UNBALANCED X CHROMOSOME MOSAICISM IN B CELLS OF MICE WITH X-LINKED IMMUNODEFICIENCY*

By MOON H. NAHM, JEFFERSON W. PASLAY,[‡] AND JOSEPH M. DAVIE

From the Departments of Pathology and of Microbiology and Immunology, Washington University
School of Medicine, St. Louis, Missouri 63110

CBA/N mice have an x-linked immunodeficiency that is mainly expressed by poor antibody production to some T-independent antigens, called TI-2 antigens (1–4) and to a few T-dependent antigens (5–8). This functional defect is associated with a failure in development of a late-appearing B lymphocyte subset (9) that can be defined in normal animals by distinctive surface antigens such as Lyb-3 (10), Lyb-5 (11), and others (12–14). Because many TI-2 antigens induce IgG antibody largely of the IgG3 subclass (15, 16), a normally minor subclass of IgG, and because CBA/N mice are deficient in IgG3 production (17), we proposed that the Lyb-3$^+$, -5$^+$ B cell subpopulation may be responsible for the bulk of IgG3 production and that IgG3 subclass response to any antigen would be defective in CBA/N (16). This has been confirmed for both T-independent and T-dependent antigens (5, 6, 16). Antibodies of other isotypes are less regularly influenced by the immunodeficiency (5).

The mutation in CBA/N mice, called *xid*, is linked to the x chromosome (1). Because of x chromosome inactivation, females heterozygous for *xid* will be mosaics, with some cells expressing the *xid*-bearing x chromosome, others not. Further, the recessive nature of the *xid* trait results in heterozygous females that are phenotypically normal (1). By examining x chromosome expression in the cells of these animals, it should be possible to determine whether the effect of *xid* on B cell development is direct or indirect. Direct effects, whereby the normal counterpart of the *xid* gene product(s) is expressed within the B cell and permits its development, would result in unbalanced x chromosome mosaicism among B lymphocytes from *xid* heterozygous females, because only B cells expressing the normal x chromosome could develop. Conversely, indirect effects, where the *xid* gene product(s) is expressed by non-B cells that subsequently influence B cell development or function, would result in balanced mosaicism among B lymphocytes. Here we examine x chromosome expression in *xid* heterozygous female mice using alloenzymes of phosphoglycerate kinase-1 (PGK-1)[1] as x chromosome markers.

Materials and Methods

Animals.   AT-10 female mice bearing *Pgk-1*ᵃ allele were obtained from Dr. V. Chapman (Roswell Park Memorial Institute, Buffalo, NY). Since AT-10 is congenic to C3H/HeHa, males with *Pgk-1*ᵃ were obtained by mating female AT-10 with male C3H/HeHa (Seneca Laboratory, West Seneca, NY). These males with *Pgk-1*ᵃ allele were used to produce the experimental animals by mating with female CBA/N (maintained in our facilities from original stocks obtained from the National Institutes of Health), CBA/J (animal F), and C57BL/10SnJ (animals G, H) (The Jackson Laboratory, Bar Harbor, ME). Animals D, E, and Q were produced by mating (AT-10 × C3H/HeHa)F₁ females with CBA/N males.

The selection of x chromosomes for inactivation is not random (18–20). Cattanach et al. (18) have found the inactivation to be influenced by an x-linked gene called *Xce*. Three alleles of *Xce* have been described (20) according to their relative preference in determining x chromosome expression: *Xce*ᶜ (AT-10 mice) is most preferred; *Xce*ᵇ (C57BL/10SnJ) is intermediate; and *Xce*ᵃ (CBA and C3H) is least preferred.

Preparation of Tissue and Hematopoietic Cells.   PGK enzyme was extracted from various hematopoietic cells by lysing them in phosphate-buffered saline (PBS) containing 1% Triton X-100 (Sigma Chemical Co., St. Louis, MO). Whole blood lysate was used as erythrocyte lysate. Using petri dishes coated with antibody to mouse IgM (21), splenocytes were separated into B cell-enriched (~81% DNL1.9⁺ [22]) and B cell-depleted (~5% surface Ig⁺) populations. The enzyme was extracted from various tissues by mincing samples in PBS followed by freeze and thaw cycles. Hybridomas were produced from heterozygous females after stimulation with 25 µg of lipopolysaccharide (LPS) (*Escherichia coli* 0128:B12; Difco Laboratories, Detroit, MI) intraperitoneally. 5 d later their spleno-cytes were hybridized with SP2/0-Ag14 cells as described elsewhere (23). The fused cells from each animal were distributed in 5–10 96-well plates. The supernates of the wells were screened for IgG1 and IgG3 production using solid-phase radioimmunoassay (17). Cells from fusions with >12% of wells showing cell growth were cloned in soft agar (24). The clones were again tested for IgG1 and IgG3 production. All the cells were maintained in culture medium containing hypoxanthine, aminopterin, and thymidine (HAT) (25) to minimize loss of the x chromosome.

Assay of PGK-1 Activity.   Because the enzyme activities of different tissue extracts were variable, tissue extracts were diluted until 10 µl of the extract gave 0.1–0.15 absorbance unit changes per minute at 340 nm when 400 µl of substrate solution was used. The substrate solution contained 2 mM ATP, 15 mM 3-phosphoglycerate, 12 mM MgSO₄, 0.7 mM disodium EDTA, 0.34 mM NADH, and 2.2 U/ml of glyceraldehyde-3-phosphate dehydrogenase (Sigma Chemical Co.) in 100 mM Tris buffer, pH 7.6 (25). The hybridoma cell lysates were obtained by lysing ~1 × 10⁶ cells in 100 µl of 1% Triton X-100 in a Tris-citrate buffer (225 mM Tris and 75 mM citrate, pH 7).

Electrophoresis of tissue extracts was performed on cellulose acetate paper (Gelman Sciences, Inc., Ann Arbor, MI) at 20 V/cm for 90 min using a pH 6.9 buffer containing 15 mM Tris, 5 mM Pipes (piperazine-*N*, *N'*-bis(2-ethanesulfonic acid); Sigma Chemical Co.), and 2.4 mM citrate. Samples (0.25 µl) were applied to the membrane using a sample applicator (Sepratek-8; Gelman Sciences, Inc.). The enzyme activity was visualized by overlaying the cellulose acetate paper with a reagent strip (Gelman Sciences, Inc.) soaked with substrate solution that contained 25% sucrose but was otherwise identical to that described above. A photographic record was made with Polaroid film (type 667) in a long wave UV box (Fotodyne, Inc., New Berlin, WI). The enzyme activity was detected as a nonfluorescent spot in the strip. Relative amounts of enzyme allelic forms were determined with this method by estimating the dilutions of the sample at which the enzyme activity in each band became undetectable.

Alternatively, electrophoresis of hybridoma cell lysates was performed in 1% agarose (equal mixture of medium and low electroendosmosis agarose [Sigma Chemical Co.]) at 10 V/cm for 6 h using the pH 7 Tris-citrate buffer over a flat-bed cooling plate (LKB, Sweden). The enzyme was located by overlaying the electrophoresis gel with a filter paper (Whatman-1; Fisher Scientific Co., St. Louis, MO) impregnated with the substrate solution without sucrose. After 15–30 min, the filter paper was dried and photographed as above.

Results

X Chromosome Expression in Hematopoietic Cells. Relative expression of the two alleles of *Pgk* among hematopoietic cells was studied. Fig. 1 shows examples of the PGK-1 isoenzyme patterns of cell lysates of splenocytes either enriched for (E) or depleted of (D) B cells. The upper and lower bands represent the variant PGK-1A and more common PGK-1B isoenzymes, respectively. By determining the dilutions of the cell lysate at which the enzymatic activity of each isoenzyme becomes undetectable, a semiquantitative estimate of the relative expression of the enzyme can be made. Thus, cells from two animals heterozygous for *Pgk* but lacking *xid* (Fig. 1, control) show either equal representation of the enzymes or slight preference of the PGK-1 alloenzyme. These patterns of mosaicism were seen in spleen cell populations depleted of or enriched for B cells as well as in thymocytes and erythrocytes (Table I). In fact, the degree of x chromosome mosaicism is largely shared among diverse hematological cell populations (27). On the other hand, cells from animals heterozygous for *Pgk* and for *xid* (Fig. 1, experimental animals and Table I) show different patterns of PGK expression. Although erythrocytes, thymocytes, and B-depleted splenocytes resemble each other, B-enriched populations of splenocytes are nearly devoid of the isoenzyme encoded by the x chromosome bearing the *xid* gene. This strongly suggests that
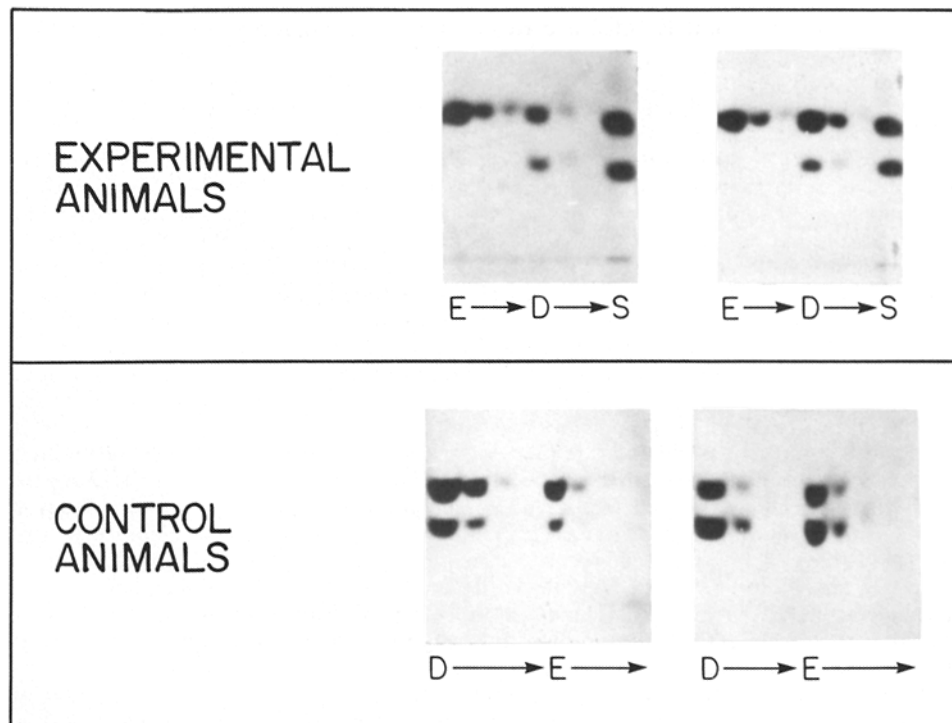

FIGURE 1. Estimation of isoenzyme activity by serial dilution. Experimental mice are heterozygous for *xid* and *Pgk-1*, whereas the control mice are heterozygous for *Pgk-1* only. S, position standards; E, splenocyte sample enriched for B cells; D, splenocyte sample depleted of B cells. Serial dilution is in the direction shown with horizontal arrows.

TABLE I

Pgk-1 Allele Expression in Hematological Samples

Cell types	Experimental animals					Control animals		
	A	B	C	D	E	F	G	H
Erythrocytes	2	4	3	3	3	2	0.5	1
Thymocytes	3	3–9	3	3	1	1–3	0.3	1
Splenocytes	9	9	9	3–9	9	3	1	1
B cell-depleted splenocytes	3	3	3	3	3	1–3	0.5	1
B cell-enriched splenocytes	>3	>9	27	>9	>9	1–3	0.5–1	1

Numbers represent the ratio of enzyme activity between PGK-1A and PGK-1B isoenzymes.

B lymphocytes with the *xid* gene defect do not develop.

The slight predominance of the *Pgk-1*[a] allele seen in many animals is probably the result of the regulation of x chromosome inactivation by *Xce*, and unconnected with *xid*, because all animals expressing this preference (A–F) are *Xce*[a]/*Xce*[c] heterozygotes, while the two with more equal expression (G and H) are *Xce*[b]/*Xce*[c] heterozygotes.

Most studies of animals expressing the *xid* trait have identified B cells as the principal target of the immunodeficiency, but only a subpopulation, approximately half of the total B cells, are thought to be involved (2, 4, 10, 11). The very marked changes in isoenzyme expression we find in isolated B cells suggests either that most B lymphocytes are affected by *xid* or that the amount of enzyme per cell produced by *Pgk-1*[a]- and *Pgk-1*[b]-bearing cells are different. To distinguish these alternatives, individual B cells must be examined. However, because of limitations in sensitivity of the enzyme assay in which 10^5–10^6 cells per determination are required, individual B lymphocytes were clonally expanded by somatic cell hybridization with myeloma cells. This technique is known to preserve the status of the inactive x chromosome (28, 29), unless a stringent selection is used (30).

We chose to compare x chromosome expression in IgG3- and IgG1-secreting hybridomas, because the *xid* trait is known to depress IgG3 production uniformly (5, 6, 16, 17), but has only variable effects on IgG1 (5, 17). We obtained 93 IgG3-producing hyridomas and 43 IgG1-producing hybridomas from nine animals; their phenotypes are summarized in Table II. Since the SP2/O-Ag14 myeloma cells have a *Pgk-1*[b] allele, a hybridoma generated from fusion with a splenocyte bearing *Pgk-1*[b] allele would express only one slow-moving enzyme band. This phenotype is labeled as CBA/N type in Table II. However, if a hybridoma was formed from a splenocyte that expressed a *Pgk-1*[a] allele, the hybridoma would have both *Pgk-1* alleles and show two bands upon electrophoresis. These hybridomas are labeled as AT-10 type in Table II. If an AT-10 type hybridoma discards the active splenocyte x chromosome, then phenotypically the hybridoma would express only the slow moving PGK-1B enzyme and be indistinguishable from the CBA/N type. Even though all the hybridomas were maintained in HAT selection media to minimize this conversion, there may be examples of the splenocyte x chromosome loss.

TABLE II

Enzyme Phenotypes of Hybridomas

Animal	IgG3-secreting clones		IgG1-secreting clones	
	AT-10 type	CBA/N type	AT-10 type	CBA/N type
I	13	1	9	0
J	9	1	8	0
K	9	1	1	0
L	13	0	2	0
M	10	1	0	10
N	7	2	3	0
O	11	0	0	3
P	4	0	2	0
Q	10	1	5	0
Total	86	7	30	13

As with splenocyte populations, the AT-10 x chromosome is preferentially active among both IgG1- and IgG3-producing hybridomas. ~70% of IgG1-secreting and ~93% of IgG3-secreting hybrid cell lines express both PGK-1 isoenzymes. Given the possibility of chromosome loss, these values may be underestimates. To rule out multiple representation of the same B cell clone among the hybridomas, only one clone was derived from a single well and electrophoretic mobilities of the IgG1 hybridoma proteins from animal M were shown to be different. However, it is clear that not all B cells express the AT-10 phenotype, because in two animals (M and O) all of the IgG1-secreting hybridomas were of the CBA/N phenotype. Interestingly, in these animals, nearly all of the IgG3-secreting cells expressed the AT-10 phenotype. These results thus suggest that the number of bone marrow stem cells for B cells is small and that at least occasionally the stem cells that generate IgG3-secreting cells are different from those that generate IgG1 secretors.

Isoenzyme Patterns in Tissue.   To determine whether other cell types in addition to B lymphocytes expressed the unbalanced x chromosome mosaicism as a result of *xid* gene expression, a survey was made of various tissues for PGK-1 isoenzyme expression in *xid* heterozygous mice. Extracts of brain, kidney, liver, muscle, and peritoneum were examined (Fig. 2). No consistent differences were seen between control and experimental groups of animals; most animals expressed both isoenzymes in similar amounts although occasional animals expressed one or the other enzyme dominantly. Further, most tissues from an individual animal had the same pattern, although striking exceptions are evident. This is clearly seen in the brain of animal B as well as in the liver and peritoneum of animal C. Further, all parts of the liver from animal C had the extreme PGK-1[a] predominance (data not shown). These examples are consistent with the notion that various organs arise from limited numbers of precursor cells (31–33). However, in this limited survey of tissues no consistent trend in unbalanced X-chromosome expression was seen between experiments and control animals. Study of individual cell types within the various tissues is required to determine whether only certain cell types express unbalanced mosaicism.
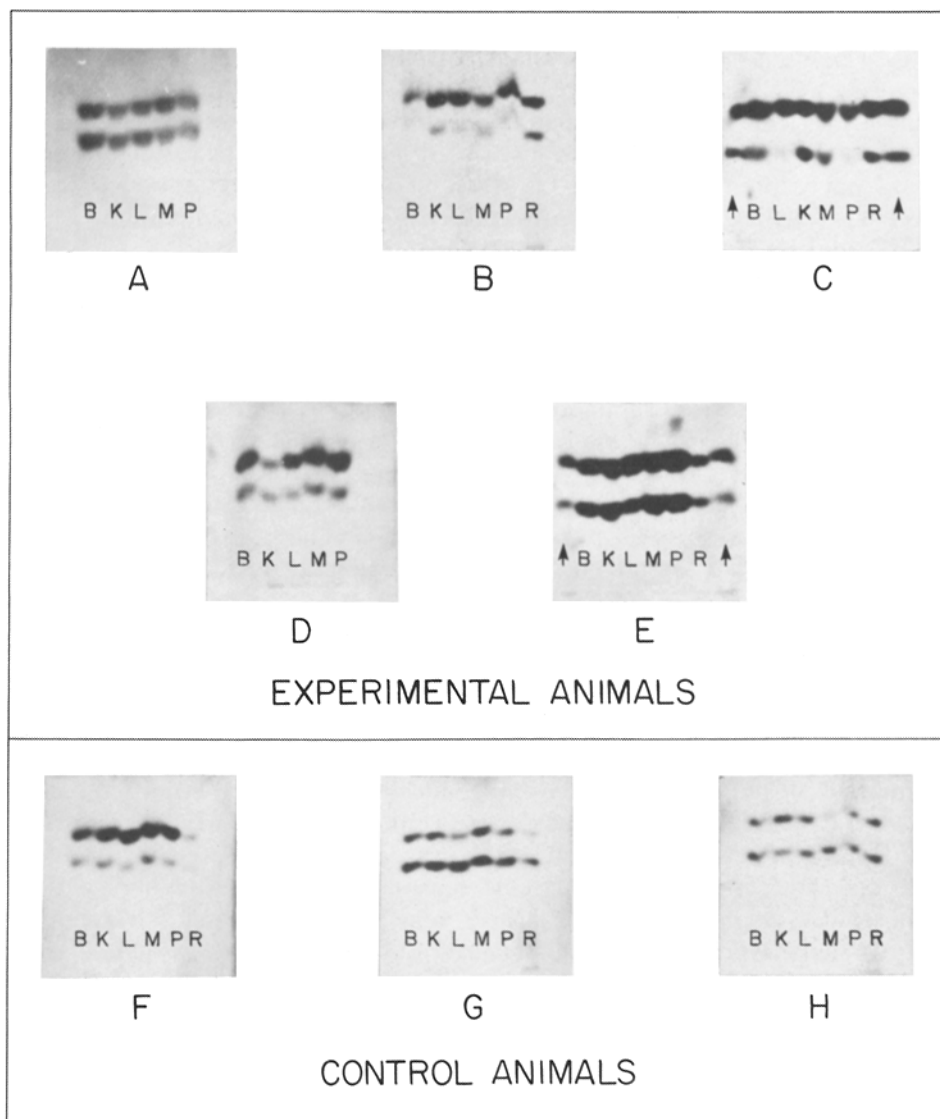
FIGURE 2. Electrophoretic patterns of PGK-1 isoenzymes from various tissues. Position standards are marked with vertical arrows. Source of tissues: B, brain; K, kidney; L, liver; M, muscle; P, peritoneum; R, erythrocytes. Experimental animals A–E are heterozygous for *xid* and *Pgk-1*. Control animals F, G, and H are heterozygous for *Pgk-1* only.

Discussion

The nature of the lesion(s) induced by *xid* has been studied extensively. Defects identified have included Lyb-3, -5, and -7-bearing B cells (10–12), Ia.W39-bearing B cells and/or macrophages (13), as well as some (34, 35), but not all (36, 37), T helper cells. These defects produce poor response to many polysaccharides (1, 3, 6, 16) and some proteins and polypeptides under immune response gene control (5), and a tendency to develop tolerance in vitro more easily than

normal (38). Because the functions involved depend on several cells, and even the development of a class of lymphocyte may depend on other cells (39, 40), it is not clear which if any of the defects is the initial lesion and which are secondary. Analysis of x chromosome mosaicism in heterozygous females is a more direct approach to this question. Cell populations directly affected by the *xid* mutation should not express the *xid*-bearing x chromosome, whereas those indirectly affected or unaffected should express this chromosome randomly.

The preliminary results reported here show clearly that B lymphocytes express unbalanced x chromosome mosaicism. Other cell populations do not, but this may reflect the failure to examine homogeneous subpopulations. Until this is possible, we cannot conclude that B lymphocytes are the only cell type directly affected by *xid*.

Examination of B cell subpopulations by hybridoma technology showed that IgG3-secreting cells nearly always expressed the normal x chromosome, while many IgG1-secreting cells expressed the *xid*-bearing x chromosome. These data support the idea that *xid* preferentially affects a subset of B lymphocytes (10, 11) that is associated with IgG3 production (16, 17). Whether the population not associated with IgG3 is unaffected by *xid* cannot be stated with assurance because only small numbers of cells have been examined. It does seem certain, however, that B lymphocyte subpopulations may express *xid*-bearing x chromosomes to different degrees.

Other examples of unbalanced x chromosome mosaicism exist. Lesch-Nyhan syndrome in man is caused by a deficiency in the x-linked enzyme, hypoxanthine-guanine phosphoribosyl transferase (41). Carriers of this recessive trait have erythrocytes exclusively derived from stem cells expressing the normal x chromosome; individual fibroblasts may express either (42). Similarly, carriers of the trait for Wiskott-Aldrich syndrome, an x-linked recessive disease involving immunodeficiency and thrombocytopenia, have unbalanced mosaicism of glucose-6-phosphate dehydrogenase (G6PD, an x-linked enzyme) among T lymphocytes and platelets particularly, and less marked effects among other hematopoietic cell types (43, 44).

Unbalanced mosaicism can occur normally because the bulk of many organs or cell subpopulations are often derived from small numbers of precursor cells. For instance, study of G6PD mosaicism suggests that human erythrocytes are derived from 8–19 precursor cells (31). Also mosaicism in skin showed mouse melanocytes are derived from 34 cells (33). Further, because x chromosome inactivation occurs early in embryogenesis and is permanent (45–47), topographic mosaicism is a normal feature in heterozygous females (18). Thus, small samples of organs or cell populations such as a patch of skin are unrepresentative of the whole, and may express unbalanced mosaicism. Our results with several organs (brain, peritoneum, and liver, Fig. 2) are consistent with either mechanism. Nevertheless finding 10 randomly selected but different CBA/N IgG1 hybridomas from one animal (Table II) supports the idea of limited precursor cells. Also the imbalance throughout the entire liver in animal C clearly supports the view that hepatocyte precursors are few. However, unlike the situations with the *xid*-mediated immunodeficiency, Wiscott-Aldrich syndrome and Lesch-Nyhan syndrome, this imbalance of normal mosaicism arises due to random expres-

sion of x chromosomes.

Having established that B lymphocytes are most likely a direct target of *xid* gene effects, is it possible to determine when in hemopoietic development that *xid* acts? We have found no gross effect of *xid* in T cells. The fact that marrow grafting from normal to nonirradiated CBA/N establishes B cell but not T cell chimerism (48) is also consistent with the notion that B cell precursors, but not T cell precursors, are defective; thus, common lymphoid stem cells are probably not affected. Kincade and co-workers (49, 50) have determined that CBA/N mice appear to develop pre-B cells with cytoplasmic μ and the 14.8 determinant (known to be expressed on B cell precursors) at the appropriate time in development (49), but that cells which give rise to B cells capable of growth in soft agar (clonable B cells) fail to develop (50). Clonable B cells appear simultaneously with surface Ig-bearing B cells at ~16–17 d of gestation. Thus, *xid* operates at least at an early stage of B cell development. Another x-linked immunodeficiency in DBA/2Ha mice has been localized to B lymphocytes. This gene appears to encode for a B cell receptor for T cell replacing factor (51). Thus, x-linked genes are involved in early and later stages of B cell differentiation.

These results are consistent with several models of B cell development. *xid* may block the development of more mature B cells such as Lyb-5$^+$ cells and clonable B cells from Lyb-5$^-$ cells. Alternatively, there may be several pathways of B cell development that diverge early and give rise to different functional subsets. The development of subsets responsive to TI-2 antigens is sensitive to *xid*. These models would suggest that the subset of B cells which develop in CBA/N animals would be normal. There is evidence, however, that the Lyb-5$^-$ B cells of CBA/N mice may not be equivalent to Lyb-5$^-$ B cells of normal animals. DeFranco and colleagues (52) noted that CBA/N B cells respond differently than normal cells to anti-μ stimulation. Although both defective and nondefective cells increase in size, defective cells fail to proliferate. Thus, it may be that even the B cells which develop in CBA/N mice may not be normal. Our results with x chromosome expression are consistent with this notion, although we do not wish to draw too fine a distinction because our data are limited. B cells from *xid* heterozygous females are more unbalanced in their x chromosome expression that would be expected if only a subpopulation were affected. It may be that *xid*-expressing B cells are at a proliferative disadvantage when compared with normal cells and that heterozygotes reflect this selection. Considerably more data are required to be confident about the extent of skewed expression of x chromosomes in heterozygotes; it is clear that it is substantial.

Finally, attention should be called to the utility of x-linked isoenzyme expression in the dissection of x-linked diseases, particularly immunodeficiencies. The x-chromosome plays an important role in the normal immune system. At least five immunodeficiency diseases in humans (53) and two in mice involve x-linked genes. The determination of the pattern of x chromosome expression in these diseases should be particularly informative.

Summary

The immunodeficiency in CBA/N mice is reflected by abnormal development of a subset of B lymphocytes. However, it is not clear how *xid*, the mutant gene

in CBA/N mice, affects the development of this subset. Specifically, it is not known if the *xid* gene influences the development of the B cell subset directly or indirectly by providing the improper developmental milieu through effects on other cells. We investigated this question using female mice heterozygous for two x chromosomal genes, *xid* and *Pgk-1* (phosphoglycerate kinase-1). Since females are mosaic because of x chromosome inactivation, their lymphocytes can be studied for the choice of the x chromosome, using the two PGK-1 isoenzymes as the cytological marker. We find that B lymphocytes in the spleen prefer the x chromosome without *xid* while the remaining splenocytes and cells from other tissues do not. This suggests that *xid* affects B lymphocytes directly and not through their developmental milieu. Furthermore, our data suggest that the precursors for IgG1- and IgG3-producing cells may be both few and different.

References

1. 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. I. Evidence that an X-linked gene plays a decisive role in determining responses. *J. Exp. Med.* **136**:931.
2. Paul, W. E., B. Subbarao, J. J. Mond, D. G. Sieckmann, I. Zitron, A. Ahmed, D. E. Mosier, and I. Scher. 1979. B lymphocyte development and activation: analysis with a mutant mouse strain. *In* Cells of Immunoglobulin Synthesis. B. Pernis, and H. J. Vogel, editors. Academic Press, Inc., New York. 383–396.
3. 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.
4. Scher, I. 1982. The CBA/N mouse strain: an experimental model illustrating the influence of the X chromosome on immunity. *Adv. Immunol.* **33**:1.
5. Press, J. L. 1981. The CBA/N defect defines two classes of T cell-dependent antigens. *J. Immunol.* **126**:1234.
6. Briles, D. E., M. Nahm, T. N. Marion, R. M. Perlmutter, and J. M. Davie. 1982. Streptococcal group A carbohydrate has properties of both a thymus independent (TI-2) and a thymus dependent antigen. *J. Immunol.* **128**:2032.
7. 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.
8. Scher, I., A. K. Berning, and R. Asofsky. 1979. X-linked B lymphocyte defect in CBA/N mice. IV. Cellular and environmental influences on the thymus dependent IgG anti-sheep red blood cell response. *J. Immunol.* **123**:477.
9. Mosier, D. E., J. J. Mond, I. M. Zitron, I. Scher, and W. E. Paul. 1977. Functional correlates of surface Ig expression for T-independent antigen triggering B cells. *In* The Immune System: Genetics and Regulation. E. E. Sercarz, L. A. Herzenberg, and C. F. Fox, editors. Academic Press, Inc., New York. 699–706.

10. Huber, B., R. K. Gershon, and H. Cantor. 1977. Identification of a B-cell surface structure involved in antigen-dependent triggering: absence of this structure on B cells from CBA/N mutant mice. *J. Exp. Med.* **145**:10.

11. Ahmed, A., I. Scher, S. O. Sharrow, A. H. Smith, W. E. Paul, D. H. Sachs, and K. W. Sell. 1977. B-lymphocyte heterogeneity: development and characterization of an alloantiserum which distinguishes B-lymphocyte differentiation alloantigens. *J. Exp. Med.* **145**:101.

12. Subbarao, B., A. Ahmed, W. E. Paul, I. Scher, R. Lieberman, and D. E. Mosier. 1979. Lyb-7, a new B cell alloantigen controlled by genes linked to the IgC$_H$ locus. *J. Immunol.* **122**:2279.

13. Rosenwasser, L. J., and B. Huber. 1981. The *xid* gene controls Ia.W39-associated immune response gene function. *J. Exp. Med.* **153**:1113.

14. Kung, J. T., S. O. Sharrow, A. Ahmed, R. Habbersett, I. Scher, and W. E. Paul. 1982. B lymphocyte subpopulation defined by a rat monoclonal antibody 14G8. *J. Immunol.* **128**:2049.

15. Perlmutter, R. M., D. Hansburg, D. E. Briles, R. A. Nicolotti, and J. M. Davie. 1978. Subclass restriction of murine anti-carbohydrate antibodies. *J. Immunol.* **121**:566.

16. Slack, J., G. DerBalian, M. Nahm, and J. M. Davie. 1980. Subclass restriction of murine antibodies. II. The IgG plaque-forming cell response to thymus-independent type 1 and type 2 antigens in normal mice and mice expressing an X-linked immunodeficiency. *J. Exp. Med.* **151**:853.

17. Perlmutter, R. M., M. Nahm, K. E. Stein, J. Slack, I. Zitron, W. E. Paul, and J. M. Davie. 1979. Immunoglobulin subclass-specific immunodeficiency in mice with an X-linked B-lymphocyte defect. *J. Exp. Med.* **149**:993.

18. Cattanach, B. M., J. N. Perez, and C. E. Pollard. 1970. Controlling elements in the mouse X chromosome. II. Location in the linkage map. *Genet. Res. Camb.* **15**:183.

19. West, J. D. and V. M. Chapman. 1978. Variation for X chromosome expression in mice detected by electrophoresis of phosphoglycerate kinase. *Genet. Res.* **32**:91.

20. Cattanach, B. M., and P. Johnston. 1981. Evidence of non-random X-inactivation in the mouse. *Hereditas.* **94**:5a. (Abst.)

21. Wysocki, L. J., and V. L. Sato. 1978. "Panning" for lymphocytes: a method for cell selection. *Proc. Natl. Acad. Sci. USA.* **75**:2844.

22. Dessner, D., and M. R. Loken. 1981. DNA 1.9: a monoclonal antibody which specifically detects all murine B lineage cells. *Eur. J. Immunol.* **11**:282.

23. Nahm, M. H., B. L. Clevinger, and J. M. Davie. 1982. Monoclonal antibodies to streptococcal group A carbohydrate. I. A dominant idiotypic determinant is located on V$_k$. *J. Immunol.* **129**:1513.

24. Coffino, P., R. Baumal, R. Laskov, and M. D. Scharff. 1972. Cloning of mouse myeloma cells and detection of rare variants. *J. Cell Physiol.* **79**:429.

25. Littlefield, J. W. 1964. Selection of hybrids from matings of fibroblasts *in vitro* and their presumed recombinants. *Science (Wash. DC).* **145**:709.

26. Nielsen, J. T., and V. M. Chapman. 1977. Electrophoretic variation for X-chromosome-linked phosphoglycerate kinase (PGK-1) in the mouse. *Genetics.* **87**:319.

27. Gandini, E., and S. M. Gartler. 1969. Glucose-6-phosphate dehydrogenase mosaicism for studying the development of blood cell precursors. *Nature (Lond.).* **224**:599.

28. Migeon, B. R. 1972. Stability of X-chromosomal inactivation in human somatic cells. *Nature (Lond.).* **239**:87.

29. Migeon, B. R., J. A. Sprenkle, and T. T. Do. 1979. Stability of the "two active X" phenotype in triploid somatic cells. *Cell.* **18**:637.

30. Kahan, B., and R. DeMars. 1975. Localized derepression on the human inactive x-chromosome in mouse human cell hybrids. *Proc. Natl. Acad. Sci. USA.* **72**:1510.

31. Fialkow, P. J. 1973. Primordial cell pool size and lineage relationships of five human cell types. *Ann. Hum. Genet.* **37:**39.

32. Nesbitt, M. N. 1971. X-chromosome inactivation mosaicism in the mouse. *Dev. Biol.* **26:**252.

33. Mintz, B. 1967. Gene control of mammalian pigmentary differentiation. I. Clonal origin of melanocytes. *Proc. Natl. Acad. Sci. USA.* **58:**344.

34. Bottomly, K., and D. E. Mosier. 1979. Mice whose B cells cannot produce the T15 idiotype also lack an antigen-specific helper T cell required for T15 expression. *J. Exp. Med.* **150:**1399.

35. Teale, J. M. 1983. Abnormalities in isotype expression in CBA/N mice due to stimulatory environment rather than B cell defect. *J. Immunol.* **130:**72.

36. Kaplan, R. B., and J. Quintans. 1979. Phosphorylcholine-specific helper T cells in mice with an X-linked defect of antibody production to the same hapten. *J. Exp. Med.* **149:**267.

37. Greenstein, J. L., E. Lord, J. W. Kapplar, and P. C. Marrack. 1981. Analysis of the response of B cells from CBA/N-defective mice to nonspecific T cell help. *J. Exp. Med.* **154:**1608.

38. Metcalf, E. S., I. Scher, and N. R. Klinman. 1980. Susceptibility to *in vitro* tolerance induction of adult B cells from mice with an X-linked B cell defect. *J. Exp. Med.* **151:**486.

39. Wortis, H. H., L. Burkly, D. Hughes, S. Roschelle, and G. Waneck. 1982. Lack of mature B cells in nude mice with x-linked immune deficiency. *J. Exp. Med.* **155:**903.

40. Mond, J. J., I. Scher, J. Cossman, S. Kessler, P. K. A. Mongini, C. Hansen, F. D. Finkelman, and W. E. Paul. 1982. Role of the thymus in directing the development of a subset of B lymphocytes. *J. Exp. Med.* **155:**924.

41. Lesch, M., and W. L. Nyhan. 1964. A familial disorder of uric acid metabolism and central nervous system function. *Am. J. Med.* **36:**561.

42. Nyhan, W. L., B. Bakay, J. D. Connor, J. F. Marks, and D. K. Keele. 1970. Hemizygous expression of glucose-6-phosphate dehydrogenase in erythrocytes of heterozygotes for the Lesch-Nyhan syndrome. *Proc. Natl. Acad. Sci. USA.* **65:**214.

43. Gealy, W. J., J. M. Dwyer, and J. B. Harley. 1980. Allelic exclusion of glucose-6-phosphate dehydrogenase in platelets and T lymphocytes from a Wiskott-Aldrich syndrome carrier. *Lancet* **I:**63.

44. Prchal, J., A. J. Carroll, J. F. Prchal, W. M. Crist, H. W. Skalka, W. J. Gealy, J. Harley, and A. Malluh. 1980. Wiskott-Aldrich syndrome: cellular impairments and their implication for carrier detection. *Blood.* **56:**1048.

45. Lyon, M. F. 1961. Gene action in the X-chromosome of the mouse (*Mus musculus* L.). *Nature (Lond.).* **190:**372.

46. Martin, G. R. 1982. X-chromosome inactivation in mammals. *Cell.* **29:**721.

47. Monk, M., and M. I. Harper. 1979. Sequential X-chromosome inactivation coupled with cellular differentiation in early mouse embryos. *Nature (Lond.).* **281:**311.

48. Volf, D., L. L. Sensenbrenner, S. J. Sharkis, G. F. Elfenbein, and I. Scher. 1978. Induction of partial chimerism in nonirradiated B-lymphocyte-deficient CBA/N mice. *J. Exp. Med.* **147:**940.

49. Kincade, P. W., H. Jyonovchi, K. S. Landreth, and G. Lee. 1982. B-lymphocyte precursors in immunodeficient, autoimmune and anemic mice. *Immunol. Rev.* **64:**81.

50. Kincade, P. W., M. A. S. Moore, G. Lee, and C. J. Paige. 1978. Colony-forming B cells in F1 hybrid and transplanted CBA/N mice. *J. Exp. Med.* **145:**249.

51. Takatsu, K., A. Tominaga, and T. Hamaoka. 1981. X-linked recessive inheritance of a defective responsiveness to T cell-replacing factor in DBA/2Ha mice. *Immunol. Lett.* **3:**137.

52. DeFranco, A. L., J. T. Kund, and W. E. Paul. 1982. Regulation of growth and proliferation in B cell subpopulations. *Immunol. Rev.* **64:**161.
53. Filipovich, A. H., and J. H. Kersey. 1983. B-lymphocyte function in immunodeficiency disease. *Immunol. Today (Amst.).* **4:**50.