Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 2454–2458, April 1987
Immunology

Adult B-cell repertoire is biased toward two heavy-chain variable-region genes that rearrange frequently in fetal pre-B cells

(immunoglobulin variable gene/subfamily/ontogeny/developmental regulation)

ANN M. LAWLER, PAUL S. LIN, AND PATRICIA J. GEARHART

Department of Biochemistry, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD 21205

Communicated by David R. Davies, December 22, 1986 (received for review September 9, 1986)

ABSTRACT **Fetal pre-B cells rearrange a very restricted set of immunoglobulin variable genes for the heavy chain (V_H). To determine whether the adult B-cell repertoire is similarly skewed, we first identified the genes that rearrange in pre-B cells from BALB/c mice and then determined their frequency of rearrangement in adult B cells. In fetal pre-B cell lines, two genes, V_H81X from the 7183 subfamily and V_HOx2 from the Q52 subfamily, comprise 75% of the rearranged alleles of an estimated 1000 genes ($P < 0.001$). Sequencing analyses revealed that rearrangements involving the two genes were both productive and nonproductive. The biased rearrangement of these two V_H genes persists in B-cell hybridomas from adult mice at a frequency of 22%, as determined by Southern gel analysis and RNA sequencing. The sequence of one V_HOx2 rearrangement from a hybridoma shows that the rearrangement is productive, suggesting that the gene encodes an antibody that could participate in the immune response. The data indicate that the adult B-cell repertoire is not random concerning usage of individual V_H genes, and it may be shaped by the unknown mechanisms that cause preferential rearrangement of certain genes early in ontogeny.**

Of some 1000 genes (1) encompassing nine known subfamilies (2–4) that encode murine immunoglobulin variable regions for the heavy chain (V_H), only a few genes are rearranged during early ontogeny. For example, six genes in the 7183 subfamily have been found rearranged in >50% of pre-B-cell lines made from fetal livers of BALB/c mice (refs. 5 and 6; this paper). This bias in gene rearrangement may be the cause of the programmed appearance of certain B cells during ontogeny (7).

The high proportion of fetal pre-B cells with preferential rearrangements suggested that B cells in the adult spleen might also be biased toward the same rearrangements. We first sequenced the V_H genes that rearrange in fetal pre-B cells, and then we determined their frequency of rearrangement in a population of splenic B-cell hybridomas. The results reveal a striking bias toward the rearrangement of two genes, V_H81X from the 7183 subfamily and V_HOx2 from the Q52 subfamily, in both fetal pre-B cells and adult B cells, indicating that the adult repertoire is not stochastic concerning usage of individual V_H genes.

MATERIALS AND METHODS

Cell Lines. Pre-B-cell lines were made from fetal livers of pregnant BALB/cJ mice (The Jackson Laboratory). Livers were taken during mid-gestation using the vaginal plug date as day 0. Pre-B cells were transformed with Abelson virus (8); cell lines BFL2, BFL6, BFL9, and BFL23 were obtained from Jacqueline Pierce (National Institutes of Health, Bethesda, MD). Each cell line was derived from an individual fetus taken from an individual mother. Hybridoma cell lines (provided by N. Levy) were made from spleen cells stimulated with the lipopolysaccharide (LPS) mitogen by fusing the cells 2–9 days after stimulation with the SP2/0 cell line. The phosphocholine-specific (*P*Cho) hybridomas have been described (9).

Southern Blots. Genomic DNA was digested with *Eco*RI and analyzed by Southern blots. DNA probes were labeled by random primer labeling (10), and the blots were washed according to Brodeur and Riblet (2) with a final high stringency wash at 65°C with 0.2× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate)/0.1% NaDodSO$_4$. Rearrangements of joining genes for the heavy chain (J_H) were detected with a 2.0-kilobase-pair (kbp) *Bam*HI/*Eco*RI probe containing J_H3 and J_H4, which detects rearrangement to all four J_H genes. Rearrangements of variable genes were detected with the following probes: the 81X probe (7183 subfamily) was an *Eco*RI/*Pst* I fragment from a germ-line gene that contains 210 bp of coding and 48 bp of 3′ noncoding sequences (provided by S. Desiderio and F. Alt; ref. 5); the Ox2 probe (Q52 subfamily) was an *Rsa* I fragment that contains 400 bp of 5′ noncoding and 100 bp of coding sequence (Fig. 1); and the S107 probe (S107 subfamily) was a cDNA clone that contains 300 bp of coding sequence (provided by U. Malipiero).

Cloning and Sequencing. The rearranged genes were cloned into λ Charon 28 vectors, restriction site maps were determined (Fig. 1), and the genes were subcloned into M13 virus for sequencing by the dideoxy method. Sequences were determined with J_H primers complementary to the last 20 nucleotides of each J_H gene (provided by L. Hood), and an 18-bp primer homologous to amino acid residues 43–48 of the V_HOx2 and $V_H23.9$ genes. Sequencing of mRNA by the primer extension method was carried out as described (11).

RESULTS

Biased Rearrangement of V_H81X and V_HOx2 in Pre-B Cells. To identify the genes that are rearranged in pre-B cells, we analyzed cells from fetal livers taken in mid-gestation, which is an early population of cells that may be in synchronous development. Pre-B cells were transformed with Abelson virus to generate seven cell lines. Fourteen rearranged heavy-chain genes were cloned and sequenced, and three were identified by Southern blots (Table 1 and Fig. 2). The sequences were compared to the nine known subfamilies of V_H genes (2–4) to identify the genes. No mutation in the V_H, diversity (D), or J_H gene segments was observed when the sequences could be directly compared to their germ-line counterparts.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: V_H, variable gene for heavy chain; D, diversity; J_H, joining gene for heavy chain; LPS, lipopolysaccharide; *P*Cho, phosphocholine.

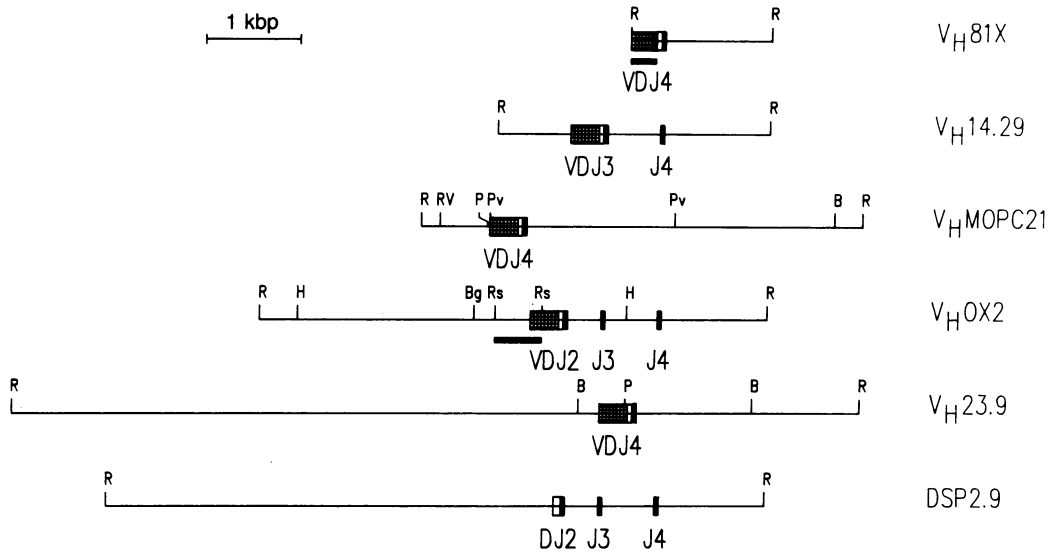

FIG. 1. Restriction maps of rearranged genes in fetal pre-B cells. Secondary deletions or rearrangements appear to have occurred on the 5′ and 3′ sides of $V_HMOPC21$ and on the 3′ side of $V_H23.9$. Solid bars below V_H81X and V_HOx2 depict fragments that were used as probes in the Southern blots in Fig. 3. R, *Eco*RI; RV, *Eco*RV; P, *Pst* I; Pv, *Pvu* II; B, *Bam*HI; H, *Hin*dIII; Bg, *Bgl* II; and Rs, *Rsa* I. Cross-hatched box, *V* gene; open box, *D* gene; solid box, *J* gene.

Table 1. Repertoire of rearranged V_H, *D*, and J_H genes in fetal pre-B cells

Cell line	*Eco*RI size, kbp	V_H	N*	*D*	N*	J_H	Type
BFL14	4.3	No *V*		*SP2.8*		*4*	DJ
(14)	2.9	*14.29*		*SP2.8*		*3*	VDJ−
	2.0	[*81X*]		ND		*3*	VDJ−
	4.9	*Ox2*		*SP2.5*, *-.7*, or *-.8*		*3*	VDJ−
BFL1	4.8	*MOPC21*	2	*SP2.5*, *-.7*, or *-.8*	3	*4*	VDJ−
(15)	1.5	[*81X*]		ND		*4*	VDJ+
BFL16	7.0	No *V*		*SP2.9*		*2*	DJ
(16)	5.4	*Ox2*		*FL16.1*		*2*	VDJ+
BFL2	7.0	No *V*		*SP2.9*		*2*	DJ
(17)	5.4	*Ox2*	2	*Q52*	2	*2*	VDJ−
	1.5	[*81X*]		ND		*4*	VDJ−
BFL6	5.4	No *V*		*FL16.1*	2	*2*	DJ
(17)	4.9	*Ox2*	7	*FL16.1* or *-.2*		*3*	VDJ−
BFL9	4.4	No *V*		*SP2.8*		*4*	DJ
(17)	2.0	*81X*		*SP2.5*, *-.7*, or *-.8*		*3*	VDJ+
BFL23	9.1	*23.9*	6	*FL16.1*		*4*	VDJ−
(17)	1.5	*81X*	3	*SP2.5*, *-.7*, or *-.8*		*4*	VDJ+

The four rearrangements in BFL14 and three rearrangements in BFL2 may be due to continued rearrangement in culture. In the three cases in brackets, V_H81X was identified by size on a Southern blot and hybridization to V_H81X and J_H probes; D segment and N region information is not available in these examples. ND, not determined. For several VDJ joins, the D segment was too short to be uniquely identified. The μ heavy-chain protein was detected by radioimmunoassay in the cytoplasm of the cell lines with productive VDJ rearrangements (VDJ+) as determined by sequencing. Incomplete rearrangements are designated DJ, and nonproductive rearrangements are called VDJ−. Six of the cell lines had unrearranged κ light-chain genes, and one line, BFL14, had one nonproductively rearranged κ allele and one germ-line allele. Numbers in parentheses designate day of gestation.

*Number of nucleotides in the N region (12).

The data reveal an extraordinary bias for the rearrangement of two genes, V_H81X of the 7183 subfamily and V_HOx2 of the Q52 subfamily. Five of 12 rearrangements utilized V_H81X, and four of 12 utilized V_HOx2 in both productive and nonproductive rearrangements (Table 1). These two genes therefore comprise 75% of the rearranged V_H genes, of a potential 1000 genes, which is highly significant compared to a random distribution ($P < 0.001$). The preferential use of V_H81X does not appear to be due to selection of certain B cells by Abelson virus because similar high frequencies have been reported in pre-B cells transformed by hybridoma fusion (5, 6) and by hybridization of the gene to mRNA from fetal livers (14). The V_HOx2 sequence (Fig. 2) is identical to a gene product in the anti-oxazalone antibody response (15) and is very similar to the V_H101 gene (16, 17). Three rearrangements involving V_H81X and one using V_HOx2 were productive and likely encode for the μ heavy-chain protein that was detected by radioimmunoassay. It is important to stress that the pre-B cells do not express light chains and therefore could not be selected by antigen.

The identification of other rearranged V_H genes in this population of pre-B cells indicated that genes in the 7183 subfamily have a very high probability of rearrangement, in accord with previous findings (5, 6). In addition to V_H81X, two genes, $V_H14.29$ and $V_HMOPC21$, were rearranged (Fig. 2). Thus, of 12 genes in this subfamily, 6 have been found rearranged in pre-B cells. In contrast, genes within the other eight subfamilies, with the exception of V_HOx2, have a low probability of rearrangement. This is most evident by the lack of rearrangement in the J558 subfamily, which contains close to 1000 genes (1) and therefore should have a very high probability of rearrangement. One gene, $V_H23.9$, from the upstream 3609 subfamily was detected in this analysis (Fig. 2).

A New *D* Gene in the *SP2* Gene Family. In the *D* and J_H gene families, certain genes were repeatedly rearranged in pre-B cells. The *D* gene segments *FL16.1*, *SP2.8*, and a new *D* gene, *SP2.9*, were frequently rearranged both in this analysis and in other studies (18–20). *SP2.9* appears to be a new *D* gene because it has a unique sequence (Fig. 2) in the coding region and the 5′ flanking sequence, which differs from the flanking sequence of other *SP2* genes by 2 of 170 nucleotides. The *J* gene segments J_H2, J_H3, and J_H4 were used equally, but J_H1

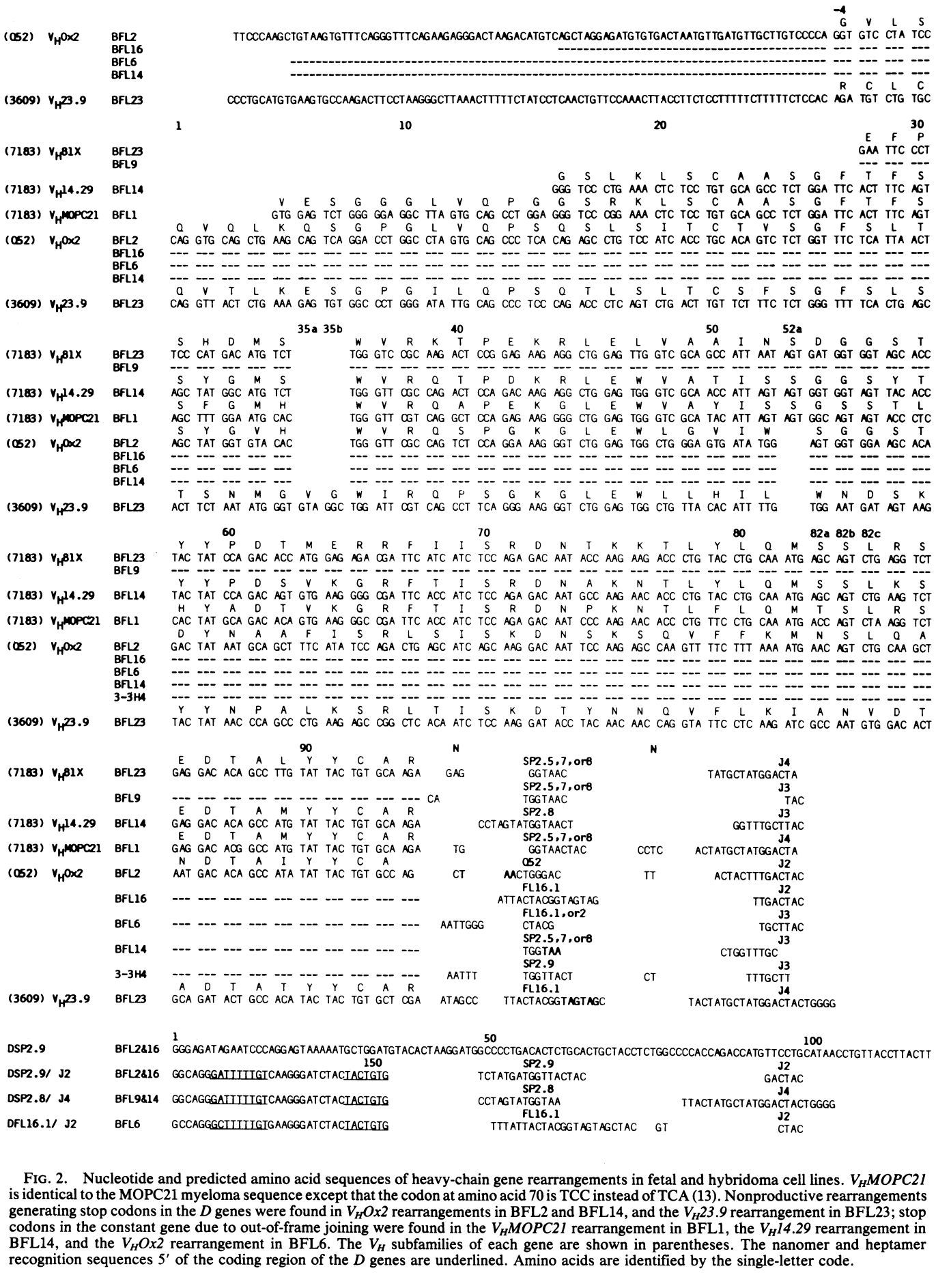

FIG. 2. Nucleotide and predicted amino acid sequences of heavy-chain gene rearrangements in fetal and hybridoma cell lines. *V_HMOPC21* is identical to the MOPC21 myeloma sequence except that the codon at amino acid 70 is TCC instead of TCA (13). Nonproductive rearrangements generating stop codons in the *D* genes were found in *V_HOx2* rearrangements in BFL2 and BFL14, and the *V_H23.9* rearrangement in BFL23; stop codons in the constant gene due to out-of-frame joining were found in the *V_HMOPC21* rearrangement in BFL1, the *V_H14.29* rearrangement in BFL14, and the *V_HOx2* rearrangement in BFL6. The V_H subfamilies of each gene are shown in parentheses. The nanomer and heptamer recognition sequences 5′ of the coding region of the *D* genes are underlined. Amino acids are identified by the single-letter code.

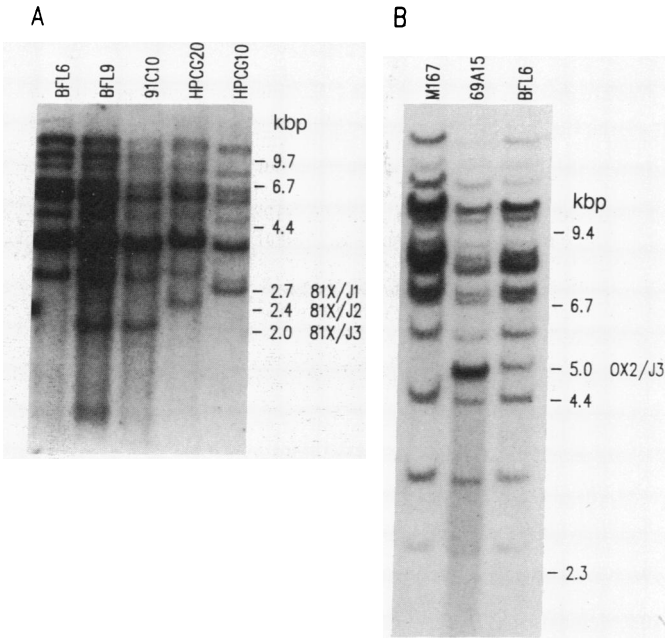

FIG. 3. Southern blot analysis of V_H81X (*A*) and V_HOx2 (*B*) rearrangements in B-cell hybridomas. (*A*) Genomic DNA was digested with *Eco*RI and hybridized with the V_H81X probe; (*B*) DNA was hybridized with the V_HOx2 probe. Gene assignments were made by size relative to the known position of the 5′ *Eco*RI site in the germ-line genes. Thus, rearrangement of V_H81X to J_H1 would produce a 2.7-kbp band; J_H2, 2.4 kbp; J_H3, 2.0 kbp; and J_H4, 1.5 kbp. Rearrangement of V_HOx2 to J_H1 would produce a 5.7-kbp band; J_H2, 5.4 kbp; J_H3, 5.0 kbp; and J_H4, 4.5 kbp. In HPCG20 and HPCG10, the appearance of the rearranged gene correlates with the loss of the 9.7-kbp band, which contains the germ-line V_H81X gene (5).

was not found in the 17 rearrangements reported here. A bias in the use of *J* genes from the κ light-chain locus in murine B cells has also been reported (21, 22).

Biased Rearrangement of V_H81X and V_HOx2 in Mature B Cells. The high proportion of fetal pre-B cells with V_H81X and V_HOx2 rearrangements suggested that these two genes may be found in a high frequency in mature B cells from adult mice. Indeed, there is evidence that the frequency of rearrangements involving V_H81X in pre-B cell lines from bone marrow is high, with 4 of 21 alleles (5) using the gene. To determine the frequency of rearrangement of these two genes in B cells from adult spleens, we performed Southern blot analyses on DNA from hybridoma cell lines and identified the rearranged genes by size with the appropriate *V* and *J* gene probes (Fig. 3). As a control, the frequency of rearrangement of the V_H11 gene in the S107 subfamily (23) was determined. Genomic DNA was obtained from the following B cells, which are summarized in Table 2: 20 hybridomas made from B cells that were stimulated with LPS, three plasmacytomas that did not bind to known antigens, and 12 hybridomas and plasmacytomas that made antibody to *P*Cho. In the latter case, only the nonproductive alleles were scored.

The results in Table 3 show preferential rearrangement of the V_H81X and V_HOx2 genes, with 5 of 36 chromosomal rearrangements using V_H81X, and 3 of 36 using V_HOx2. There was one rearrangement of V_H11, which could have occurred preferentially or by chance. The probability of observing a random rearrangement of V_H11 would be greater if <1000 genes have the potential to rearrange. Nonetheless, even if the number of genes that can rearrange is only 100, the observance of three repeated rearrangements of V_HOx2 and five of V_H81X is significant ($P < 0.006$ and $P < 0.00003$, respectively). The validity of the Southern blot analysis to detect rearranged genes was confirmed by sequencing the mRNA from the 33H4 cell line. The sequence, shown in Fig. 2, indicated that the rearranged gene was V_HOx2, which had rearranged productively to J_H3, as predicted. Two other cell lines with V_HOx2 rearrangements produced RNA that hybridized with the *Ox2* probe, suggesting that the rearranged gene is expressed.

Table 2. *Eco*RI size (kbp) of rearranged alleles in mature B-cell lines

Line	B-cell alleles	SP2/0 allele	Line	B-cell alleles	*P*Cho allele	SP2/0 allele
LPS lines			*P*Cho lines			
21B3	*2.4(81X)*	*5.5*	S107.3.4	*4.9, 2.0(81X)*	*7.3*	
21B51	*5.8*	*5.5*	MCPC603	*4.5, 4.1*	*7.3*	
21E6	*2.0*	*5.5*	MOPC167	*5.5*	*7.3*	
33B1	*18, 5.0*	*5.5*	HPCM2	*2.2*	*7.3*	*5.5*
33B4	*7.0*	*5.5*	HPCM25	*7.0*	*7.3*	
33C111	*9.0, 3.7(V11)*	*5.5*	HPCG10	*2.7(81X)**	*7.3*	
33F21	*4.2*	*5.5*	HPCG11	*4.8*	*7.3*	
33H4	*6.7, 5.0(Ox2)†*		HPCG14	*2.4*	*7.3*	
69A15	*5.9, 5.0(Ox2)‡*	*5.5*	HPCG17	*4.9*	*7.3*	*5.5*
	4.5		HPCG20	*2.4(81X)**	*7.3*	
69B10	*8.0*	*5.5*	HPCG22	*4.6*	*7.3*	
69B12	*4.5, 4.1*	*5.5*	HPCG32	*5.3*	*7.3*	
69C11	*7.4*	*5.5*				
610A2	*5.0*	*5.5*	Others			
610A51	*5.1*	*5.5*	45.6	*2.9*		
610D52	*5.1*	*5.5*	A202J	*4.3*		
610F61	*5.1*	*5.5*	SP2/0			*5.5*
610F65	*3.6*	*5.5*				
91C10	*2.0(81X)*	*5.5*				
92E8	*5.4(Ox2)‡*	*5.5*				
92G10	*4.7, 3.7*	*5.5*				

One allele in each LPS line is assumed to be a productive rearrangement (VDJ+), because the lines secreted immunoglobulin, as detected by radioimmunoassay, with the exception of 91C10.
* V_H81X assignment confirmed by loss of germ-line band (Fig. 3).
† V_HOx2 assignment confirmed by sequencing.
‡ RNA hybridized to a V_HOx2 probe.

Table 3. Biased use of two V_H genes in adult B cells

V_H gene	Pre-B cells from fetal liver	Pre-B cells from adult bone marrow	B-cell hybridomas from adult spleen*
81X	5/12	4/21†	5/36
Ox2	4/12	ND	3/36
V11	0/12	ND	1/36

Results are expressed as gene rearrangements per VDJ+ or VDJ− allele. ND, not done.

*The data were corrected for alleles with incomplete rearrangements containing no variable gene by assuming that 39% of the nonproductive alleles in functional B cells were incomplete DJ rearrangements (24).

†Data from ref. 5.

DISCUSSION

The data suggest that the adult B-cell repertoire is not random concerning usage of individual V_H genes and may be shaped by the unknown mechanisms that cause preferential rearrangement of certain genes. One mechanism may be the proximity of genes to the *D* and *J* gene cluster, since the 7183 subfamily is the most 3′ of the subfamilies (25), and V_H*81X* is one of the most 3′ genes in that family (5). The most 3′ variable gene encoding a chicken λ light chain also rearranges at a very high frequency (26). Although the position of V_H*Ox2* in BALB/c mice is not precisely known, it is 5′ of V_H*81X* and 3′ of the *S107* subfamily as determined by Southern blot analysis (unpublished data). It is possible that both genes are physically located near each other because genes in the Q52 and 7183 subfamilies are interspersed in other strains of mice (27, 28). Another mechanism may be the increased affinity of local nucleotide sequences around the genes for the recombinase enzyme. Indeed, one of the most intriguing aspects of these data is the repeated selection of V_H*81X* and V_H*Ox2* at a much higher frequency than their closely linked neighbor genes, which are 6 and 9 kbp away, respectively (5, 16). Thus, these two genes appear to be targeted for frequent rearrangement.

V_H genes continue to rearrange throughout the lifetime of an animal in pre-B cells from bone marrow, and biased rearrangement of V_H*81X* appears to occur in these cells as well (ref. 5; Table 3). The frequency may be lower in bone marrow than in fetal pre-B cells because the latter may be in a more synchronous stage of development. In addition, the lower frequency could be due to continued V_H gene replacement of the initial *V–D–J* join (28, 29). Preferential rearrangement of V_H*81X* and V_H*Ox2* in adult B-cell hybridomas therefore mirrors the bias in bone marrow pre-B cells. In at least one case, V_H*Ox2* rearranged productively in a hybridoma stimulated with LPS, suggesting that it encodes an antibody that could participate in the immune response. A function for biased rearrangements is not clear, as it is not known what antigens these two proteins bind, with the exception of the oxazalone hapten.

The persistence of V_H*81X* and V_H*Ox2* rearrangements in B cells from BALB/c adult mice is unexpected since some other genes and subfamilies have been reported to be expressed randomly in the A/J (30) and C57BL/6 (31) strains of mice. In strains such as BALB/c that show preferential rearrangement of genes during ontogeny, the repertoire may not become random in adult life. However, these mice will still be able to produce a heterogeneous immune response when antigen selects the B cells expressing the infrequently rearranged genes.

We are grateful to D. Martin and A. Quinn for technical help and to N. Levy, U. Malipiero, L. Hood, and S. Desiderio for critical comments. This work was supported by National Institutes of Health Grant CA34127 and March of Dimes Grant 1-965.

1. Livant, D., Blatt, C. & Hood, L. (1986) *Cell* **47**, 461–470.
2. Brodeur, P. H. & Riblet, R. (1984) *Eur. J. Immunol.* **14**, 922–930.
3. Dildrop, R. (1984) *Immunol. Today* **5**, 85–86.
4. Winter, E., Radbruch, A. & Krawinkel, U. (1985) *EMBO J.* **4**, 2861–2867.
5. Yancopoulos, G. D., Desiderio, S. V., Paskind, M., Kearney, J. F., Baltimore, D. & Alt, F. W. (1984) *Nature (London)* **311**, 727–733.
6. Perlmutter, R. M., Kearney, J. F., Chang, S. P. & Hood, L. E. (1985) *Science* **227**, 1597–1601.
7. Sigal, N. H., Gearhart, P. J., Press, J. L. & Klinman, N. R. (1976) *Nature (London)* **259**, 51–52.
8. Rosenberg, N. & Baltimore, D. (1976) *J. Exp. Med.* **143**, 1453–1463.
9. Gearhart, P. J., Johnson, N. D., Douglas, R. & Hood, L. (1981) *Nature (London)* **291**, 29–34.
10. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132**, 6–13.
11. Hamlyn, P. H., Brownlee, G. G., Cheng, C., Gait, M. J. & Milstein, C. (1978) *Cell* **15**, 1067–1075.
12. Alt, F. W. & Baltimore, D. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4118–4122.
13. Bothwell, A. L. M., Paskind, M., Reth, M., Imanishi-Kari, T., Rajewsky, K. & Baltimore, D. (1981) *Cell* **24**, 625–637.
14. Blackwell, T. K., Yancopoulos, G. D. & Alt, F. W. (1984) *UCLA Symp. Mol. Cell. Biol. New Ser.* **19**, 537–547.
15. Kaartinen, M., Gillian, G. M., Markham, A. F. & Milstein, C. (1983) *Nature (London)* **304**, 320–324.
16. Kataoka, T., Nikaido, T., Miyata, T., Moriwaki, K. & Honjo, T. (1982) *J. Biol. Chem.* **257**, 277–285.
17. Yaoita, Y., Matsunami, N., Choi, C. Y., Sugiyama, H., Kishimoto, T. & Honjo, T. (1983) *Nucleic Acids Res.* **11**, 7303–7316.
18. Reth, M. G. & Alt, F. W. (1984) *Nature (London)* **312**, 418–423.
19. Desiderio, S. V., Yancopoulos, G. D., Paskind, M., Thomas, E., Boss, M. A., Landau, N., Alt, F. W. & Baltimore, D. (1984) *Nature (London)* **311**, 752–755.
20. Hagiya, M., Davis, D. D., Takahashi, T., Okuda, K., Raschke, W. C. & Sakano, H. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 145–149.
21. Wood, D. L. & Coleclough, C. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 4756–4760.
22. Nishi, M., Kataoka, T. & Honjo, T. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6399–6403.
23. Crews, S., Griffin, J., Huang, H., Calame, K. & Hood, L. (1981) *Cell* **25**, 59–66.
24. Alt, F. W., Yancopoulos, G. D., Blackwell, T. K., Wood, C., Thomas, E., Boss, M., Coffman, R., Rosenberg, N., Tonegawa, S. & Baltimore, D. (1984) *EMBO J.* **3**, 1209–1219.
25. Brodeur, P. H., Thompson, M. A. & Riblet, R. (1984) *UCLA Symp. Mol. Cell. Biol. New Ser.* **18**, 445–453.
26. Reynaud, C. A., Anquez, V., Dahan, A. & Weill, J. C. (1985) *Cell* **40**, 283–291.
27. Reth, M. G., Jackson, S. & Alt, F. W. (1986) *EMBO J.* **5**, 2131–2138.
28. Kleinfield, R., Hardy, R. R., Tarlington, D., Dangl, J., Herzenberg, L. A. & Wiegert, M. (1986) *Nature (London)* **322**, 843–846.
29. Reth, M., Gehrmann, P., Petrac, E. & Wiese, P. (1986) *Nature (London)* **322**, 840–842.
30. Near, R. I., Manser, T. & Gefter, M. L. (1985) *J. Immunol.* **134**, 2004–2009.
31. Dildrop, R., Krawinkel, U., Winter, E. & Rajewsky, K. (1985) *Eur. J. Immunol.* **15**, 1154–1156.