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# Nonrandom X chromosome inactivation in B cells from carriers of X chromosome-linked severe combined immunodeficiency

**(B-cell differentiation/somatic cell hybrids/restriction fragment length polymorphism/carrier detection)**

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**ABSTRACT** **X chromosome-linked severe combined immunodeficiency (XSCID) is characterized by markedly reduced numbers of T cells, the absence of proliferative responses to mitogens, and hypogammaglobulinemia but normal or elevated numbers of B cells. To determine if the failure of the B cells to produce immunoglobulin might be due to expression of the XSCID gene defect in B-lineage cells as well as T cells, we analyzed patterns of X chromosome inactivation in B cells from nine obligate carriers of this disorder. A series of somatic cell hybrids that selectively retained the active X chromosome was produced from Epstein–Barr virus-stimulated B cells from each woman. To distinguish between the two X chromosomes, the hybrids from each woman were analyzed using an X-linked restriction fragment length polymorphism for which the woman in question was heterozygous. In all obligate carriers of XSCID, the B-cell hybrids demonstrated preferential use of a single X chromosome, the nonmutant X, as the active X. To determine if the small number of B-cell hybrids that contained the mutant X were derived from an immature subset of B cells, lymphocytes from three carriers were separated into surface IgM positive and surface IgM negative B cells prior to exposure to Epstein–Barr virus and production of B-cell hybrids. The results demonstrated normal random X chromosome inactivation in B-cell hybrids derived from the less mature surface IgM positive B cells. In contrast, the pattern of X chromosome inactivation in the surface IgM negative B cells, which had undergone further replication and differentiation, was significantly nonrandom in all three experiments [logarithm of odds (lod) score >3.0]. These results suggest that the XSCID gene product has a direct effect on B cells as well as T cells and is required during B-cell maturation.**

Severe combined immunodeficiency (SCID) is a term used to describe a heterogeneous group of disorders characterized by profound defects of both cellular and humoral immunity (1, 2). In man there are several different genetic defects that result in SCID. Approximately 15% of affected children have inherited autosomal recessive defects in the production of adenosine deaminase or purine nucleoside phosphorylase (1–3). The abnormal or absent gene products in the remaining cases have not yet been identified. However, the fact that affected boys outnumber affected girls by a ratio of 4:1 (1, 2) suggests that X chromosome-linked forms of severe combined immunodeficiency (XSCID) account for a high percentage of cases. In the past, most investigators have not distinguished between XSCID and other forms of the disease because most affected boys do not have a positive family history, carrier detection has only recently become available (4), and there are no clinical or laboratory findings that differentiate XSCID from other forms of the disease.

Most children with SCID have hypogammaglobulinemia, markedly reduced numbers of T cells, and absent mitogen responses, but many have normal or elevated numbers of B cells (1, 2). The failure of B cells to differentiate into immunoglobulin-secreting cells in children with SCID may be explained by either (*i*) stringent dependence of B-cell differentiation on normal T-cell function or (*ii*) expression of the gene defect in B-lineage cells as well as T cells. Several observations have supported the former explanation. The B cells from affected children can sometimes be induced to synthesize and secrete immunoglobulin if they are cultured with normal T cells and mitogen (5–7). Children with SCID characterized by markedly reduced numbers of T cells but normal numbers of B cells usually engraft T cells but not B cells after bone marrow transplantation (2, 8, 9). In some of these patients, with time, the host B cells begin to function and secrete antigen-specific antibody (2, 9). However, there is also some evidence that supports the hypothesis that there are B-cell as well as T-cell defects in children with SCID. Development of B-cell function in engrafted patients is slow and unpredictable (2). Also, children with SCID who have B cells but no T cells are usually profoundly hypogammaglobulinemic, whereas nude mice, which lack a thymus and T cells, have an altered pattern of immunoglobulin isotypes but are not panhypogammaglobulinemic (10, 11). This difference suggests either that B-cell function is more T-cell dependent in humans than in the mouse or that the SCID gene products are expressed in B cells as well as T cells but are less crucial for B-cell differentiation.

To examine this question we have analyzed patterns of X chromosome inactivation in B cells of carriers of XSCID. Early in embryogenesis of the female, random inactivation of one X chromosome occurs in each somatic cell and is transmitted to all descendants of that cell (12). Thus, in every cell lineage from a normal woman, approximately half the cells use the maternally derived X as the active X and half use the paternally derived X. The exceptions to this random pattern of X inactivation indicate origin from a common precursor, such as a monoclonal malignancy (13), or a selective advantage of cells expressing the genes on one X over cells expressing the genes on the other X (4, 14–16). By combining the production of somatic cell hybrids that selectively retain the active human X chromosome with the use of restriction fragment length polymorphisms that distinguish

Abbreviations: SCID, severe combined immunodeficiency; XSCID, X chromosome-linked severe combined immunodeficiency; $sIgM^+$, surface IgM positive; $sIgM^-$, surface IgM negative; EBV, Epstein–Barr virus; HPRT, hypoxanthine phosphoribosyltransferase.

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between the two X chromosomes, we have recently demonstrated that T cells from obligate carriers of XSCID exhibit nonrandom X chromosome inactivation (4). The X chromosome that does not carry the gene defect is preferentially used as the active X. If the XSCID gene defect were detrimental to B cells as well as T cells, one would expect that B cells from obligate carriers of XSCID would also demonstrate nonrandom X chromosome inactivation.

## METHODS AND MATERIALS

**Subjects.** Nine obligate carriers from eight unrelated families demonstrating X-linked inheritance of SCID were studied. Carrier 2 is the mother of carrier 3. The proband male in each family had typical XSCID with markedly reduced numbers of T cells, hypogammaglobulinemia, and normal or elevated numbers of B cells. The sons of carriers 1 and 8 died of an Epstein–Barr virus-related lymphoma after bone marrow transplantation. The sons of carriers 3, 4, and 5 were transplanted with T-cell-depleted bone marrow from their mothers. These boys are all clinically well 2–4 years after transplantation. All three have engrafted T cells and have normal numbers of host B cells, although none make normal concentrations of serum immunoglobulins. The son of carrier 6 received a bone marrow transplant from his histoidentical sister less than 1 year ago. The sons of carriers 2, 7, and 9 died of infection in infancy. The families of carriers 1, 2, and 3 (ref. 4) and the sons of carriers 4 and 5 (ref. 2) and carrier 8 (ref. 17) have been reported.

**Cell Separations.** Mononuclear cells were separated from peripheral venous blood by centrifugation through a Ficoll/Hypaque gradient. The cells at the interface were washed and then allowed to adhere to plastic Petri dishes. Over 95% of the adherent cells were shown to be monocytes by nonspecific esterase staining. The nonadherent cells were separated into T cells and T-depleted cells by rosetting techniques using aminoethylisothiouronium hydrobromide-treated sheep erythrocytes (AET-SRBC) as previously described (18). In some experiments the T-depleted cells were further separated into surface IgM positive ($sIgM^+$) B cells and surface IgM negative ($sIgM^-$) cells using a two-step panning procedure as previously described (18). Cells were first allowed to adhere to a plastic Petri dish coated with goat anti-human IgM at 2 $\mu$g/ml. Nonadherent cells were removed by gentle pipetting and were incubated on a second dish coated with anti-IgM at 10 $\mu$g/ml. The cells adhering to the first plate were removed by vigorous pipetting and were designated as $sIgM^+$ B cells. The cells adhering to the second plate were discarded, and the cells that were not adherent to the second plate were designated as $sIgM^-$ B cells.

**Production of Epstein–Barr Virus (EBV)-Transformed Cell Lines.** The B-cell populations were incubated for 1 hr with filtered spent media from the EBV-producing cell line B95-8 (19). The cells were then washed and cultured in multiple aliquots in RPMI 1640 medium with 20% (vol/vol) fetal calf serum. The cells were fed and expanded for 3–6 weeks, until 30–50 million B-lineage cells could be harvested.

**Immunofluorescence Staining.** EBV-stimulated B cells were centrifuged onto slides by cytocentrifugation, fixed, rehydrated, and then stained as previously described (18, 19) either with fluorescein-conjugated anti-IgM, -IgG or -IgA and rhodamine-conjugated anti-$\kappa$ or with rhodamine-conjugated anti-IgM, -IgG or -IgA and fluorescein-conjugated anti-$\lambda$. The rhodamine-conjugated anti-IgG and the fluorescein-conjugated anti-$\lambda$ were obtained from Southern Biotechnology (Birmingham, AL). The other reagents were purified and fluorochrome-conjugated as previously described (20, 21).

**Production of Somatic Cell Hybrids.** B- and T-cell hybrids were produced as previously described (4). Briefly, $3–5 \times 10^7$ T cells or EBV-expanded B cells were fused to $10^7$ cells from the Chinese hamster fibroblast cell line RJK88. This cell line readily forms human–rodent hybrids that tend to lose human chromosomes; however, because the RJK88 cell line has a deletion of the X-linked enzyme hypoxanthine phosphoribosyltransferase (HPRT) (22), hybrids grown in selective (hypoxanthine/azaserine) medium must retain the active human X chromosome to survive. Monocyte hybrids were produced by layering $3 \times 10^6$ RJK88 cells over each dish of approximately $1–2 \times 10^6$ monocytes that had been purified by plastic adherence. The combined monocytes and RJK88 cells were incubated for 24 hr in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum at 37°C in 8% $CO_2$. The plates were then washed three times with serum-free medium, flooded with 2.8 ml of 50% polyethylene glycol for 1 min, rinsed with serum-free medium, then cultured an additional 24 hr in DMEM with 10% fetal calf serum before the addition of selective medium. Hybrids were macroscopically identified 10–20 days after the fusions. Individual clones were isolated using cloning rings and were expanded until $10^8$ cells could be harvested for DNA preparation.

**DNA Analysis.** The preparation of high molecular weight DNA, digestion of DNA using the restriction enzyme *Taq* I, electrophoresis, and Southern blotting were performed as previously described (4). The X chromosome probes used in these experiments and the polymorphisms recognized by these probes are as follows: 36B-2, DXS10; 19-2, DXS3; and St14, DXS52 (4).

## RESULTS

To investigate the cell lineages affected by the XSCID gene defect, somatic cell hybrids were produced using the Chinese hamster cell line RJK88 and T cells, monocytes, or B-lineage cells from obligate carriers of XSCID. Freshly isolated adherent monocytes were used to make monocyte hybrids. To obtain sufficient numbers of B cells to produce hybrids, peripheral blood B cells were exposed to EBV and then allowed to proliferate in culture in 4–12 separate aliquots for 3–6 weeks. The validity of this approach was tested in two ways. First, to demonstrate that the cells used to produce the hybrids were polyclonal in origin, immunofluorescence staining for cytoplasmic immunoglobulin was performed on EBV-stimulated cells from eight women the same day that the cells were used to produce B-cell hybrids. Aliquots from each cell preparation were stained for cytoplasmic IgM, IgG, or IgA. A contrasting fluorochrome was used to stain the cells for either $\kappa$ or $\lambda$ immunoglobulin light chain. The percentage of cells that was positive for cytoplasmic IgM was $43.2 \pm 19.4$ (mean $\pm$ SD), $6.4 \pm 9.3$% were positive for IgG, and $9.1 \pm 16.2$% were positive for IgA. Both $\kappa$ and $\lambda$ positive cells could be detected in each immunoglobulin class.

Second, the X inactivation patterns of B-cell hybrids from EBV-stimulated cells from three women not at risk of being carriers of XSCID were determined. One woman, the mother of both a boy and a girl with SCID, was a carrier of an autosomal recessive form of the disease. B-cell hybrids from this woman demonstrated a normal pattern of X chromosome inactivation when analyzed with probe 19-2 (Fig. 1). One allele detected by this probe is a 5-kilobase (kb) DNA fragment and the other allele has an extra restriction site resulting in a 2.2/2.8-kb two-fragment allele. Seven B-cell hybrids were positive for the 5-kb allele and 16 were positive for the allele with an extra restriction site. B-cell hybrids from two additional control women also demonstrated normal, random X chromosome inactivation with allele distributions of 5 and 4 for one woman and 9 and 7 for the other.

The patterns of X chromosome inactivation in the T cells, monocytes, and B cells from the three obligate carriers originally reported (4) and from six additional unrelated obligate carriers of XSCID are shown in Table 1. T-cell hybrids from all

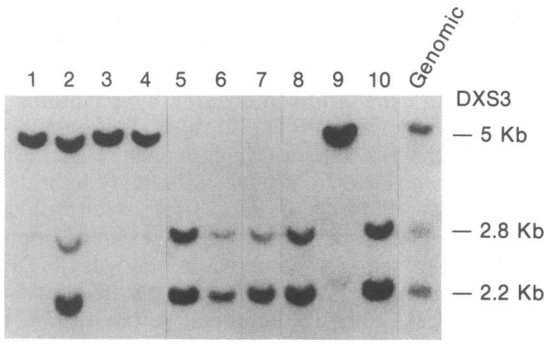


FIG. 1. EBV-stimulated B cells from a control woman who was a carrier of an autosomal recessive form of SCID were fused to the Chinese hamster cell line RJK88. The resulting human–hamster hybrids were analyzed with the human X chromosome probe 19-2. One allele detected by this probe is a 5-kb fragment, and the other allele has an extra restriction site resulting in a 2.2/2.8-kb two-fragment allele. The woman's genomic DNA is shown at the right. The hybrids in lanes 1, 3, 4, and 9 contain the X chromosome bearing the 5-kb allele. The hybrids in lanes 5, 6, 7, 8, and 10 contain the other X chromosome, and the hybrid in lane 2 contains both X chromosomes.

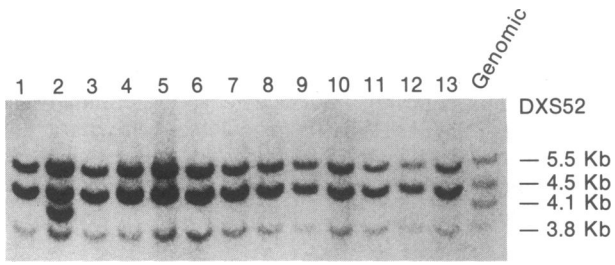


FIG. 2. EBV-stimulated B cells from carrier 7 were used to produce human–hamster hybrids. The hybrids were analyzed with the X chromosome probe St14, which detects constant fragments of 3.8 and 5.5 kb. One allele recognized by this probe has an extra 4.5-kb fragment and another allele has a 4.1-kb fragment. The woman's genomic DNA is shown at the right. The hybrid in lane 2 contains both X chromosomes. All other hybrids contain the X chromosome bearing the 4.5-kb allele.

of the women studied exhibited preferential use of a single X chromosome as the active X. As previously reported, family studies demonstrated that the X chromosome used as the active X in T-cell hybrids was the nonmutant, paternally derived X (4). Although the number of monocyte hybrids analyzed from each individual was small, the pooled data clearly indicate that monocytes from obligate carriers of XSCID exhibit normal, random X chromosome inactivation. In contrast, B-cell hybrids from these women demonstrated preferential use of the nonmutant X as the active X chromosome. A representative Southern blot from carrier 7 is shown in Fig. 2. Of a total of 13 B-cell hybrids, 1 hybrid (lane 2) contained both X chromosomes; the other 12 hybrids all used the X with the 4.5-kb DXS52 allele as the active X.

Although B-cell hybrids from all of the women studied demonstrated skewed patterns of X chromosome inactivation, in carriers 3, 5, 7, and 8 there was exclusive use of the same X that was used as the active X in T cells, whereas in carriers 1, 2, 6, and 9 a small number of B-cell hybrids used the mutant X as the active X. This heterogeneity has at least three possible explanations. First, there may be more than one genetic defect resulting in the clinical disorder XSCID. One type of defect might result in more profound skewing of X chromosome inactivation in B cells than others. To explore this possibility, B-cell hybrids from several members of a single family were examined. One out of 10 B-cell hybrids from carrier 2 had the mutant X as the active X, whereas all 22 B-cell hybrids from her daughter, carrier 3, had the normal X as the active X. Further, another daughter of carrier 2 (identified as II-6 in ref. 4) was found to be a carrier of XSCID based on analysis of her T-cell hybrids. All 13 T-cell hybrids from this woman used the paternally derived X as the active X. However, of the 13 B-cell hybrids derived from this woman, 8 had a paternally derived active X and 5 had a maternally derived active X. These findings indicate that there is heterogeneity within families as well as between families.

A second explanation for the rare exceptions that use the mutant X as the active X in B-cell hybrids is that the EBV-stimulated cell population used to make the hybrids may have been contaminated with a non-B-cell population demonstrating normal random X chromosome inactivation. If this were so, then one might expect that there would also be exceptions in the B-cell hybrids from carriers of X-linked agammaglobulinemia, a disorder in which we have shown that there is exclusive use of the normal X chromosome as the active X in B cells but normal patterns of X chromosome inactivation in platelets, T cells, and neutrophils (16). In a total of 115 B-cell hybrids derived from seven obligate carriers of X-linked agammaglobulinemia (produced using exactly the same methods), there were no exceptions to the use of a single X

Table 1. Patterns of X chromosome inactivation in hybrids from obligate carriers of XSCID

| | Number of hybrids containing each X chromosome | | | | | | |
|---|---|---|---|---|---|---|---|
| | T cells | | Monocytes | | B cells | | |
| Carrier | Normal* | Mutant | Normal | Mutant | Normal | Mutant | Polymorphic locus (probe)† |
| 1 | 21 | 2 | 3 | 4 | 14 | 3 | DXS3 (19-2) |
| 2 | 29 | 2 | — | — | 9 | 1 | DXS3 (19-2) |
| 3 | 18 | 0 | 2 | 1 | 22 | 0 | DXS10 (36B-2) |
| 4 | 20 | 0 | 8 | 4 | — | — | DXS3 (19-2) |
| 5 | 30 | 0 | 3 | 11 | 9 | 0 | DXS10 (36B-2) |
| 6 | 19 | 0 | 1 | 1 | 16 | 1 | DXS3 (19-2) |
| 7 | 7 | 0 | 1 | 0 | 19 | 0 | DXS52 (St14) |
| 8 | 6 | 2 | — | — | 13 | 0 | DXS3 (19-2) |
| 9 | 14 | 0 | — | — | 10 | 1 | DXS3 (19-2) |
| Total hybrids | 164 | 6 | 18 | 21 | 112 | 6 | |
| % hybrids using the mutant X as the active X | 3.5 | | 53.8 | | 5.1 | | |

*Family studies have previously shown that the normal, nonmutant X chromosome is used as the active X in the majority of T-cell hybrids.

†Locus (probe) used to distinguish the two chromosomes of each woman tested.

as the active X (41). These results suggest that the use of the mutant X as the active X in B-cell hybrids from carriers of XSCID cannot be attributed to contamination of the B cells used to make the hybrids.

A third explanation for the occurrence of B-cell hybrids containing the mutant X is that the gene defect that causes XSCID might result in a relative, but not absolute, disadvantage in B-cell proliferation or survival. If this were so, then B cells that had undergone more rounds of replication might be expected to show more marked skewing of X chromosome inactivation. To examine this possibility, B cells from carriers 4 and 8 and the daughter of carrier 2, whose unseparated B-cell hybrids demonstrated multiple exceptions, were separated into cells that were $sIgM^+$ and cells that were $sIgM^-$ prior to exposure to EBV. The proportions of B-lineage cells that contained cytoplasmic IgM, IgG, or IgA in each cell population on the day the B-lineage cells were fused to RJK88 cells are shown in Table 2. In each experiment, the B cells derived from the $sIgM^-$ population demonstrated a marked enrichment for IgG and IgA, the immunoglobulin classes that are produced after a greater number of cycles of B-cell replication. As shown in Table 2, the hybrids derived from the $sIgM^+$ B cells from all three carriers demonstrated random X chromosome inactivation. In contrast, the $sIgM^-$ hybrids from each experiment (Fig. 3) exhibited preferential use of the nonmutant X as the active X that was statistically significant [logarithm of odds (lod) score >3.0 for each experiment].

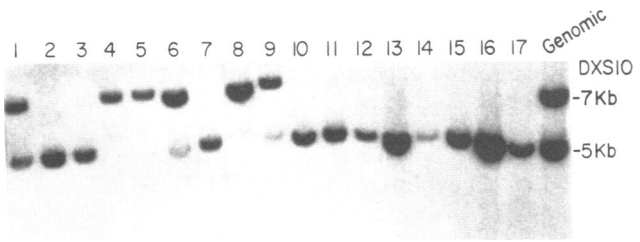


FIG. 3. EBV-stimulated $sIgM^+$ and $sIgM^-$ B cells from the daughter of carrier 2 were used to produce human–hamster hybrids. The hybrids were analyzed with the X chromosome probe 36B-2. One allele detected by this probe is a 7-kb fragment; the other is a 5-kb fragment. The woman's genomic DNA is shown at the right. The hybrids in lanes 1–9 were derived from $sIgM^+$ B cells and demonstrate random X chromosome inactivation. Those in lanes 10–17 were derived from $sIgM^-$ B cells and demonstrate exclusive use of a single X chromosome as the active X.

## DISCUSSION

In a previous report we demonstrated that T cells from obligate carriers of XSCID exhibit nonrandom patterns of X chromosome inactivation (4). The X chromosome not carrying the gene defect is selectively used as the active X. The data in this paper demonstrate that the B cells from these women also exhibit preferential use of the normal X as the active X. The most straight-forward interpretation of these data is that the gene defect that causes XSCID has a direct effect on B cells or B-cell precursors as well as T cells. The gene product is expressed in B-lineage cells, and a defective gene product is detrimental to proliferation or survival of B-lineage cells. Further, the gene product is probably not transportable between cells. If the gene product were transportable between cells or were not expressed in B-lineage cells, then one would expect that the cells that did synthesize the normal gene product could transport it to B cells or B-cell precursors with either the normal or the mutant X as the active X, and the B cells from obligate carriers of XSCID would demonstrate normal random X chromosome inactivation. The data presented here do not indicate if the XSCID gene defect is expressed at a single point in B-cell differentiation or at multiple points.

EBV-expanded B cells were used as a source of B-lineage cells in these experiments. Although the majority of peripheral blood B cells express receptors for EBV (21, 23, 24), only about 10% are activated to proliferate in response to this virus (25, 26). After prolonged periods in culture, a small number of clones with a growth advantage may predominate. Care was taken to ensure that B-lineage cells used in these experiments were polyclonal in origin; however, the cells used do represent a selected subset of B cells. The majority of EBV-responsive cells are mature, resting B cells that express sIgM (27, 28), and in most EBV-stimulated cultures, IgM-producing cells predominate (25, 26). However, B cells at almost all stages of differentiation can be induced to proliferate in response to EBV (29, 30). We took advantage of this fact to study patterns of X chromosome inactivation in B cells from two different stages of differentiation.

B cells from three carriers of XSCID were separated into $sIgM^+$ and $sIgM^-$ B cells. In all three experiments, the hybrids from the mature IgG- and IgA-producing B-lineage cells showed significant skewing, whereas the hybrids from the less mature IgM-producing B cells exhibited random X chromosome inactivation. In fact, a surprising number of hybrids from the IgM-producing cells used the mutant X as the active X, in view of the rare exceptions in hybrids from the unseparated B cells. This finding may be explained by the technique used to purify the $sIgM^+$ B cells. The $sIgM^+$ B cells were isolated from plastic Petri dishes that had been coated with very low concentrations of anti-human IgM. This procedure is likely to select out a subset of $sIgM^+$ cells with a high density of sIgM, a population previously shown to represent immature B cells in both mice and humans (21, 31).

Table 2. Patterns of X chromosome inactivation in hybrids from immature and mature B-cell subsets from carriers of XSCID

| Subject | B-cell subset fused | % cells positive for cytoplasmic immunofluorescence* | | | Hybrids containing each X chromosome, no. | | Lod score† |
|---|---|---|---|---|---|---|---|
| | | IgM | IgG | IgA | Normal | Mutant | |
| Carrier 4 | $sIgM^+$ | 46 | 19 | 11 | 11 | 5 | 0.50 |
| | $sIgM^-$ | 14 | 44 | 22 | 20 | 3 | 3.05 |
| Carrier 8 | $sIgM^+$ | 62 | 4 | 6 | 8 | 6 | 0.06 |
| | $sIgM^-$ | 9 | 31 | 6 | 16 | 0 | 4.82 |
| Daughter of carrier 2 | $sIgM^+$ | 66 | 6 | 2 | 5 | 10 | −1.13 |
| | $sIgM^-$ | 2 | 49 | 4 | 14 | 0 | 4.21 |

*Cytoplasmic immunofluorescence staining was done on the day the B-lineage cells were fused to the Chinese hamster cell line RJK88.

†Maximum likelihood ratio test was used to calculate lod (logarithm of odds) scores as previously described (4). A lod score greater than 3.0 indicates significant skewing; a lod score less than 1.5 indicates no evidence of skewed inactivation.

The finding of random X inactivation in the hybrids from the $sIgM^+$ B cells rules out the possibility that the XSCID gene defect is expressed only in a precursor common to both T cells and B cells. If the gene defect were expressed only in a precursor, then all cells beyond this early stage of differentiation should demonstrate the same degree of skewing. The observation that hybrids from $sIgM^+$ B cells exhibit random X chromosome inactivation supports the hypothesis that the XSCID gene defect results in a selective disadvantage of B cells at several stages of differentiation so that there may be progressively more skewing as B cells from the obligate carrier proliferate and differentiate.

The observation that B cells from affected patients can be stimulated to synthesize and secrete immunoglobulin both *in vitro* when cultured with normal T cells and mitogen and *in vivo* after bone marrow transplant and successful T-cell engraftment indicates that the B-cell defect can be at least partially overcome. A similar situation is seen in HPRT deficiency, the X-linked disorder known as Lesch–Nyhan syndrome. T cells from obligate carriers of this disorder demonstrate selective use of the nonmutant X as the active X (32), indicating that the gene for HPRT is expressed in T cells and that a defect in this gene results in a selective disadvantage for these cells. However, boys with HPRT deficiency do not have demonstrable T-cell abnormalities (33), suggesting that the presence of a normal HPRT gene is not a prerequisite for T-cell proliferation or function. Even a minor advantage in either cell survival or proliferation of one cell population over another will allow the first cell population to predominate after multiple rounds of cell replication.

Animal models may also be helpful in interpreting the data in this paper. The CBA/N mouse has an X-linked defect (*xid*) resulting in decreased B-cell numbers, an apparent lack of a mature population of B cells, and poor antibody production to certain T-independent antigens that usually elicit an IgM or IgG3 antibody response (34, 35). Analysis of patterns of X chromosome inactivation in female mice carrying this disorder indicates that there is preferential use of the normal X chromosome as the active X in all B cells and not just in the B cells that bear mature markers or give rise to IgG3-producing cells (36, 37). Bone marrow from CBA/N mice is defective in its ability to reconstitute all B-cell populations in an irradiated host (38). Further, if a mouse not only carries the *xid* gene defect but is also homozygous for the gene defect that gives rise to the athymic nude mouse (*nu/nu*), then this doubly affected mouse will have much more profound B-cell defects than are usually seen in mice that carry the *xid* defect alone. Mice that express both the *xid* and the *nu/nu* mutations have almost no B cells and have less than 10% of the normal concentrations of serum immunoglobulins (39, 40). Taken together, these observations suggest that the *xid* gene defect is intrinsic to the B-cell lineage, affects all mature B cells, and can be partially overcome in the presence of normal T cells.

Although it is not clear that all cases of XSCID represent defects at the same gene locus, our studies suggest a consistent pattern in obligate carriers from eight kindreds. The heterogeneity that occurs in the degree of skewing of X chromosome inactivation in B cells is seen within families as well as between families. To determine whether XSCID represents a single gene disorder, it will be necessary to do mapping studies to ascertain if the gene defect maps to the same locus in all families.

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