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MAPPING OF THE X-LINKED IMMUNE DEFICIENCY MUTATION (*xid*) OF CBA/N MICE[1]

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CBA/N mice carry a recessive mutation at the X-linked immune deficiency (*xid*) locus that precludes their response to certain thymus-independent (TI) antigens. We have determined that *xid* is situated on the X-chromosome between the genes *Ta* (tabby) and *Hyp* (hypophosphatemia), genetic distances being *Ta* − 6.6 ± 1.8 − *xid* − 12.2 ± 2.3 − *Hyp*.

CBA/N mice possess an immune deficiency characterized by a profound but discrete defect in B lymphocyte function. This defect is intrinsic to the B lymphocyte lineage, rather than the result of a T lymphocyte or microenvironmental abnormality (1, 2). CBA/N mice fail to produce antibody when immunized with certain thymus-independent (TI)[3] antigens, such as polyriboinosinic-polyribocytidilic acid (Poly I·C), type III pneumococcal polysaccharide, and nitrophenyl conjugates of Ficoll (2–5). Such antigens are designated TI-2 antigens to distinguish them from another group of TI antigens to which CBA/N mice respond (6, 7), the TI-1 antigens. In addition, CBA/N mice have reduced responses to certain thymus-dependent antigens (8), low serum IgM and IgG_3 levels (4, 9), fail to form B lymphocyte colonies in soft agar (10), and lack both the B lymphocyte minor lymphocyte stimulating-defined antigens and a set of differentiation antigens, Lyb 3, 5, and 7 (11–14). Further analysis of CBA/N cell populations by using rapid flow microfluorometry has indicated a diminished number of B lymphocytes, an immature pattern of B lymphocyte surface Ig density distribution, and an abnormally high ratio of B lymphocyte membrane IgM-to-membrane IgD (15, 16). Virtually all of these defects can be accounted for if an entire subpopulation of B lymphocytes is absent in CBA/N mice (11, 15).

The immune abnormalities described above for CBA/N mice are caused by an X-linked recessive mutation (3, 4), hereafter called X-linked immune deficiency (gene symbol *xid*). In this report we present evidence that the *xid* gene is located between the loci tabby (*Ta*) and hypophosphatemia (*Hyp*).

MATERIALS AND METHODS

Animals. CBA/N mice were obtained from the Small Animal Section, Division of Research Services, National Institutes of Health, Bethesda, Md. Their origin has been previously described (4). F_1 female mice obtained from matings involving CBA/N females to males carrying two dominant X-linked mutations, Ta^{By} (tabby-By) and *Hyp*, were crossed to CBA/N males to obtain the backcross mice used to determine the position of *xid* on the X-chromosome. The Ta^{By} mutation was found in 1961 by Donald Bailey in an F_1 male produced from the mating of a BALB/c female to an irradiated (522 rads) C57BL/6By male. The F_1 mutant male was mated to a C57BL/6By female. Each generation thereafter, either a Ta^{By}/+ or Ta^{By}/Y individual was mated to a C57BL/6By individual. At backcross generation N14, the line was split and two separate lines were continued. One Ta^{By}/+ N19 female from one line was mated to a Ta^{By}/Y N18 male from the other line. Homozygous Ta^{By} females in the next generation, called N18R1, were recovered and mated to their Ta^{By}/Y brothers. The line was propagated thereafter by Ta^{By}/Ta^{By} by Ta^{By}/Y sib matings. We received several Ta^{By}/Y males from the N18R1F16 generation. Since the Ta^{By} mutation originated in a male whose X-chromosome came from a BALB/c female, the C57BL/6By-Ta^{By} strain is actually a congenic line and can be designated C57BL/6By.BALB/c-Ta^{By}, or more simply B.C-Ta^{By}.

The origin of the *Hyp* mutation has been presented elsewhere (17). The Ta^{By}*Hyp*/Y males used to mate to the CBA/N females were produced by mating a C57BL/6J-*Hyp*/+ female to a B.C-Ta^{By}/Y male. The Ta^{By}+/+ *Hyp* F_1 females were mated to C57BL/6J males and the Ta^{By} and *Hyp* mutations recovered in coupling in male offspring (Ta^{By} *Hyp*/Y).

Phenotyping of progeny. Twenty micrograms of 2,4,6-trinitrophenylaminoethyl carbamylmethyl-Ficoll (TNP-Ficoll), containing an average of 56 TNP groups per molecule of Ficoll, were injected i.p. into 8-week-old mice. Antigen was prepared by the method of Inman (18). Four days after immunization, mice were killed by cervical dislocation and a single-cell sus-

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[3] Abbreviations used in this paper: TI, thymus-independent; Poly I·C, polyriboinosinic-polyribocytidilic acid; Ta^{By}, tabby-By; *Hyp*, hypophosphatemia; *xid*, X-linked immune deficiency; *spf*, sparse fur; *Pi*, inorganic phosphate.

pension of their spleens was prepared (2). Anti-TNP plaque-forming cells (PFC) were detected by using a modification of the Jerne hemolytic plaque technique, employing TNP-coated sheep erythrocytes as indicator cells (19). Defective mice (*xid*/*xid* or *xid*/Y) produce 500 or fewer anti-TNP PFC per spleen, whereas immunologically normal mice (*xid*/+ or +/Y) develop 40,000 to 80,000 anti-TNP PFC per spleen.

The presence of the Ta^{By} mutation was detected by direct inspection of the coat, a striping indicating the presence of the Ta^{By} mutation in females and a darkening of the coat in the mid-back region, lack of zigzag hairs, and a slit-like eye, indicating the presence of the mutation in males.

The presence of the *Hyp* mutation was determined in 7-week-old mice by measuring their tail lengths and serum inorganic phosphate (*Pi*) concentrations (20). Values obtained from the backcross progeny, together with control values, are seen in Figure 1. The *Hyp* mutation causes a rachitic condition, evident by a reduced body and tail length and low levels of serum *Pi*. All mice whose tails were equal to or less than 7.8 cm and whose serum *Pi* levels were equal to or less than 6.3 mg% were considered hypophosphatemic, thus *Hyp*/+ or *Hyp*/Y. Mice whose tail lengths were greater than 7.8 cm and whose serum *Pi* levels were greater than 6.9 mg% were considered normal, thus +/+ or +/Y. Those animals with serum *Pi* levels between 6.3 and 6.9 mg% were classified as hypophosphatemic or normal, respectively, if their tail lengths were shorter than 7.1 cm or longer than 8.5 cm. The remaining mice that had intermediate values for both parameters or showed poor correlation between serum *Pi* level and tail length were discarded from the study.

RESULTS

A total of 239 progeny from the cross of (CBA/N × [Ta^{By} *Hyp*/Y])F_1 × CBA/N parents were classified for the *Ta*, *xid*, and *Hyp* traits. Of this number, 24 mice were discarded from the study due to inconclusive typing for the presence of the *Hyp* mutation. Of the eight phenotypes theoretically possible, two are the parental, nonrecombinant types and six are the recombinant types. Table I presents the frequency of these classes in the order: parental (1 and 2), recombinants between Ta^{By} and *xid* (3 and 4), recombinants between *xid* and *Hyp* (5 and 6), and double recombinants (7 and 8).

Before considering recombinational frequency and gene order between these loci, it should be noted that whereas only 18 of the 99 male mice inherited the Ta^{By} allele, 56 of the 116 females inherited this allele. We suspect that the observed deficiency of backcross Ta^{By}/Y males was due to the effect on dentition of the mutant *Ta* allele, leading to poor nutrition and death before the age at which they were typed. Consequently, we have computed the recombination frequency using data from either all females, all non-Ta^{By} males, or both groups combined.

A summary of the recombinational frequencies between each pair of genetic markers can be seen in Table II. The recombinational frequency between *Ta* and *xid* was 6.0 ± 2.0 when the data of females were analyzed, 7.4 ± 2.9 using non-Ta^{By} male data, and 6.6 ± 1.8 using both sets of data. The frequency of crossovers between *xid* and *Hyp* was 12.9 ± 3.3 with female data, 11.1 ± 3.5 with non-Ta^{By} male data, and 12.2 ± 2.3 with data from both. Finally, the recombinational frequency between the *Ta* and *Hyp* genes was 19.0 ± 3.6 with female data, 18.5 ± 4.3 for non-Ta^{By} male data, and 18.8 ± 2.6 with the combined data. If we assume that the frequency of the double recombinant class is less than the frequency of either single recombinant class, we can place the *xid* gene between the *Ta* and *Hyp* loci. Using the combined data, we calculate the genetic distances as (percent recombination ± standard error): *Ta* − 6.6 ± 1.8 − *xid* − 12.2 ± 2.3 − *Hyp*.

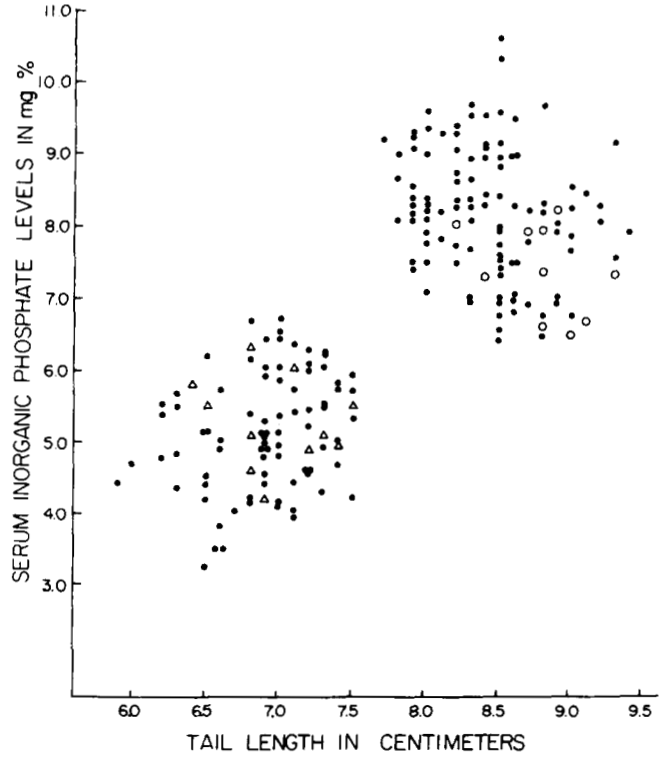

Figure 1. Assessment of backcross progeny and control mice for hypophosphatemia (*Hyp*) by tail measurement and serum inorganic phosphate determination. ●, backcross progeny; △, hypophosphatemic controls; ○, normal controls.

TABLE I

*Progeny produced from the cross: (Ta^{By} + *Hyp*/+ *xid* +)F_1 females to + *xid* +/Y CBA/N males*

No.[a]	Chromosome Inherited from F_1 Female[b]	No. of Female	No. of Male	Total	
1	Ta^{By} + *Hyp*	50	12	62	172
2	+ *xid* +	44	66	110	
3	Ta^{By} *xid* +	2	4	6	17
4	+ + *Hyp*	5	6	11	
5	Ta^{By} + +	4	1	5	25
6	+ *xid Hyp*	11	9	20	
7	Ta^{By} *xid Hyp*	0	1	1	1
8	+ + +	0	0	0	
		116	99	215	
			(81 non-Ta^{By} males)		

[a] Groups 1 and 2 are parental phenotypes; groups 3 to 8 are recombinant phenotypes.

[b] The phenotypes of Ta^{By}, *Hyp*, and *xid* were determined as given in the *Materials and Methods* sections.

TABLE II

Recombinational frequencies

Site of Recombination	Females	Non-Ta^{By} Males	Combined Estimate
Between Ta^{By} and *xid*	6.0 ± 2.0 (7/116)	7.4 ± 2.9 (6/81)	6.6 ± 1.8 (13/197)
Between *xid* and *Hyp*	12.9 ± 3.3 (15/116)	11.1 ± 3.5 (9/81)	12.2 ± 2.3 (24/197)
Between Ta^{By} and *Hyp*	19.0 ± 3.6 (22/116)	18.5 ± 4.3 (15/81)	18.8 ± 2.6 (37/197)

DISCUSSION

CBA/N mice have an X-linked mutation causing immunologic defects in a wide range of B lymphocyte functions. In this report we have determined that the location of the responsible gene, *xid*, is between the *Ta* and *Hyp* loci. Further confirmation of this finding was obtained from a separate breeding study utilizing mice that possessed the sparse fur (*spf*) mutation, which is a recessive gene located at the centromeric end of the X-chromosome. By making appropriate crosses between such animals and CBA/N mice, it was found that recombinants were recovered between the *spf* and *xid* genes 44% of the time, placing *xid* far away from the centromere and thus in the same general area as was obtained from the data presented herein.

Efforts made thus far to segregate the various manifestations caused by the *xid* mutation have been unsuccessful, even though large numbers of backcross progeny have been tested (unpublished observations, Berning, Ahmed and Scher). Successful separation of such characteristics, of course, would indicate that two or more very tightly linked genes are responsible for this series of abnormalities. At this time, the simplest hypothesis is that the immunologic defects observed in CBA/N mice are due to a mutation at a single locus, the *xid* locus.

Whether the *xid* gene lies in an as-yet-to-be-discovered region of the X-chromosome dealing with immune regulation awaits further analysis. That this may be true is suggested by the fact that a number of X-linked immune disorders are known in man. In contrast to this hypothesis is the possibility that the numerous defects observed when *xid* is present in the homozygous or hemizygous condition are caused by the interaction of *xid* with other loci located elsewhere in the genome; that is, the genetic background influences the expression of the *xid* gene. In favor of this hypothesis is our observation that the introduction of the *xid* mutation onto another genetic background can lead to an even more profound immune defect than observed in CBA/N mice (21). One now wonders whether the supposed distinct X-linked human diseases are the action of a single defective X-chromosomal gene interacting with loci in other parts of the genome. Further investigations are necessary to shed light on these possibilities.

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