STRAIN VARIATION IN THE FREQUENCY OF ABELSON MURINE LEUKEMIA VIRUS-TRANSFORMED FETAL LIVER PRE-B CELLS BEARING COMPLETE IMMUNOGLOBULIN HEAVY CHAIN REARRANGEMENTS

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The genes that encode the murine IgH chain variable region are encoded by three clusters of germline DNA segments; variable segments (V_H), diversity segments (D_H), and joining segments (J_H) (1). A complete VDJ_H variable region gene is assembled by somatic rearrangement during the differentiation of B cells: D_H to J_H joinings occur first on both chromosomes, followed by V_H to DJ_H rearrangements (2).

Abelson murine leukemia virus (A-MuLV),[1] a replication defective retrovirus, has the unique property of transforming very early progenitors of the B cell lineage in vitro. These progenitors can be found in fetal liver, adult bone marrow, and spleen (3). A-MuLV-transformed pre-B cell lines have provided a very useful model system for the study of early B cell development and Ig gene rearrangements. For example, Alt and coworkers (2) observed that the vast majority of Abelson fetal liver transformants analyzed (9 of 11) are initially DJ_H/DJ_H and continue to assemble their IgH genes in culture. In contrast, the predominant phenotype among Abelson bone marrow-derived pre-B cell lines (14 of 16) exhibits VDJ_H rearrangement on at least one allele. These findings led the authors to conclude that while fetal liver-derived transformants generally represent the earliest defined B cell progenitors (pre pre-B cell, cytoplasmic μ-negative), transformants isolated from adult bone marrow usually represent a more mature pre-B cell phenotype (pre-B cell, cytoplasmic μ-positive). Moreover, a third A-MuLV transformant phenotype has also been identified in bone marrow of CBA/Tufts and BALB/c mice independently by our group and others (Ramakrishnan, L., and N. Rosenberg, personal communication). These bone marrow A-MuLV transformants have a DJ_H/DJ_H configuration but do not rearrange V_H gene segments in culture.

It is not known why fetal liver transformants are usually DJ_H/DJ_H rearranging while bone marrow transformants have a more mature phenotype. One possibility is that the fetal liver environment per se, determines the frequency of the IgH rear-

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[1] *Abbreviation used in this paper:* A-MuLV, Abelson murine leukemia virus.

rangement phenotypes (DJ_H vs. VDJ_H). We have recently generated A-MuLV pre-B cell lines from fetal livers of our two congenic mouse strains, CBA/Tufts and CBA/Tufts.*xid*, to examine the effect of the X-linked immune deficiency (*xid*) mutation on the V_H repertoire. While analyzing IgH rearrangements in both sets of cell lines, we were able to address this issue.

Materials and Methods

Mice. C57BL/10J mice were purchased from The Jackson Laboratory, Bar Harbor, ME. BALB/c.Ann and BALB/c.Ann.*xid* congenic strains were obtained from Dr. Carl Hansen, The Small Animal Section at the National Institutes of Health, Bethesda, MD. The CBA/Tufts.*xid* mouse strain was constructed by using the CBA/N as the donor of the *xid* locus (3) and was maintained as inbred stock at Tufts University School of Medicine, Boston, MA.

Cell Lines. Pre-B cell lines were made from fetal livers taken on day 16–17 of gestation using the vaginal plug date as day 1. Individual fetal livers were transformed in vitro with Abelson virus (4). Fetuses were sexed at the time of dissection and the sex was confirmed by hybridizing DNA from cell lines with a Y chromosome-specific probe (pY2) (5).

Southern Blots. Genomic DNA preparations were digested with Eco RI and electrophoresed through 0.7% agarose gels. Fractionated DNA was then blotted onto Nytran filters (Schleicher & Schuell, Inc., Keene, NH). DNA probes were labeled by random primer labeling (Boehringer Manheim Biochemicals, Indianapolis, IN) and the blots were hybridized and washed as previously described (6). Rearrangements of IgH loci were detected with a 1.9-kbp Bam HI/Eco RI fragment isolated from pJ_{11} (7). This probe contains J_H3, J_H4, and 3′ flanking sequence, and it detects rearrangement to all four J_H segments. To distinguish DJ_H from VDJ_H rearrangements, we used a mixture of ~3.0-kbp Eco RI/Bgl II 5′ D_HFL16 and ~3.3-kbp Eco RI/Bgl II 5′ D_HSP2 (2) fragments as a probe.

Results and Discussion

Frequency of A-MuLV Target Cells in CBA/Tufts and CBA/Tufts.xid Fetal Livers. We have previously reported that the *xid* gene does not alter the frequency of Abelson virus target cells in adult bone marrow (8). We have now determined the frequency of A-MuLV target cells in the fetal livers of CBA/Tufts and CBA/Tufts.*xid* mice to be 19.8 ± 3.4 and 19.5 ± 2.0 foci/10^6 nucleated cells, respectively. Therefore, the introduction of the *xid* mutation did not alter the frequency of Abelson target cells in the fetal liver of the CBA/Tufts strain. In addition, we found no difference in A-MuLV transformation frequencies between fetal liver cells from BALB/cAnn and BALB/cAnn.*xid*, both frequencies being about fourfold greater than those of the two CBA strains (Table I). We conclude, therefore, that while the frequency of A-MuLV targets varies between strains, as previously reported (4), target cell frequency is not affected by the *xid* mutation.

IgH Rearrangements in A-MuLV Pre-B Cell Lines Obtained from Fetal Livers. To prove that the cell lines were of clonal origin, we took advantage of the fact that the A-MuLV genome is not cleaved by Eco RI and the viral sequence is seen in the context of unique flanking cellular DNA (9). Southern blot analysis using an Abelson virus probe (*p v-abl*) revealed that each CBA/Tufts fetal liver cell line had a single fragment of unique size and therefore is clonal (data not shown). Using a J_H probe, each cell line exhibits a unique pattern of fragments on a Southern blot, two or three strongly hybridizing bands, and multiple bands with varying densities (Fig. 1, lane *1* in each cell line). Similar multiple J_H hybridizing fragments were also observed for fetal liver pre-B cell lines derived from BALB/cAnn mice (data not shown). This pattern resembles that previously reported for fetal liver A-MuLV-transformed pre-B cells

TABLE I

Strain Comparison of IgH Locus Rearrangements in Fetal Liver A-MuLV Transformants

Exp.	Strain	Sex	Foci*	Number of cell lines DJ‡	VDJ§
1	CBA/Tufts	ND	19.8 ± 3.4	9	3
2	CBA/Tufts.*xid*	ND	19.5 ± 2	3	9
3	BALB/c.Ann	ND	89.7 ± 10	14	1
4	BALB/c.Ann.*xid*	ND	90.3 ± 11.6	14	1
5	(BALB/c.*xid* x B10)	F_1♂	91 ± 0.9	10	2
		F_1♀	100.5 ± 13.9	11	0
6	(CBA/Tufts.*xid* x CBA/Tufts)	F_1♂	19 ± 1.8	2	12
		F_1♀	18.5 ± 1.3	0	12

* The frequency of A-MuLV target cells was determined as previously reported (4). Each number represents the mean ± SD of three different experiments.

‡ As described in the text, cell lines with DJ_H rearrangement showed two to three strongly hybridizing bands and multiple bands with varying densities using a J_H probe. Two (or more) of these fragments hybridized to a 5′ D_H as well.

§ Cell lines designated "VDJ" exhibit only two strongly hybridizing Eco RI fragments uusing a J_H probe and lack the multiple, faintly hybridizing fragments associated with active in vitro IgH locus rearrangements (2). Each cell line has at least one complete VDJ rearrangement; i.e., one that hybridizes to the J_H probe but not with the 5′ D_H probe.

(10) and has been shown to result from in vitro V_H to DJ_H rearrangements (2). In contrast, most cell lines (9 of 12) derived from CBA/Tufts.*xid* fetal livers have stably rearranged IgH loci; i.e., each has two fragments that strongly hybridize to the J_H probe and lack the multiple hybridizing fragments associated with active in vitro IgH rearrangement (Fig. 2, lane *1* in F10, 1C3, F5, 1B3, 1A3, 2B2, F12, F15, F20). This finding raises the following questions: Do CBA/Tufts.*xid* derived cell lines have a DJ_H/DJ_H configuration and yet fail to rearrange their heavy chain genes in culture or do they have VDJ_H rearrangements? To distinguish a DJ_H from VDJ_H rearrangement we used a mixture of 5′ flanking D_HFL16 and D_HSP2 probes. Only the DJ_H type of rearrangement with intact 5′ flanking sequences will hybridize to the mixture of 5′ flanking D_H probes. As shown in Fig. 2 (lane *2* in each cell line), all pre-B cell lines derived from CBA/Tufts.*xid* fetal livers are VDJ_H on at least one allele. This observation was confirmed by using the V_H81X probe (11), which detects the most D_H-proximal V_H gene family (V_H7183). As illustrated in Fig. 2, cell lines with VDJ_H rearrangements on both chromosomes either partially or completely deleted this family (Fig. 2, lane *3*, 1C3, 1B3, F12, 1A3, 2B2, and F20). By contrast, most fetal liver CBA/Tufts-derived cell lines (9 of 12) exhibited continuing rearrangement and were positive for hybridization to the 5′ D_H probe. These cell lines usually contain two (or more) Eco RI fragments that hybridized to both the 5′ D_H- and J_H-specific probes (Fig. 1, 2A1, 5B6, F35, 6A3, 2D5, 7C1, 7B5, 7B6, 6B6), and are therefore DJ_H/DJ_H.

Genetic Analyses of A-MuLV Target Cells in Different Mouse Strains. To determine whether the VDJ_H A-MuLV pre-B cells found in CBA/Tufts.*xid* fetal livers resulted from the expression of the *xid* mutation, or an *xid*-linked gene, several additional panels of

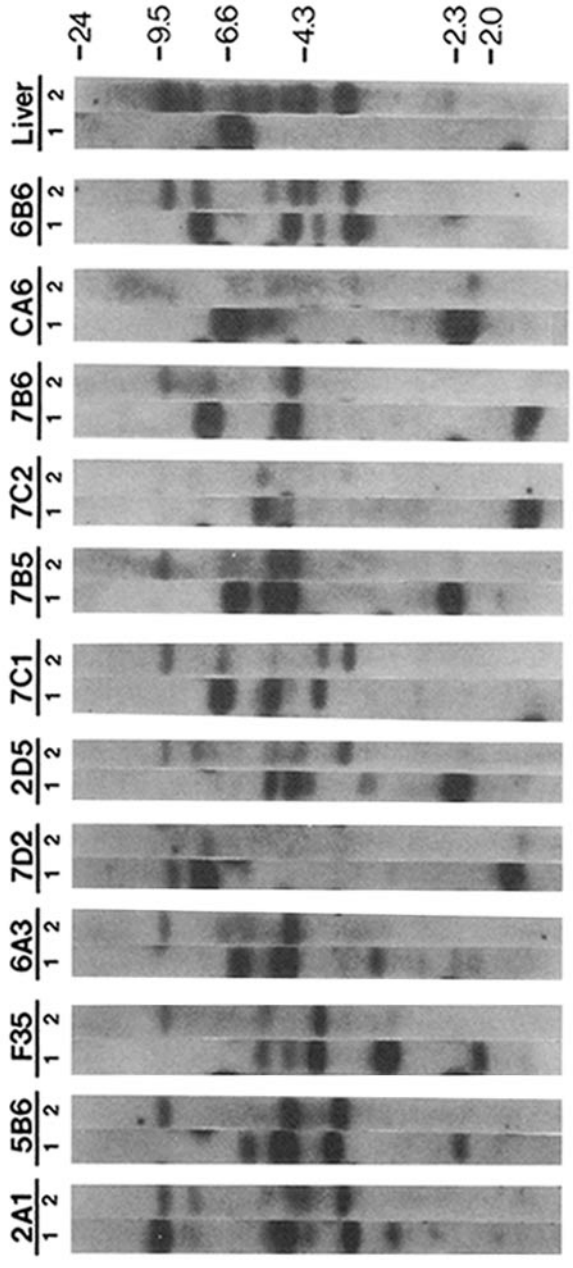

FIGURE 1. Assignment of DJ_H and VDJ_H rearrangements in A-MuLV transformants from CBA/Tufts fetal livers. Approximately 10 μg of Eco RI-digested DNA from each cell line was electrophoresed through a 0.7% agarose gel, transferred to Nytran membrane, and hybridized with labeled J_H probe (lane *1*). After autoradiography, the probe was removed (0.4 N NaOH, 42°C for 30 min). Probe removal was confirmed by autoradiography for 3 d, −80°C with intensifying screen. The Southern blot filter was then hybridized with labeled 5′ D_H probe (lane *2*).

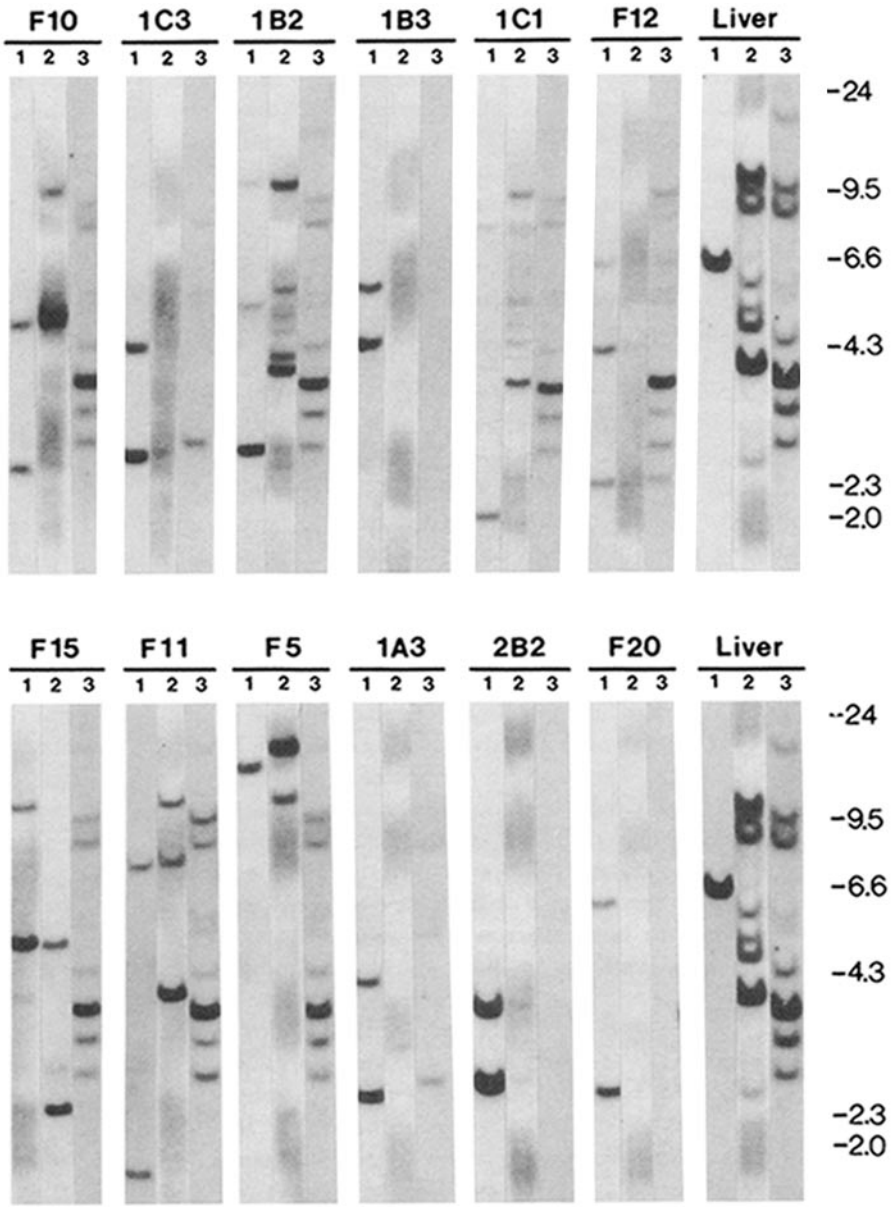

FIGURE 2. Assignment of DJ_H and VDJ_H rearrangements in A-MuLV transformants from CBA/Tufts.*xid* fetal livers. Southern blot was hybridized with J_H (lane *1*), 5′ D_H (lane *2*), or V_H81X probe (lane *3*). See Fig. 1 legend for details. Partial or complete deletion of members of the V_H7183 family (the most D_H-proximal V_H gene family) indicates that a given cell line exhibits VDJ_H rearrangements on both chromosomes (lane *3*: 1C3, 1B3, F12, 1A3, 2B2, and F20).

transformants were generated and analyzed. As shown in Table I, fetal liver pre-B cell lines derived from either CBA/Tufts.*xid* or (CBA/Tufts.*xid* × CBA/Tufts)F_1 mice are VDJ_H on at least one allele at the initial stage of culture. In contrast, transformants obtained from BALB/c.Ann.*xid* initially have DJ_H/DJ_H rearrangements and continue to assemble complete VDJ_H rearrangements in culture. Thus, for *xid* to be involved, BALB/cAnn.*xid* mice must carry a gene (or genes) that inhibits this manifestation of the *xid* phenotype. Furthermore, for *xid* or any X-linked gene to be involved, our panel of pre-B cell lines from (CBA/Tufts.*xid* × CBA/Tufts)F_1 female fetuses must, by chance, not include any cell lines with an active paternal (CBA/Tufts) X-chromosome. We believe that these explanations are unlikely, since, in general B cells with a normal X-chromosome have a selective growth advantage in vivo over cells expressing the *xid* gene (12–14). Taken together, our data suggest that the generation of fetal liver pre-B cell lines having a mature, VDJ_H phenotype is not due to *xid* or any other X-linked gene derived from the CBA/Tufts.*xid*. We have also considered the possible influence of a maternal effect of CBA/Tufts.*xid* mothers. However, there is no precedent for such an effect. Therefore, we favor the simplest explanation, that is CBA/Tufts.*xid* mice carry an autosomal dominant gene(s) and the donor was the CBA/N mouse strain that was originally used as a donor of the *xid* locus (3). Therefore, this dominant gene (at least one) is responsible for the VDJ_H bearing pre-B cell lines found in the fetal livers of (CBA/Tufts.*xid* × CBA/Tufts)F_1 mice. However, we do not know whether this gene (at least one) affects the intrinsic development of B cells or the fetal microenvironment, expanding pre-B cells of the "more mature" VDJ_H phenotype.

The influence of the microenvironment has been studied in vitro by Denis et al. (15) by using bone marrow stromal adherent cells and fetal liver nonadherent cells. They failed to obtain any continuously rearranging A-MuLV transformants from long-term fetal cultures. Therefore, it is possible that the bone marrow microenvironment influenced the differentiation of the early fetal liver B cell lineage. Together, these findings suggest that the microenvironment may influence the phenotype of A-MuLV transformants. Accordingly, it is possible that the dominant gene(s) that we identified increases the rate of B cell development and, in most strains, may only be expressed in the bone marrow.

Analyses of V_H gene family usage have revealed a preferential utilization of the V_H7183 gene family among Abelson fetal liver transformants (98% of the VDJ_H alleles analyzed) (11) as well as fetal liver pre-B cell hybridomas (78%) (16). On the other hand, studies performed with cell lines and hybridomas derived from adult bone marrow indicated that the V_H7183 gene family is much less frequently used. For instance, Yancopoulos et al. (11) reported that while 38% of the VDJ_H alleles of bone marrow Abelson transformants rearranged members of the V_H7183 family, the remaining 62% used V_H gene segments from a variety of other families. Similarly, Yoshida et al. (17) established pre-B cell hybridomas from Whitlock-Witte long-term bone marrow culture and documented that 33% used the V_H7183 family (17). In contrast to most adult bone marrow–derived lines, there are two unusual but well-studied pre-B cell lines, an NIH/Swiss bone marrow–derived line (300-19) and a BALB/c neonatal spleen–derived line (AT11-2), which continue to rearrange their IgH loci in culture. The majority of subclones obtained from both of these lines have rearranged members of the V_HQ52 gene family (18, 19). Interpretation of the

preferential utilization of the most D_H-proximal V_H gene segments, V_H7183 and V_HQ52 family members by actively rearranging pre-B cell lines has been clouded by the possibility that such restricted V_H gene segments usage is an in vitro phenomenon, since the V_H genes analyzed were assembled in culture.

Despite the profound effects of the autosomal dominant gene(s) on the development of B cells in the fetal liver, we have not found an alteration in V_H gene usage. The majority of A-MuLV transformants derived from (CBA/Tufts.*xid* × CBA/Tufts)F_1 females use the most D_H-proximal V_H gene families (V_H7183 and V_HQ52) (data to be published elsewhere). In addition, the majority of A-MuLV transformants and subclones obtained from fetal livers of CBA/Tufts mice also use the same restricted V_H gene families (V_H7183 and V_HQ52). Therefore, we believe that fetal liver pre-B cells, both with DJ_H/DJ_H and VDJ_H/DJ_H rearrangements, may belong to a distinct early B cell lineage that preferentially uses the most D_H-proximal V_H7183-V_HQ52 gene families.

Summary

Fetal liver Abelson pre-B cell lines obtained from CBA/Tufts.*xid* and (CBA/Tufts.*xid* × CBA/Tufts)F_1 mice have complete VDJ_H rearrangements on at least one allele. Such high frequencies of VDJ_H rearrangements have previously been observed in adult derived but not fetal liver derived Abelson pre-B cell lines. Genetic analyses suggest that CBA/Tufts.*xid* carries an autosomal dominant gene(s) that determines the predominance of VDJ_H rearrangements among transformants. This autosomal gene(s) might affect the intrinsic development of the early B cell lineage in the fetus or the fetal microenvironment, expanding pre-B cells of the "more mature" VDJ_H phenotype.

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References

1. Tonegawa, S. 1983. Somatic generation of antibody diversity. *Nature (Lond.)* 302:575.
2. Alt, F., G. Yancopoulos, T. Blackwell, C. Wood, E. Thomas, M. Boss, R. Coffman, N. Rosenberg, S. Tonegawa, and D. Baltimore. 1984. Ordered rearrangement of immunoglobulin heavy chain variable region segments. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:1209.
3. Wortis, 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.
4. Rosenberg, N., and D. Baltimore. 1976. A quantitative assay for transformation of bone marrow cells by Abelson murine leukemia virus. *J. Exp. Med.* 143:1453.
5. Lamar, E., and E. Palmer. 1984. Y-Encoded, species-specific DNA in mice: Evidence that the Y chromosome exists in two polymorphic forms in inbred strains. *Cell.* 37:171.
6. Brodeur, P., and R. Riblet. 1984. The immunoglobulin heavy chain variable region (IgH-V) in the mouse. I. One hundred IgH-V genes comprise seven families of homologous genes. *Eur. J. Immunol.* 14:922.

7. Marcu, K., J. Banerji, N. Penncavage, R. Lang, and N. Arnheim. 1980. 5' Flanking region of immunoglobulin heavy constant region genes displays length heterogeneity in germlines of inbred mouse strains. *Cell.* 22:187.
8. Karagogeos, D., N. Rosenberg, and H. Wortis. 1986. Early arrest of B cell development in nude, X-linked immune-deficient mice. *Eur. J. Immunol.* 16:1125.
9. Goff, S., E. Gilboa, O. Witte, and D. Baltimore. 1980. Structure of the Abelson murine leukemia virus genome and the homologous cellular gene: Studies with cloned viral DNA. *Cell.* 22:777.
10. Alt, F., N. Rosenberg, S. Lewis, E. Thomas, and D. Baltimore. 1981. Organization and reorganization of immunoglobulin genes in A-MuLV transformed cells: rearrangement of heavy chain but not light chain genes. *Cell.* 27:381.
11. Yancopoulos, G., S. Desiderio, M. Paskind, J. Kearney, D. Baltimore, and F. Alt. 1984. Preferential utilization of the most J_H-proximal V_H gene segments in pre-B-cell lines. *Nature (Lond.).* 311:727.
12. Naham, M., J. Paslay, and J. Davie. 1983. Unbalanced X-chromosome mosaicism in B cells of mice with X-linked immunodeficiency. *J. Exp. Med.* 158:319.
13. Sprent, J., and J. Bruce. 1984. Physiology of B cells in mice with X-linked immunodeficiency (*xid*). III. Disappearance of *xid* B cells in double bone marrow chimeras. *J. Exp. Med.* 160:711.
14. Forrester, L., J. Ansell, and H. Micklem. 1987. Development of B lymphocytes in mice heterozygous for the X-linked immunodeficiency (*xid*) mutation. *xid* inhibits development of all splenic and lymph node B cells at at stage subsequent to their initial formation in bone marrow. *J. Exp. Med.* 165:949.
15. Denis, K., L. Treiman, J. Claire, and O. Witte. 1984. Long-term cultures of murine fetal liver retain very early B lymphoid phenotype. *J. Exp. Med.* 160:1087.
16. Perlmutter, R., J. Kearney, S. Chang, and L. Hood. 1985. Developmentally controlled expression of immunoglobulin V_H genes. *Science (Wash. DC).* 227:1597.
17. Yoshida, N., A. Radbruch, and K. Rajewsky. 1987. Ig gene rearrangement and expression in the progeny of B-cell progenitors in the course of clonal expansion in bone marrow cultures. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:2735.
18. Reth, M., S. Jackson, and F. Alt. 1986. V_HDJ_H formation and DJ_H replacement during pre-B differentiation: non-random usage of gene segments. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:2131.
19. Sugiyama, H., T. Maeda, Y. Tani, S. Miyake, Y. Oka, T. Komori, H. Ogawa, T. Soma, Y. Minami, N. Sakato, and S. Kishimoto. 1987. Selective use of the V_HQ52 family in functional V_H to DJ_H rearrangements in a B precursor cell line. *J. Exp. Med.* 166:607.