined defects in immune function have provided valuable models for the elucidation of the mechanisms of normal immune responses. Thus, the CBA/N mouse, which bears a mutant X chromosomal gene (*xid*), has been of great value in understanding functional and developmental heterogeneity among B lymphocytes (1). Mice which are homozygous or hemizygous for the *xid* gene are unresponsive to a set of thymus-independent (TI)[1] antigens, of which trinitrophenylated (TNP)-Ficoll is the prototype (2). The TI antigens to which such mice are unresponsive have been designated TI-2 antigens (3). By contrast, these mice can respond to another set of TI antigens, TI-1 antigens, of which TNP-*Brucella abortus* (TNP-BA) is the prototype, and to many thymus-dependent (TD) antigens (2–5). Furthermore, splenic B lymphocytes from defective mice are stimulated to proliferate and to synthesize antibody by polyclonal B-cell activators, such as lipopolysaccharide (LPS) (2, 4) and Nocardia water-soluble mitogen (NWSM) (6), although they tend to do so most efficiently at high cell density and in the presence of fetal calf serum (2). B lymphocytes from mice with the CBA/N immune defect have low ratios of membrane IgD (mIgD) to membrane IgM (mIgM) when compared with normal controls (7) and fail to express the B-lymphocyte differentiation antigens Lyb3 (8), Lyb5 (9), and Lyb7 (10). It has been proposed that their defect stems from the absence of a subset of B lymphocytes which are either mature members of a single, B lymphocyte lineage or which are in a lineage separate from the B lymphocytes that respond to TI-1 antigens (1).

The C3H/HeJ mouse possesses a mutant gene Lps^d (11), located on chromosome 4 (12), that leads to unresponsiveness to the immunological and pharmacological

[1] *Abbreviations used in this paper:* BC, backcross; CFA, complete Freund's adjuvant; Con A, concanavalin A; DNP-KLH, 2,4-dinitrophenyl-conjugated keyhole limpet hemocyanin; F^-, unresponsive to trinitrophenylated Ficoll; F^+, responsive to trinitrophenylated Ficoll; IC, intercross; K^-, unresponsive to lipopolysaccharide from *Escherichia coli* K235; K^+, responsive to lipopolysaccharide from *E. coli* K235; LPS, lipopolysaccharide; LPS 0111:B4, LPS from *E. coli* 0111:B4; LPS K235, LPS from *E. coli* K235; mIgD, membrane IgD; mIgM, membrane IgM; NWSM, Nocardia water-soluble mitogen; PFC, plaque-forming cell(s); RBC, reverse backcross; SD, synergistic defect; SD^-, lacking the SD phenotype; SD^p, partial SD phenotype; TD, thymus dependent; TI, thymus independent; TNP, trinitrophenyl; TNP-BA, trinitrophenylated *Brucella abortus*; TNP-Ficoll, trinitrophenylated Ficoll; TNP-LPS, trinitrophenylated LPS 0111:B4; TNP-levan, trinitrophenylated levan.

effects of endotoxin. Thus, C3H/HeJ lymphocytes fail to proliferate or synthesize antibody when exposed to LPS preparations which are not contaminated with mitogenic bacterial lipoproteins or proteins (13). In addition, these mice are insensitive to the macrophage-mediated adjuvant activities of LPS (14). Immunological activities stimulated by agents other than LPS are normal in these mice.

We have recently begun to investigate the immune responsiveness of mice that are crosses between the CBA/N and C3H/HeJ strains. We observed that some F_1 male mice from the cross of CBA/N female and C3H/HeJ male parents and many backcross male mice resulting from matings of F_1 female and C3H/HeJ male parents exhibit more profound immune defects than either parental strain. Such mice are unresponsive, in vitro, to TI-1 as well as to TI-2 antigens, do not exhibit proliferative responses to several B-cell mitogens, and have an abnormality in the mIgD:mIgM ratio on their B lymphocytes which is substantially more striking than that of CBA/N (J. J. Mond, C. Bona, S. Kessler, I. Scher, and W. E. Paul. Manuscript in preparation.).

This synergistic, genetically determined immune defect of B lymphocytes does depend on the *xid* gene as crosses of CBA/CaN female mice, which are +/+ at the *xid* locus, to C3H/HeJ males and subsequent backcrossing to C3H/HeJ males fails to yield progeny which are unresponsive to TI-1 antigens or to B-cell mitogens. By contrast, the defective gene at the *Lps* locus (Lps^d) is not required for the expression of the synergistic defect as backcrosses obtained from matings of (CBA/N × C3H/HeN)F_1 mice × C3H/HeN mice are as defective as similar mice prepared using C3H/HeJ parents, although the C3H/HeN strain has a normal allele at the *Lps* locus (Lps^n).

Materials and Methods

Mice. CBA/N, CBA/CaN, and C3H/HeN mice were obtained from the Division of Research Services, National Institutes of Health, Bethesda, Md. C3H/HeJ mice were purchased from The Jackson Laboratory (Bar Harbor, Maine). F_1 hybrids from the matings of CBA/N females with C3H/HeJ males [(CBA/N × C3H/HeJ)F_1], from CBA/CaN females with C3H/HeJ males [(CBA/CaN × C3H/HeJ)F_1], and from CBA/N females with C3H/HeN males [(CBA/N × C3H/HeN)F_1] were prepared in our animal facility. F_1 female mice were mated with C3H/HeJ or C3H/HeN male mice to yield backcross mice. Mice from the backcross [(CBA/N × C3H/HeJ)F_1 × C3H/HeJ] are referred to BC.1 mice. This breeding scheme and the genotypes of progeny are presented in Fig. 1.

Antigens and Mitogens. TNP derivatives of aminoethylcarbamylmethyl Ficoll, (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.), *B. abortus*, *Escherichia coli* 0111:B4 LPS (TNP-LPS), and NWSM (TNP-NWSM) were prepared as previously described (4, 5, 15–17). 2,4-dinitrophenyl-conjugated keyhole limpet hemocyanin (DNP-KLH) was provided by Dr. Kathryn Stein, National Institute of Allergy and Infectious Diseases, National Institutes of Health; it contained 10 groups per 100,000 daltons.

LPS from *E. coli* K235, (LPS K235), the kind gift of Dr. David Rosenstreich, National Institute of Dental Research, National Institutes of Health, was prepared according to the procedure of Westphal et al. (18). This preparation lacks detectable quantities of mitogenic lipoprotein and protein and fails to stimulate spleen cells from C3H/HeJ mice. LPS from *E. coli* 0111:B4, (LPS 0111:B4), prepared according to the Boivin procedure, was purchased from Difco Laboratories (Detroit, Mich.). It has been reported to be contaminated with lipoprotein mitogen and can stimulate proliferative and plaque-forming cell (PFC) responses by C3H/HeJ spleen cells. Lipoprotein extracted from *E. coli* was a gift from Dr. Bressler, University of Tübingen, Tübingen, West Germany. NWSM was prepared according to the technique of

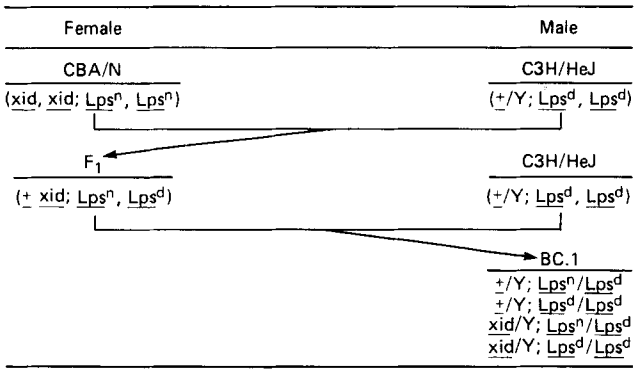

FIG. 1. Breeding scheme for the production of BC.1 mice.

Ciorbaru et al. (19). Concanavalin A (Con A) was obtained from Pharmacia Fine Chemicals, Div. of Pharmacia, Inc.

In Vitro Antibody and Proliferative Responses. Spleen cells (5×10^5) from various mice were cultured in microtiter plates (tissue culture, cluster 36, Costar, Data Packaging, Cambridge, Mass.) with TNP-Ficoll (10^{-3} μg/ml), TNP-BA (1:100 dilution of stock), TNP-LPS (5 μg/ml), and TNP-NWSM (3 μg/ml), as described previously (5, 17). Anti-TNP PFC responses to TNP-Ficoll and trinitrophenylated levan (TNP-levan) were measured on day 4; responses to TNP-BA, TNP-LPS, and TNP-NWSM were measured either on day 3 or day 4.

Proliferative responses were measured by culturing 2.5×10^5 spleen cells in microtiter wells with LPS K235 (25 μg/ml), LPS 0111:B4 (25 μg/ml), NWSM (50 μg/ml), lipoproteins (3 μg/ml), or Con A (2 μg/ml), as previously described (5). Uptake of tritiated thymidine was determined on the third day of culture.

Results

Responses of BC.1 Male Spleen Cells to TI Antigens and Mitogens. BC.1 male mice were prepared by backcrossing (CBA/N × C3H/HeJ)F_1 female mice to C3H/HeJ male mice, as shown in Fig. 1. Male mice from two consecutive litters were killed and their spleen cells tested in vitro for anti-TNP antibody responses to TNP TI antigens and for proliferative responses to various B-cell mitogens. As controls, cells from C3H/HeJ males, CBA/N females, and F_1 females were also studied.

As shown in Table I, F_1 female spleen cells yield substantial PFC responses to TNP-Ficoll, TNP-BA, TNP-LPS, and TNP-NWSM. They develop strong proliferative responses to both LPS K235 and LPS 0111:B4, lipoprotein, NWSM, and Con A. CBA/N spleen cells fail to respond to TNP-Ficoll and C3H/HeJ cells to LPS K235, as anticipated from their known genetic defects. Both CBA/N and C3H/HeJ spleen cells respond quite normally to the other stimulants. Spleen cells from four of seven BC.1 male mice make no anti-TNP antibody response to TNP-Ficoll. This is expected because one-half of these mice should be hemizygous for the *xid* gene, the hallmark of which is unresponsiveness to TNP-Ficoll. However, spleen cells from three of these mice also failed to give in vitro anti-TNP responses to any of the TNP TI antigens. Spleen cells from six of seven BC.1 male mice failed to proliferate in response to LPS K235. Of these, four were also unresponsive to LPS 0111:B4, lipoprotein, and NWSM. Three of the four LPS 0111:B4-unresponsive mice were the animals that were unresponsive to all TNP TI antigens. Thus, we concluded that four of the seven BC.1 male mice have a more profound defect than either parent and that three of these are

TABLE I

Responses of BC.1 Male Spleen Cells to TI Antigens and Mitogens

	Response to TNP TI antigens (No. of anti-TNP PFC/culture)					Response to B-lymphocyte mitogens (uptake of tritiated thymidine)*					
	Medium	TNP-Ficoll	TNP-BA	TNP-LPS	TNP-NWSM	Medium	K235	LPS 0111:B4	Lipoprotein	NWSM	Con A
						cpm					
C3HHeJ male	6	152	396	166	154	(5,887)‡	2,338	82,190	80,551	29,199	351,296
CBA/N female	0	0	154	78	310	(4,242)	25,230	90,482	49,723	37,870	279,988
F_1 female	20	138	584	146	248	(10,879)	88,417	101,321	106,121	48,830	356,471
BC.1 male No.											
1	0	0	4	10	0	(1,034)	0	1,746	39	0	233,254
2	20	104	372	50	230	(2,228)	80,910	138,922	102,872	26,054	291,576
3	4	0	52	92	106	(277)	0	2,915	2,994	694	400,789
4	0	88	190	62	12	(2,276)	1,014	29,767	30,585	5,657	150,155
5	0	0	0	6	2	(563)	0	709	261	362	52,737
6	0	0	0	0	0	(407)	27	2,223	1,171	1,020	58,805
7	0	26	126	38	72	(1,113)	0	62,647	46,295	97,325	218,242

* Counts per minute of tritiated thymidine taken up by cells from cultures exposed to a mitogen minus the counts per minute of tritiated thymidine taken up by cells from cultures exposed to medium only.

‡ Values in parentheses are uptake of tritiated thymidine by cells exposed to medium only.

unresponsive to any of the B-cell stimulants we tested. We refer to the failure of BC.1 mice to respond to B-cell mitogens and to TI-1 antigens as a synergistic defect (SD). Mice such as animals 1, 5, and 6, which are unresponsive to all B-cell mitogens and TI-1 antigens, are designated SD^+, whereas mice, such as animal 3, which respond to TI-1 antigens but either not at all or only poorly to LPS 0111:B4, lipoproteins, and NWSM, are considered to express a partial SD phenotype (SD^p).

All the BC.1 mice tested responded to Con A, indicating that SD^+ mice are responsive to some lymphocyte stimulants.

Because of these results, we examined in vitro proliferative and antibody responses in a large series of BC.1 male mice as well as in the parental strains, F_1 hybrids, and BC.1 females. In general, spleen cells from C3H/HeJ, CBA/N, (C3H/HeJ × CBA/N)F_1 females, and BC.1 females gave good proliferative responses to LPS 0111:B4 and NWSM (Table II) and good antibody responses to TNP-BA and TNP-NWSM (Table III). As expected, spleen cells from C3H/HeJ mice did not respond to LPS K235, whereas CBA/N and F_1 female cells responded well to this stimulant. CBA/N spleen cells did not produce anti-TNP antibody to TNP-Ficoll, but C3H/HeJ, F_1 female, and BC.1 females were all good responders. F_1 male mice, which are hemizygous for the *xid* gene, were unresponsive to TNP-Ficoll. However, approximately one-half were also unresponsive to LPS K235 and LPS 0111:B4, and only weakly responsive to NWSM, TNP-BA, and TNP-NWSM. Of BC.1 males, 32 of 51 were unresponsive to TNP-Ficoll, and approximately one-half failed to respond to the other stimulants. In general, individual BC.1 male mice expressed a good correlation in their proliferative response to LPS 0111:B4 and in their anti-TNP antibody response to TNP-BA (Fig. 2). This indicates that either of the two responses could be used to assign the SD phenotype. Wherever possible, we have evaluated both characteristics in all animals studied and we assign the SD^+ phenotype only to those in which both responses are absent.

Using this approach, we phenotyped 41 BC.1 male mice upon whom all of our tests were performed. Because one-half of the males should have inherited an *xid* gene, we

TABLE II
Responsiveness of Spleen Cells from BC.1 Mice to B-Cell Mitogens

Mice	No. of responders/total*		
	E. coli LPS		NWSM
	LPS K235	LPS 0111:B4 (Boivin)	
C3H/HeJ	1/13	11/14	9/9
CBA/N	11/11	13/13	8/8
F_1 female	9/9	9/9	8/8
F_1 male	10/20	9/19	16/16
BC.1 female	ND	4/6	6/6
BC.1 male	8/44	17/46	25/48

* Responders are defined as cultures in which the counts per minute were >3,000. ND, not done.

TABLE III
Responsiveness of Spleen Cells from BC.1 Mice to TI Antigens

Mice	No. of responders/total*		
	TNP-Ficoll	TNP-BA	TNP-NWSM
C3H/HeJ	14/15	15/15	12/12
CBA/N	1/16	15/15	11/12
F_1 female	8/8	10/10	9/9
F_1 male	0/11	18/20	16/21
BC.1 female	6/6	7/7	7/7
BC.1 male	19/51	23/41	25/44

* Responders are defined as cultures in which 20 or more anti-TNP PFC per culture were obtained.

anticipated that 50% would be unresponsive to TNP-Ficoll (F^-). In fact, 24 of 41 (58%) were F^- (Table IV). Similarly, we anticipated that one-half of the mice would be homozygous for the Lps^d gene and thus unresponsive to LPS K235 (K^-). In fact, 32/41 (78%) were K^-. Among mice responsive to TNP-Ficoll (F^+), approximately one-half (9/17) were K^-. However, among F^- mice, 23/24 were K^- and all 23 K^- individuals expressed either the SD^+ or SD^p phenotype. This suggests that in the presence of the *xid* defect, a C3H gene present in the heterozygous form can lead to the expression of the SD and that such animals may fail to respond to LPS K235 because of their SD phenotype even if they possess an Lps^n gene. Indeed, the finding in Tables II and III that approximately one-half of the F_1 males expressed an SD^p or SD^+ phenotype is consistent with this view. It does appear however that the expression of the SD phenotype in F^- BC.1 male mice is much more frequent than in F^- F_1 male mice.

Mice with SD^+ phenotype, although unresponsive in vitro to B-cell mitogens and to both TI-1 and TI-2 antigens, can make secondary anti-TNP antibody responses to the TD antigen DNP-KLH. Thus, a group of 16 BC.1 male mice were immunized with 10 μg of DNP-KLH emulsified in complete Freund's adjuvant (CFA). 1 mo later, they received a secondary immunization with 10 μg of DNP-KLH in saline. Direct and facilitated splenic anti-TNP PFC were measured 5 d later. These mice were also

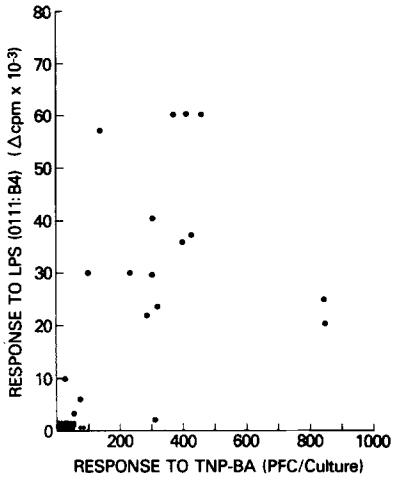

FIG. 2. Responses of spleen cells from individual BC.1 male mice to TNP-BA and to LPS 0111:B4.

TABLE IV

Frequency of Phenotypes in BC.1 Male Mice

Genotype	Anticipated		Observed ($n = 41$)	
	Phenotype	Frequency	Phenotype	Frequency
$+/Y, Lps^n/Lps^d$	F^+K^+	0.25	F^+K^+	0.20
$+/Y, Lps^d/Lps^d$	F^+K^-	0.25	F^+K^-	0.22
$xid/Y, Lps^n/Lps^d$	F^-K^+	0.25	F^-K^+	0.02
$xid/Y, Lps^d/Lps^d$	F^-K^-	0.25	F^-K^-	0.56
			F^-K^-, SD^+	0.39
			F^-K^-, SD^p	0.17

typed for the SD phenotype by measuring the proliferative response of their spleen cells to LPS 0111:B4. As shown in Table V, the SD^+ BC.1 male mice made responses equivalent to those of their littermates without the SD^+ phenotype (SD^-).

The xid but not the Lps^d Gene is Required for the Expression of the SD Phenotype. The observation that 23 of 24 F^- BC.1 male mice were also K^- and expressed either a partial or complete SD and that some F_1 male mice were SD^+, suggested that the C3H gene(s) involved did not have to be present in the homozygous form. To examine this more closely, we first wished to determine whether the SD phenotype depended on the presence of the Lps^d gene in the homozygous form. Accordingly, a BC.1 SD^+ male was crossed to a C3H/HeJ female (Table VI). Because none of the progeny of this cross should be F^-, it should be possible to evaluate the presence of the Lps^n gene simply by determining proliferative response of the progeny to LPS K235. In fact, all seven of the progeny were F^+, as expected, and of the six tested, all were responsive to LPS K235 (K^+). Thus, the BC.1 SD^+ male parent must have possessed a Lps^n gene and homozygosity for the defective gene at the *Lps* locus is not required for the SD phenotype.

To determine whether the Lps^d gene, even in a heterozygous form, was required for the SD phenotype, we prepared BC.1 male mice by crossing (CBA/N × C3H/HeN)F_1

TABLE V

Secondary Antibody Responses of BC.1 Male Mice to DNP-KLH

Mice	Responsiveness to LPS 0111:B4	Anti-TNP PFC/10^6 cells	
		Direct	Indirect
C3H/HeJ male	+	10	108
CBA/N female	+	39	220
F_1 male	+	30	353
BC.1 male [8]*	+	60 (1.5)‡	309 (1.5)
BC.1 male [8]	−	51 (1.8)	646 (1.5)

Individual C3H/HeJ, CBA/N, F_1 male, and 16 BC.1 male mice were immunized with 10 μg of DNP-KLH emulsified in CFA. 1 mo later, they received a secondary immunization with 10 μg of DNP-KLH in saline. 5 d later, the mice were killed and their spleens removed. A portion was used to measure direct and indirect anti-TNP PFC and the remainder were cultured with LPS 0111:B4. Of the 16 BC.1 mice, 8 were responsive and 8 were unresponsive to this LPS.

* Number of animals is in brackets.

‡ Geometric mean; relative SE in parentheses.

TABLE VI

Responsiveness of Spleen Cells from Various Backcross Progeny to TNP TI-1 and TI-2 Antigens and B-Cell Mitogens

Male progeny from crosses of	Responders/total			
	TNP-Ficoll	TNP-BA	LPS K235	LPS 0111:B4
C3H/HeJ × BC.1 SD^+ (+/+, Lps^d/Lps^d)(*xid*/Y, Lps^d/Lps^d or Lps^d/Lps^n)	7/7	6/6	6/6	5/5
(CBA/N × C3H/HeN)F_1 × C3H/HeN (*xid*/+, Lps^n/Lps^n) (+/Y, Lps^n/Lps^n)	11/24	16/24	13/24	13/24
(CBA/CaN × C3H/HeJ)F_1 × C3H/HeJ (+/+, Lps^n/Lps^d) (+/Y, Lps^d/Lps^d)	11/11	10/10	8/10	10/10

females with C3H/HeN males. The C3H/HeN strain is similar to the C3H/HeJ strain but it possesses normal allelles at the *Lps* locus. As shown in Table VII, 13 of 24 BC.1 male mice were F^-, as expected. However, 11 of 24 were unresponsive to LPS K235 and LPS 0111:B4 and 8 of 24 failed to respond to TNP-BA. Thus, the Lps^d gene is not required for either the SD^p or SD^+ phenotypes. However, we should note that none of the (CBA/N × C3H/HeN)F_1 males we have studied expressed an SD phenotype, suggesting that the Lps^d gene may make some contribution to the SD. By contrast, the *xid* gene appears essential for the expression of the synergistic defect. BC.1 male mice were prepared by crossing (CBA/CaN × C3H/HeJ)F_1 female mice to C3H/HeJ males. CAB/CaN mice are genetically similar to CBA/N mice but they possess a + allele at the *xid* locus. BC.1 male mice from this cross respond normally to TNP-Ficoll, TNP-BA, and to LPS 0111:B4. 2 of 10 were unresponsive to LPS K235, presumably because they were homozygous for the Lps^d gene.

As an initial step in attempting to determine the nature of the C3H influence which led to the appearance of the SD phenotype in the presence of the *xid* defect, we crossed a BC.1 SD^+ male mouse to a CBA/N female and then intercrossed the progeny. We

TABLE VII

Responsiveness of Spleen Cells from Reverse Backcross and Intercross Progeny to TNP TI-1 and TI-2 Antigens and B-Cell Mitogens

Progeny of crosses of*	Sex	No. of responders/total			
		TNP-Ficoll	TNP-BA	LPS K235	LPS 0111:B4
CBA/N × BC.1 SD^+	M	0/2	0/2	0/2	0/2
	F	0/4	4/4¶	4/4¶	4/4¶
RBC.SD^p × RBC.SD^+‡	M	0/5	4/5	2/5	2/5
	F	0/5	4/5	3/5	3/5
IC.1 SD^p × IC.1 SD^p§	M	0/5	2/4	0/5	0/5
	F	0/6	3/6	0/6	0/6
IC.2 $SD^{+(p)}$ × IC.2 SD^+‖	M	0/4	0/1	0/4	0/4
	F	0/7	0/5	0/7	0/7

* The phenotypes of parents were determined after they had been mated and had produced progeny.

‡ RBC mice are progeny from CBA/N × BC.1 SD^+ mating.

§ IC.1 mice are progeny from matings of RBC.SD^p × RBC.SD^+ mice. IC.2 mice are progeny from matings of IC.1 SD^p × IC.1 SD^p mice.

‖ A single IC.2 SD^+ male was crossed to two IC.2 females, one of which was later typed as SD^+ and the other as SD^p. Two litters were studied for responsiveness to TNP-Ficoll and to LPS K235 and LPS 0111:B4. Only the first litter was tested for responsiveness to TNP-BA.

¶ Two of the four mice gave very weak, although positive, responses to TNP-BA, LPS K235, and LPS 0111:B4. They were typed as SD^p.

wished to determine whether the SD phenotype could be expressed outside of an intact C3H genome and, in addition, to begin the development of an inbred strain expressing the synergistic defect. As shown in Table VII, a CBA/N female crossed to a BC.1 SD^+ male gives rise to male and female progeny, referred to as reverse backcross (RBC) mice, which are F^- as expected, because the mother is homozygous and the father hemizygous for the *xid* gene. Both RBC males were SD^+. Of the four females, two responded normally to TNP-BA, LPS K235, and LPS 0111:B4, and two responded poorly. The latter were classed as SD^p. One SD^p female and one SD^+ male RBC mouse were intercrossed, before their typing, to yield intercross (IC).1 mice. All of the IC.1 mice were F^-, two (one male and one female) were SD^+ and several were SD^p. An SD^p IC.1 male was crossed to an SD^p IC.1 female. All the IC.2 progeny expressed some SD and one-half expressed the SD^+ phenotype. Two litters of IC.3 mice were studied. All expressed an SD phenotype. This suggests that a gene or genes from the C3H background has been fixed in the intercrossed animals and is required for the expression of the SD.

Discussion

We have shown here that the introduction of the *xid* gene, in a hemizygous form, into the C3H genetic environment leads to a more profound immune defect than is expressed by either the CBA/N mouse or the C3H/HeJ mouse. This synergistic defect, in its full form, it characterized by unresponsiveness to a series of B-lymphocyte mitogens and by a failure to respond, in vitro, to TI-1 as well as TI-2 antigens. A partial form of the synergistic defect also occurs, generally characterized by unresponsiveness to B-cell mitogens and by weak responses to TI-1 antigens.

The nature of the C3H genes involved in the expression of the SD phenotype is not known but the *Lps*^{d} gene is not required although it is possible that this gene contributes to the defect. The finding that the SD$^+$ phenotype can be established in intercrossed mice resulting from a cross of a BC.1 SD$^+$ male mouse to a CBA/N female strongly suggests that the C3H influence is genetic rather than environmental in nature. Whether the difference between the SD^P and SD$^+$ phenotypes is simply one of penetrance or reflects the action of two distinct C3H genes is not known.

The establishment of strains of mice with the SD phenotype should shed considerable light on the ontogeny, heterogeneity, and function of B lymphocytes. The CBA/N defect had been considered to reflect either a maturation arrest in a single lineage of B lymphocytes or a defect limited to one of two lines (1). The latter interpretation is supported by data from Kincade (20) and Johnson et al. (21) which indicate that CBA/N mice are defective in a B-cell function which is normally expressed as early as 17 d of fetal life, namely the capacity to form B-lymphocyte colonies in agar. If the CBA/N defect is thought of as a deletion or a very early developmental arrest in one of two B-lymphocyte sublines, then responsiveness to TI-1 antigens would, most logically, be a function of the alternative cell line because CBA/N mice respond quite well to these antigens. The finding that the introduction of the *xid* gene into the C3H genetic background leads to the SD phenotype, in which there is no responsiveness to TI-1 antigens, would then require that the SD involved both B-cell sublines. An alternative possibility is that responsiveness to TI-1 antigens is a very primitive function of the subline which will eventually respond to TI-2 antigens and that the synergizing C3H genes cause the maturation arrest in that subline to occur at an even earlier stage than that at which TI-1 antigen responsiveness is acquired.

The effect of the SD on TD responses had not been fully evaluated. The finding that secondary responses to DNP-KLH are not obviously altered does not exclude the possibility that serious abnormalities in other TD responses may occur. Until a more complete study of TD responses in mice with SD phenotype has been carried out, it would be premature to attempt to place responsiveness to TD antigens in this developmental scheme. These issues should be possible to address when inbred and congenic lines expressing the SD phenotype are available. We are currently preparing two such lines.

Our results could be restated to say that in the presence of the synergizing C3H genes(s), the immunologic defect caused by the *xid* gene is much more profound than that observed in CBA/N mouse. In a sense, the clinical syndrome exhibited by BC.1 male mice is much more severe than that exhibited by CBA/N mice or by F_1 males from crosses between CBA/N females and DBA/2, BALB/c, or C57BL/6 males, although all these mice possess the same defective X chromosomal gene. This raises the possibility that distinct human X-linked immunodeficiency diseases (e.g., X-linked agammaglobulinemia, severe combined immunodeficiency, and Wiskott-Aldrich syndrome) may not all involve distinct defective X chromosomal genes. Rather, the same X chromosomal gene in different autosomal genetic environments might lead to quite distinct immunologic deficiencies.

Summary

CBA/N female mice, which express an X-linked defect in B-lymphocyte function, were mated with C3H/HeJ male mice, which are unresponsive to lipopolysaccharide

(LPS). The resulting F_1 hybrid females were mated to C3H/HeJ males. Approximately one-half of the backcross (BC.1) males obtained from this mating expressed a more profound immunologic defect than either of the parental strains. Spleen cells from these mice were unresponsive to a series of B-cell mitogens including LPS prepared from *Escherichia coli* K235 and from *E. coli* 0111:B4, lipoprotein mitogen from *E. coli*, and Nocardia water-soluble mitogen (NWSM). They failed to give in vitro antibody responses to the thymus-independent type 2 (TI-2) antigen trinophenylated Ficoll and most were unresponsive to the TI-1 antigens trinitrophenylated *Brucella abortus*, trinitrophenylated LPS, and trinitrophenylated NWSM. This synergistic defect in B-lymphocyte function depended on the presence of the CBA/N *xid* gene but the critical gene(s) from the C3H strain was not the defective Lps gene (Lps^d). These mice should provide a valuable tool for the elucidation of B-lymphocyte ontogeny, heterogeneity, and function.

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