# Restricted κ Chain Expression in Early Ontogeny: Biased Utilization of $V_\kappa$ Exons and Preferential $V_\kappa$-$J_\kappa$ Recombinations

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## Summary

To determine the extent of κ chain diversity in the preimmune repertoire early in development, κ cDNA libraries were analyzed from 15-d old fetal omentum, 18-d-old fetal liver, and 3-wk old bone marrow. An anchored polymerase chain reaction approach was used to avoid bias for particular $V_\kappa$ families. From the sequence analysis of 27 bone marrow clones, 10 different families and 20 unique $V_\kappa$ genes were identified. In contrast, the $V_\kappa$ expression in the fetus is highly restricted and clearly differs from the broader distribution seen in 3-wk-old bone marrow. Although several $V_\kappa$ families were represented in the fetal library including $V_\kappa$9, $V_\kappa$10, $V_\kappa$4,5, $V_\kappa$8, and $V_\kappa$1, one or two members of individual families were observed repeatedly. The fetal liver and omentum libraries were found to be largely overlapping. Given the $V_\kappa$ families/exons identified in the fetal sequences, the mechanism of κ rearrangements in the early repertoire appears to occur predominantly by inversion. Importantly, the fetal repertoire was further restricted by dominant $V_\kappa$-$J_\kappa$ combinations such as $V_\kappa$4,5-$J_\kappa$5, $V_\kappa$9-$J_\kappa$4, and $V_\kappa$10-$J_\kappa$1. Since in some cases independent rearrangements could be established, the results indicate a bias for particular $V_\kappa$-$J_\kappa$ joins. The results also suggest that clonal expansion/selection in the fetal repertoire takes place after light chain rearrangement as opposed to at the pre-B cell level in the bone marrow. The restriction observed in κ light chain expression together with known restrictions in gene usage and junctional diversity at the heavy chain level indicate a remarkably conserved fetal repertoire.

Diversity of the antibody repertoire is created by several genetic mechanisms. These include recombination of discrete DNA segments (V, $D_H$, J) that encode the variable region genes of both H and L chains, addition of nontemplated nucleotides during joining (N insertions), P additions, deletion of nucleotides during recombination, somatic mutation, and H and L chain pairing (for a review see reference 1). Together, such mechanisms allow for enormous repertoires with estimates of $10^7$–$10^9$ different antibody specificities (1).

Early in ontogeny, B lymphopoiesis occurs in the fetal liver, bone marrow, spleen, and omentum, whereas in the adult, B cell generation occurs mainly in bone marrow (2–4). Early interest in the developing antibody repertoire focused on the reproducible patterned appearance of specificities, suggesting a genetic program (5, 6). Subsequent studies revealed a nonrandom use of $V_H$ gene families with preference for D-proximal families 7183 (7, 8) and Q52 (9). This contrasts with the adult where $V_H$ family usage in bone marrow correlates with the complexity of the families in the germline (9, 10) suggesting distinct differences in mechanisms of diversity. Importantly, fetal B cell progenitors propagated on adult bone marrow stromal cells still gave rise to fetal-like V gene repertoires, indicating that fetal B cell/progenitors are distinct from their adult counterparts (11). Accumulating evidence continues to support this hypothesis (12–15), underscoring the importance of defining associated mechanisms and biological significance.

Among murine antibodies, κ light chains dominate and therefore contribute significantly to diversity (16). In the mouse, the κ locus has been classified into 24 $V_\kappa$ groups according to amino acid similarities up to Cys 23 (17). More recently, 14–16 $V_\kappa$ families have been defined based upon 80% nucleotide similarity and RFLP analysis (18, 19). $V_\kappa$32, $V_\kappa$33, and $V_\kappa$20 are among the new families described by several groups (20, 21). Importantly, up to 40% of the $V_\kappa$ genes appear to be in the opposite transcriptional orientation from J (22), and rearrangement by inversion appears to take place as efficiently as deletion mechanisms (23–26) that dominate H chain rearrangements. Moreover, secondary $V_\kappa$ gene rearrangements are common and take place even when the initial rearrangement is a productive one (27, 28). Therefore, $V_\kappa$ replacements may play a critical role in increasing the diversity of $V_\kappa$ gene usage.

Comparative analyses of adult and fetal/neonatal $V_\kappa$ gene family usage have been done (29–31). No evidence was found for a bias in J-proximal families early in ontogeny. Among

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the studies, some differences were noted between adult and fetal $V_\kappa$ family usage. But, in general, the results were not dramatic and were somewhat conflicting in terms of differences in expression of particular families (29–31). Much of the discrepancy probably relates to the early use of $V_\kappa$ family probes containing the more conserved 3′ portions of $V_\kappa$ exons resulting in detection of more than one family (32, 33). In general, $V_\kappa$ families are more homologous in sequence to each other than $V_H$ families, making $V_\kappa$ family analyses by probe hybridization less reliable (32).

To address more definitively $V_\kappa$ usage early in ontogeny, cDNA libraries were constructed and analyzed from fetal liver, fetal omentum, and 3-wk-old bone marrow. An anchored PCR was used to minimize bias and allow for the detection of all $V_\kappa$ gene families. The results indicate a consistent preference during fetal life for a small set of $V_\kappa$ exons from multiple families. Diversity appeared to be further restricted by the repeated use of particular $V_\kappa$-$J_\kappa$ combinations.

## Materials and Methods

*Mice.* All experiments were performed with BALB/c mice obtained from Sprague-Dawley, Inc. (Indianapolis, IN). Animals were bred and maintained at the animal facility of the University of Texas Health Science Center and were routinely evaluated for pathogens. Livers from four 18-d-old fetuses were pooled. Omental tissue was obtained from a pool of six 15-d-old fetuses. The gestational age was determined by considering the day of mating as day 0 or by the presence of vaginal plugs. Extreme care was taken when dissecting the omentum to avoid contamination from spleen or liver. Furthermore, tissues were transferred multiple times to petri plates containing fresh solutions of BSA in HBSS to avoid carry-over of individual contaminating cells. Bone marrow cell suspensions were prepared from the femurs and tibias of 3-wk-old mice. Cells from three animals were pooled.

*Preparation of cDNA Libraries.* Total cellular RNA was isolated from tissues by lysis with guanidinium isothiocyanate followed by centrifugation over a cesium chloride gradient. First strand cDNA was generated using reverse transcriptase (SuperScript RNase $H^-$; GIBCO BRL, Gaithersburg, MD) and oligo-dT priming. Unincorporated nucleotides and primers were removed from the reaction mixture by separation through columns (Elutip-d; Schleicher & Schuell, Inc., Keene, NH). Instructions provided by the manufacturer were followed except that all the solutions were prepared using potassium salts, as sodium is known to inhibit terminal deoxynucleotide transferase (TdT)[1] activity. RNA–DNA hybrids were disrupted by alkaline hydrolysis with 0.2N KOH followed by neutralization with 1 N HCl. Poly-dG tailing of the cDNA molecules using TdT (Stratagene, La Jolla, CA) was performed as described by Roth et al. (34). After phenol-chloroform extraction of the reaction mixture, DNA was precipitated, resuspended in 0.1 × TBE and used as template for PCR.

*PCR Amplification, Cloning, and Sequencing.* PCR was performed in 50-μl reactions containing 2.5 U Taq polymerase (Promega Corp., Madison, WI), 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin, 25 pmole of each primer (Genosys Biotechnologies, Inc., The Woodlands, TX), and 5 μl of each cDNA template. To prevent mispriming events, all the components were added to the reaction except the polymerase. After one cycle of 7 min at 95°C, the temperature of the thermocycler was lowered to 72°C. At this time, 2.5 U of the Taq polymerase was added to each reaction and samples were overlaid with 50 μl of mineral oil. The samples were subjected to 30 cycles of 1 min at 94°C, 2 min at 47°C, and 1.5 min at 72°C. All PCR were terminated with a 15-min extension. Extreme precautions were taken to prevent contamination. All reactions were carried in a laminar hood, and equipment and tubes were UV irradiated. Mock samples that were subjected to all the enzymatic treatments served as negative controls.

The sequences of the primers used in the amplifications were as follows: 5′ end, 5′-CAC-GAT-CCG-CGG-TGC-CCC-CCC-CCC-CCC-3′; 3′ end, 5′-CAC-CAT-ATC-GAT-TTG-GTG-CAA-CAT-CAG-3′. To facilitate cloning, the primer at the 5′ end included a SacII restriction site and the one at the 3′ end ($C_k$) a ClaI site.

PCR bands were resolved in 1% agarose gels and the appropriate size products were excised. DNA was eluted from the agarose gels by centrifugal membrane filtration through 0.45-μm low binding membranes (Durapore; Millipore Corp., Bedford, MA). Agarose contaminants were removed by treatment with glass milk (Bio 101, Inc., Vista, CA). Purified fragments were cloned into p-Bluescript SK–vector (Stratagene) using the SacII and ClaI restriction sites. DH5αF′ or DH11S competent cells (GIBCO BRL) were transformed with the ligation reaction and plated onto nitrocellulose filters.

To screen for positive clones, a consensus $J_\kappa$ and the $C_\kappa$ primer used in the amplifications were endlabeled with [$^{32}$P]ATP using T7 polynucleotide kinase (New England Biolabs, Inc., Beverly, MA). Double positive ($J_\kappa^+$, $C_\kappa^+$) clones were expanded, and single stranded DNA obtained by superinfection with M13K07 helper phage (GIBCO BRL). DNA sequencing was performed using the dideoxy nucleotide termination method with Sequenase 2 (United States Biochem. Corp., Cleveland, OH).

*Sequence Analysis.* Nucleic acid similarities between sequences in GenBank and EMBL data banks and our sequences were determined using the FASTDB program (35).

## Results and Discussion

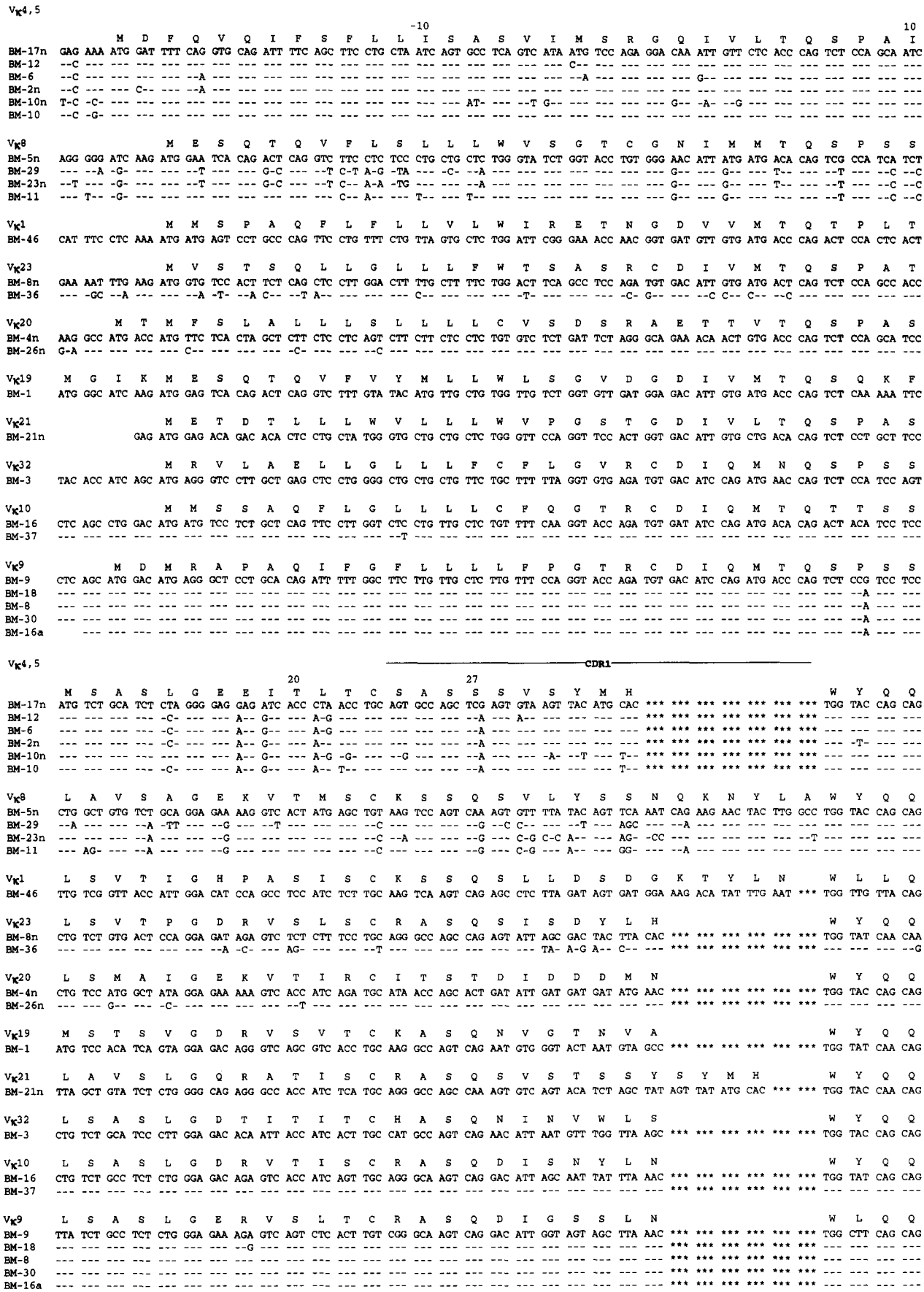
*Multiple $V_\kappa$ Exons Are Expressed in 3-wk-old Bone Marrow.* To evaluate the expressed repertoire of κ light chains, bone marrow was pooled from three 3-wk-old BALB/c mice. Immunocytochemical staining with anti-Ig showed that the proportion of plasma cells in the cell preparation was very low (<0.1%). 3 wk of age was chosen since it was anticipated from previous $V_H$ gene analyses that the repertoire would be mostly adultlike with the possibility for some overlap with the early repertoire. cDNA templates were amplified using anchored PCR to avoid bias for particular $V_\kappa$ families. $V_\kappa$-$J_\kappa$ rearrangements were identified after cloning and sequencing of the amplified cDNA fragments. All of the rearrangements analyzed appeared to be productive ones. The data are summarized in Table 1. Clones representing probable independent rearrangements are listed separately. If two or fewer substitutions were found between individual clones they were grouped as identical since two substitutions approximated the error calculated for the Taq polymerase. An exception to this was when an identical substitution was found in more than one clone or a different library making an error with the Taq polymerase unlikely. In this case, the clones were considered as independent rearrangements.

[1] *Abbreviation used in this paper:* TdT, deoxynucleotide transferase.

**Table 1.** *3-wk-old Bone Marrow Clones*

| Clone | $V_\kappa$ | $J_\kappa$ | Frequency | Percent match | Specificity | Reference |
|---|---|---|---|---|---|---|
| BM-17N | 4,5 | 1 | 1/27 | 99.6* (84.CON)‡ | Germline, $V_\kappa$Ox§ | 36 |
| BM-12 | 4,5 | 1 | 1/27 | 100 (R9) | Germline, $V_\kappa$Ox | 54 |
| BM-6 | 4,5 | 1 | 1/27 | 98.9 (H3, CH5) | Germline, $V_\kappa$Ox | 54, 39 |
| BM-2n | 4,5 | 4 | 1/27 | 99.3 (76.CON) | Germline, $V_\kappa$Ox | 36 |
| BM-10n | 4,5 | 2 | 1/27 | 99 (R13) | Germline, $V_\kappa$Ox | 54 |
| BM-10 BM-41 | 4,5 | 5 | 2/27 | 100 (H4, T6-416) | Germline, $V_\kappa$Ox, Hemagglutinin | 54, 49 |
| BM-5n | 8 | 1 | 1/27 | 100 (T5-626) | Hemagglutinin | 49 |
| BM-29 | 8 | 2 | 1/27 | 99.7-100 (220GL, NC12-H5, PC3609) | Hemagglutinin Plasmacytoma | 55, 56, 25 |
| BM-23n BM-15 | 8 | 2 | 2/27 | 98.3 (MRB2) | Histone | 57 |
| BM-11 | 8 | 5 | 1/27 | 100 (M603) | PC | 58 |
| BM-46 | 1 | 1 | 1/27 | 99.7 ($V_\kappa$1.6, DNA14, 42-4B-12, 1210.7) | Germline, DNA, dextran, Arsonate | 31, 59 |
| BM-8n | 23 | 2 | 1/27 | 99.3 ($V_\kappa$23.32) | Germline | 31 |
| BM-36 | 23 | 1 | 1/27 | 84.6 ($V_\kappa$23.32) | Germline | 31 |
| BM-4n | 20 | 5 | 1/27 | 100 (C8.5) | DNA | 21 |
| BM-26n | 20 | 2 | 1/27 | 99.6 (33.28) | Colon-carcinoma antigen | 60 |
| BM-1 | 19 | 5 | 1/27 | 99.6 (PC7043) | Plasmacytoma | 25 |
| BM-21N | 21 | 5 | 1/27 | 99.0-99.7 ($V_\kappa$21 E1.6, BrM8) | Germline, multireactive | 61, 62 |
| BM-3 | 32 | 1 | 1/27 | 99.6-99.3 (AN04K, NC6-C8) | DNP | 20, 56 |
| BM-16 BM-37 | 10 | 1 | 2/27 | 99.6-100 (KL2.21, CH12) | Germline, $V_\kappa$Ars‖, Multireactive | 63, 64, 39 |
| BM-9 | 9 | 1 | 1/27 | 99.3-99.6 (MOPC41, S2-14.2, H220-23) | Germline, RBC and T cells, Hemagglutinin | 65, 66, 67 |
| BM-8 BM-18 | 9 | 1 | 2/27 | 99.3-99.6 (MOPC41, S2-14.2, H220-23) | Germline, RBC and T cells, Hemagglutinin | 65, 66, 67 |
| BM-30 | 9 | 2 | 1/27 | 99.6-100 (MOPC41, S2-14.2, H220-23) | Germline, RBC and T cells, Hemagglutinin | 65, 66, 67 |
| BM-16a | 9 | 4 | 1/27 | 99.6-99.3 (MOPC41, S2-14.2, H220-23) | Germline, RBC and T cells, Hemagglutinin | 65, 66, 67 |

* Percent homology through the coding regions of the variable gene.
‡ Germline gene designation or hybridoma or cell line from which the gene was cloned and/or sequenced.
§ Anti-2-phenyloxazolone–related germline gene.
‖ Antiarsonate-related germline gene.


Vκ4,5
-10
10
M D F Q V Q I F S F L L I S A S V I M S R G Q I V L T Q S P A I
BM-17n GAG AAA ATG GAT TTT CAG GTG CAG ATT TTC AGC TTC CTG CTA ATC AGT GCC TCA GTC ATA ATG TCC AGA GGA CAA ATT GTT CTC ACC CAG TCT CCA GCA ATC
BM-12 --C --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- C-- --- --- --- --- --- --- --- --- --- --- --- --- ---
BM-6 --C --- --- --- --- --A --- --- --- --- --- --- --- --- --- --- --- --- --- --A --- --- --- --- G-- --- --- --- --- --- --- --- ---
BM-2n --C --- --- C-- --- --A --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BM-10n T-C -C- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- AT- --- --T G-- --- --- --- --- G-- -A- --G --- --- --- --- ---
BM-10 --C -G- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Vκ8
M E S Q T Q V F L S L L L W V S G T C G N I M M T Q S P S S
BM-5n AGG GGG ATC AAG ATG GAA TCA CAG ACT CAG GTC TTC CTC TCC CTG CTG CTC TGG GTA TCT GGT ACC TGT GGG AAC ATT ATG ATG ACA CAG TCG CCA TCA TCT
BM-29 --- --A -G- --- --- --T --- --- G-C --- --T C-T A-G -TA --- -C- --A --- --- --- --- --- --- --- G-- --- G-- --- T-- --- --T --- --C --C
BM-23n --T --- -G- --- --- --T --- --- G-C --- --T C-- A-A -TG --- --- --A --- --- --- --- --- --- --- G-- --- G-- --- T-- --- --T --- --C --C
BM-11 --- T-- -G- --- --- --- --- --- --- --- --- C-- A-- --- T-- --- T-- --- --- --- --- --- --- --- G-- --- G-- --- --- --- --T --- --C --C
Vκ1
M M S P A Q F L F L L V L W I R E T N G D V V M T Q T P L T
BM-46 CAT TTC CTC AAA ATG ATG AGT CCT GCC CAG TTC CTG TTT CTG TTA GTG CTC TGG ATT CGG GAA ACC AAC GGT GAT GTT GTG ATG ACC CAG ACT CCA CTC ACT
Vκ23
M V S T S Q L L G L L L F W T S A S R C D I V M T Q S P A T
BM-8n GAA AAT TTG AAG ATG GTG TCC ACT TCT CAG CTC CTT GGA CTT TTG CTT TTC TGG ACT TCA GCC TCC AGA TGT GAC ATT GTG ATG ACT CAG TCT CCA GCC ACC
BM-36 --- -GC --A --- --- --A -T- --A C-- --T A-- --- --- --- C-- --- --- --- -T- --- --- --- -C- G-- --- --C C-- C-- --C --- --- --- --- ---
Vκ20
M T M F S L A L L L S L L L L C V S D S R A E T T V T Q S P A S
BM-4n AAG GCC ATG ACC ATG TTC TCA CTA GCT CTT CTC CTC AGT CTT CTT CTC CTC TGT GTC TCT GAT TCT AGG GCA GAA ACA ACT GTG ACC CAG TCT CCA GCA TCC
BM-26n G-A --- --- --- --- C-- --- --- --- -C- --- --- --C --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Vκ19
M G I K M E S Q T Q V F V Y M L L W L S G V D G D I V M T Q S Q K F
BM-1 ATG GGC ATC AAG ATG GAG TCA CAG ACT CAG GTC TTT GTA TAC ATG TTG CTG TGG TTG TCT GGT GTT GAT GGA GAC ATT GTG ATG ACC CAG TCT CAA AAA TTC
Vκ21
M E T D T L L L W V L L L W V P G S T G D I V L T Q S P A S
BM-21n GAG ATG GAG ACA GAC ACA CTC CTG CTA TGG GTG CTG CTG CTC TGG GTT CCA GGT TCC ACT GGT GAC ATT GTG CTG ACA CAG TCT CCT GCT TCC
Vκ32
M R V L A E L L G L L L F C F L G V R C D I Q M N Q S P S S
BM-3 TAC ACC ATC AGC ATG AGG GTC CTT GCT GAG CTC CTG GGG CTG CTG CTG TTC TGC TTT TTA GGT GTG AGA TGT GAC ATC CAG ATG AAC CAG TCT CCA TCC AGT
Vκ10
M M S S A Q F L G L L L L C F Q G T R C D I Q M T Q T T S S
BM-16 CTC AGC CTG GAC ATG ATG TCC TCT GCT CAG TTC CTT GGT CTC CTG TTG CTC TGT TTT CAA GGT ACC AGA TGT GAT ATC CAG ATG ACA CAG ACT ACA TCC TCC
BM-37 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --T --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Vκ9
M D M R A P A Q I F G F L L L L F P G T R C D I Q M T Q S P S S
BM-9 CTC AGC ATG GAC ATG AGG GCT CCT GCA CAG ATT TTT GGC TTC TTG TTG CTC TTG TTT CCA GGT ACC AGA TGT GAC ATC CAG ATG ACC CAG TCT CCG TCC TCC
BM-18 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --A --- ---
BM-8 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --A --- ---
BM-30 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --A --- ---
BM-16a --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --A --- ---
Vκ4,5
CDR1
20
27
M S A S L G E E I T L T C S A S S S V S Y M H W Y Q Q
BM-17n ATG TCT GCA TCT CTA GGG GAG GAG ATC ACC CTA ACC TGC AGT GCC AGC TCG AGT GTA AGT TAC ATG CAC *** *** *** *** *** *** *** TGG TAC CAG CAG
BM-12 --- --- --- --- -C- --- --- A-- G-- --- A-G --- --- --- --- --- --A --- A-- --- --- --- --- *** *** *** *** *** *** *** --- --- --- ---
BM-6 --- --- --- --- -C- --- --- A-- G-- --- A-G --- --- --- --- --- --A --- --- --- --- --- --- *** *** *** *** *** *** *** --- --- --- ---
BM-2n --- --- --- --- -C- --- --- A-- G-- --- A-- --- --- --- --- --- --A --- --- --- --- --- --- *** *** *** *** *** *** *** --- -T- --- ---
BM-10n --- --- --- --- --- --- --- A-- G-- --- A-G -G- --- --- --G --- --- --A --- --- --A- --T --- T-- *** *** *** *** *** *** *** --- --- --- ---
BM-10 --- --- --- --- -C- --- --- A-- G-- --- A-- T-- --- --- --- --- --A --- --- --- --- --- T-- *** *** *** *** *** *** *** --- --- --- ---
Vκ8
L A V S A G E K V T M S C K S S Q S V L Y S S N Q K N Y L A W Y Q Q
BM-5n CTG GCT GTG TCT GCA GGA GAA AAG GTC ACT ATG AGC TGT AAG TCC AGT CAA AGT GTT TTA TAC AGT TCA AAT CAG AAG AAC TAC TTG GCC TGG TAC CAG CAG
BM-29 --A --- --- --A -TT --- --G --- --T --- --- --- --C --- --- --- --G --C C-- --- --T --- AGC --- --A --- --- --- --- --- --- --- --- ---
BM-23n --- --- --- --A --- --- --G --- --- --- --- --- --C --A --- --- --G --- C-G C-C A-- --- AG- -CC --- --- --- --- --- --T --- --- --- ---
BM-11 --- AG- --- --A --- --- --G --- --- --- --- --- --C --- --- --- --G --- C-G --- A-- --- GG- --- --A --- --- --- --- --- --- --- --- ---
Vκ1
L S V T I G H P A S I S C K S S Q S L L D S D G K T Y L N W L L Q
BM-46 TTG TCG GTT ACC ATT GGA CAT CCA GCC TCC ATC TCT TGC AAG TCA AGT CAG AGC CTC TTA GAT AGT GAT GGA AAG ACA TAT TTG AAT *** TGG TTG TTA CAG
Vκ23
L S V T P G D R V S L S C R A S Q S I S D Y L H W Y Q Q
BM-8n CTG TCT GTG ACT CCA GGA GAT AGA GTC TCT CTT TCC TGC AGG GCC AGC CAG AGT ATT AGC GAC TAC TTA CAC *** *** *** *** *** *** TGG TAT CAA CAA
BM-36 --- --- --- --- --- --- --A -C- --- AG- --- --- --T --- --- --- --- --- --- --- TA- A-G A-- C-- --- *** *** *** *** *** *** --- --- --- --G
Vκ20
L S M A I G E K V T I R C I T S T D I D D D M N W Y Q Q
BM-4n CTG TCC ATG GCT ATA GGA GAA AAA GTC ACC ATC AGA TGC ATA ACC AGC ACT GAT ATT GAT GAT GAT ATG AAC *** *** *** *** *** *** TGG TAC CAG CAG
BM-26n --- --- G-- --- -C- --- --- --- --- --T --- --- --- --- --- --- --- --- --- --- --- --- --- --- *** *** *** *** *** *** --- --- --- ---
Vκ19
M S T S V G D R V S V T C K A S Q N V G T N V A W Y Q Q
BM-1 ATG TCC ACA TCA GTA GGA GAC AGG GTC AGC GTC ACC TGC AAG GCC AGT CAG AAT GTG GGT ACT AAT GTA GCC *** *** *** *** *** *** TGG TAT CAA CAG
Vκ21
L A V S L G Q R A T I S C R A S Q S V S T S S Y S Y M H W Y Q Q
BM-21n TTA GCT GTA TCT CTG GGG CAG AGG GCC ACC ATC TCA TGC AGG GCC AGC CAA AGT GTC AGT ACA TCT AGC TAT AGT TAT ATG CAC *** *** TGG TAC CAA CAG
Vκ32
L S A S L G D T I T I T C H A S Q N I N V W L S W Y Q Q
BM-3 CTG TCT GCA TCC CTT GGA GAC ACA ATT ACC ATC ACT TGC CAT GCC AGT CAG AAC ATT AAT GTT TGG TTA AGC *** *** *** *** *** *** TGG TAC CAG CAG
Vκ10
L S A S L G D R V T I S C R A S Q D I S N Y L N W Y Q Q
BM-16 CTG TCT GCC TCT CTG GGA GAC AGA GTC ACC ATC AGT TGC AGG GCA AGT CAG GAC ATT AGC AAT TAT TTA AAC *** *** *** *** *** *** TGG TAT CAG CAG
BM-37 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- *** *** *** *** *** *** --- --- --- ---
Vκ9
L S A S L G E R V S L T C R A S Q D I G S S L N W L Q Q
BM-9 TTA TCT GCC TCT CTG GGA GAA AGA GTC AGT CTC ACT TGT CGG GCA AGT CAG GAC ATT GGT AGT AGC TTA AAC *** *** *** *** *** *** TGG CTT CAG CAG
BM-18 --- --- --- --- --- --- --- --G --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- *** *** *** *** *** *** --- --- --- ---
BM-8 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- *** *** *** *** *** *** --- --- --- ---
BM-30 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- *** *** *** *** *** *** --- --- --- ---
BM-16a --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- *** *** *** *** *** *** --- --- --- ---

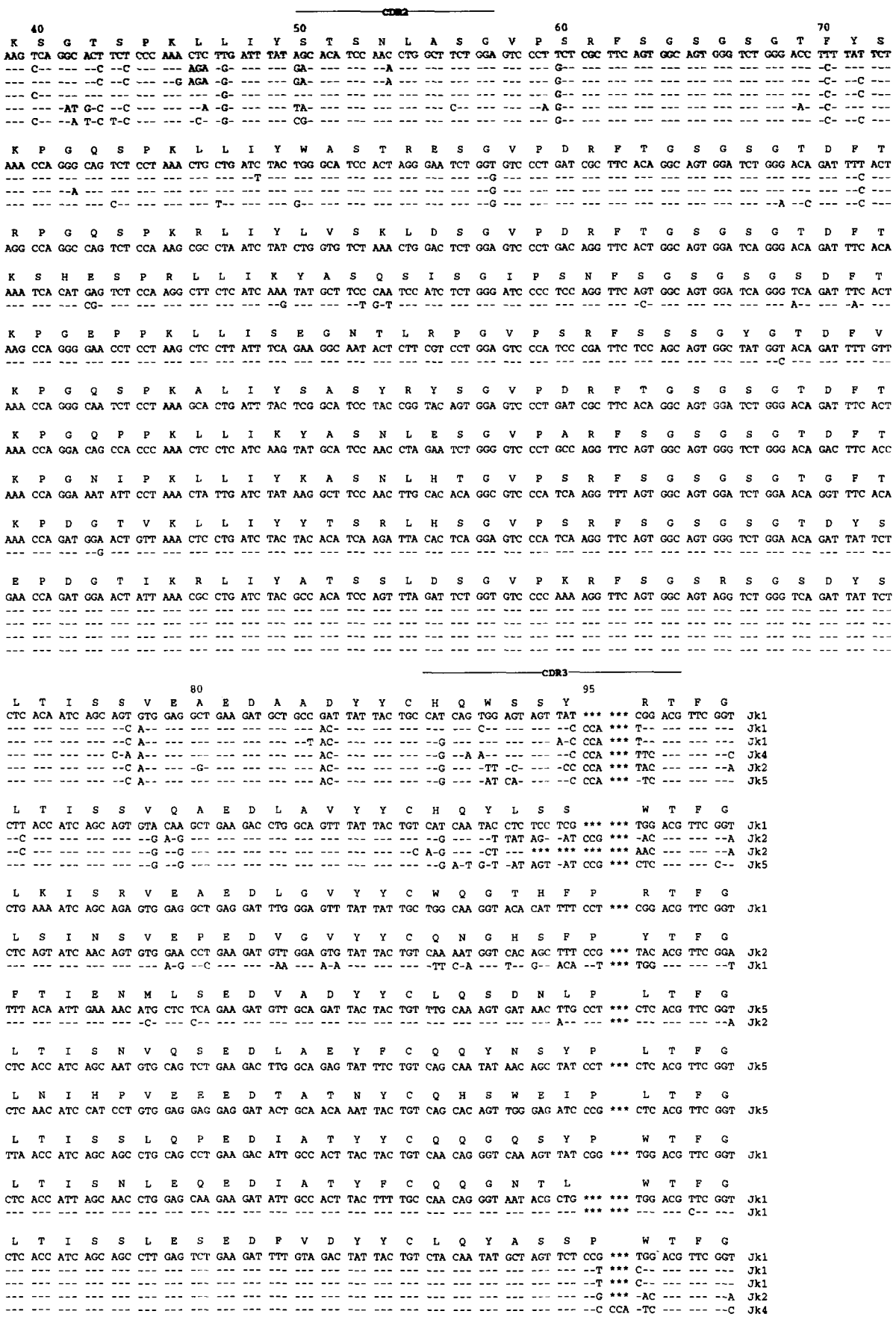



**Figure 1.** Nucleotide sequences of bone marrow κ clones. Sequences are grouped according to $V_\kappa$ gene family and are shown relative to one member of the family. (---) Identity. (*) Gaps were introduced to facilitate alignment of the sequences. The deduced amino acid sequences for the representative member of each family is numbered according to Kabat et al. (69) and the locations of CDRs are shown. Sequence data for BM36 and FL-31 are available from EMBL under accession numbers Z17400 and Z17401, respectively.

**Table 2.** *Summary of $J_\kappa$ Utilization*

| cDNA library | No. sequences analyzed | Percent sequences expressing | | | |
|---|---|---|---|---|---|
| | | $J_\kappa 1$ | $J_\kappa 2$ | $J_\kappa 4$ | $J_\kappa 5$ |
| 3-wk-old bone marrow | 27 | 44.4* | 25.9 | 7.4 | 22.2 |
| Fetal liver | 26 | 30.8 | 7.7 | 34.6 | 26.9 |
| Omentum | 22 | 40.9 | 4.5 | 18.2 | 36.4 |

* Data taken from Tables 1, 3, and 4.

The data in Table 1 reveals a pattern of considerable $V_\kappa$ sequence diversity. In all, 10 different families and 20 unique $V_\kappa$ genes were identified among the 27 clones sequenced. Even though the presence of plasma cells cannot be ruled out, their contribution appears minimal based upon the frequency of independent rearrangements and the diversity of $V_\kappa$ exons found. Some of the same $V_\kappa$ genes identified have been previously described for LPS-stimulated bone marrow cells (29, 30) and bone marrow pre-B cell lines (31). Rearrangements involving identical $V_\kappa$ genes but different $J_\kappa$ segments added to the diversity of the $\kappa$ chains.

The bone marrow sequences are shown in Fig. 1. Of particular interest is BM-36, a $V_\kappa 23$, which only shows 83% homology to the closest gene, $V_\kappa 23.32$. Therefore, it likely represents a new $V_\kappa 23$ germline gene.

As discussed previously (33), it is difficult to accurately assess the extent of $V_\kappa$ family usage in terms of family complexity since the number of functional genes for most of the families is not known. Nevertheless, it is interesting that the family estimated to be the largest is $V_\kappa 4,5$ ($\geq$16 members), and this family was represented most frequently in the bone marrow cDNA library (26%). Recently, analysis of a genomic library of the $V_\kappa 4,5$ family ($V_\kappa Ox$) confirms multiple, functional $V_\kappa$ genes (36). Based upon use of the more specific 5′ $V_\kappa$ family probes used by Kalled and Brodeur (33), $V_\kappa 8$ (4–7 members) and $V_\kappa 9$ (5–10 members) are also relatively large families and are represented prominently in the library shown here. In contrast, $V_\kappa 21$ (13 members) and $V_\kappa 19$ (5 members) were found infrequently. However, the splenic cDNA libraries analyzed by Kalled and Brodeur (33) for $V_\kappa$ gene family utilization also showed unexpectedly low frequencies of $V_\kappa 21$ (3.8%). None of the smaller $V_\kappa$ families with one, two, or three members were found with unexpectedly high frequencies in the bone marrow library. In general, these data support the lack of bias for particular $V_\kappa$ families using the anchored PCR.

*Nonrandom Features of the $V_\kappa$ Bone Marrow Repertoire.* Although multiple $V_\kappa$ families and exons were found in the library analyzed, indicating considerable diversity, the utilization of $V_\kappa$ genes was not random. One member of $V_\kappa 9$ was preferentially used in the bone marrow library. Although independent rearrangements were likely in these clones, there appeared to be a clear bias for the MOPC41 germline gene, the same gene that is used preferentially in the fetal repertoire as described below. Whether this is due to remnants of the fetal repertoire in 3-wk-old animals is not clear. However, repeats were also observed in other sets of clones, e.g., BM-16/BM37 (KL2.21) and BM23n/BMB15 (MRB2), and these genes were not found in the fetal libraries.

Also nonrandom was the utilization of individual $J_\kappa$ segments with $J_\kappa 1$ preferentially used and $J_\kappa 4$ underutilized (Table 2). Similar frequencies of the use of individual $J_\kappa$ segments have been reported by others (33) further validating that the anchored PCR approach was not introducing a bias. A possible mechanism for the nonrandom use of $J_\kappa 1$ was recently reported (37). The DNA binding protein KLP was shown to bind at a site 5′ of the $J_\kappa 1$ segment, an event that could target the recombinase to this region and increase the frequency of rearrangements involving $J_\kappa 1$.

*Restricted $V_\kappa$-$J_\kappa$ Gene Expression in Fetal Liver.* $V_\kappa$-$J_\kappa$ rearrangements were also examined in 18-d-old fetuses by generating a cDNA library from fetal liver using anchored PCR. The data are summarized in Table 3 with representative sequences shown in Fig. 2. Most of the sequences are highly homologous to known germline genes. However, FL-31 showed only 96% homology with $V_\kappa 1.6$ (11 substitutions in the coding region). Therefore, FL-31 may represent a new $V_\kappa 1$ germline gene.

The results indicate a highly restricted $V_\kappa$ repertoire in the 18-d-old fetal liver compared with that observed with 3-wk-old bone marrow. In a collection of 26 clones, some sequences were noted repeatedly. Several families were represented in the library including $V_\kappa 4,5$, $V_\kappa 9$, $V_\kappa 10$, $V_\kappa 8$, and $V_\kappa 1$, and presumably a more extensive library would have revealed additional $V_\kappa$ families. However, only one or two members of each family were identified, suggesting a highly restricted repertoire. Even more striking was the repeated use of particular $V_\kappa$-$J_\kappa$ combinations such as $V_\kappa 9$-$J_\kappa 4$, $V_\kappa 10$-$J_\kappa 1$, and $V_\kappa 4,5$-$J_\kappa 5$. This is unlikely to be artifact since the same combinations found in the fetal library were also found in two separate omentum libraries described below. Moreover, in several cases among the three libraries, independent rearrangements could be established. This suggests that these particular $V_\kappa$-$J_\kappa$ joins are genetically favored or that B cells undergoing such rearrangements are selected.

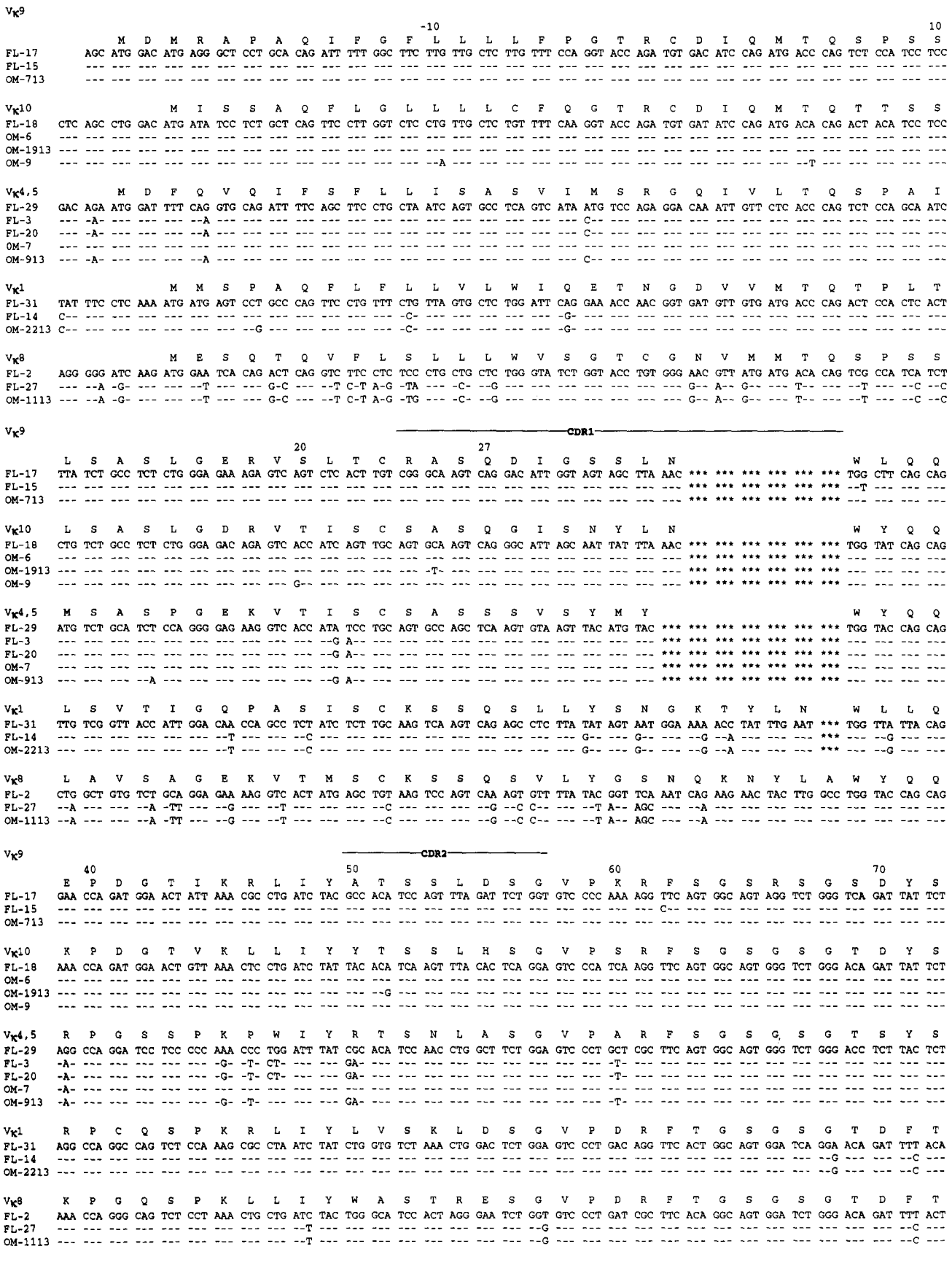
*Analysis of the $V_\kappa$-$J_\kappa$ Repertoire in Omentum.* Recent re-

**Table 3.** *Fetal Clones*

| Clone | $V_\kappa$ | $J_\kappa$ | Frequency | Percent match | Specificity | Reference |
|---|---|---|---|---|---|---|
| FL-17 | | | | | | |
| FL-23 | | | | | | |
| FL-1 | | | | | | |
| FL-15 | | | | | | |
| FL-7 | 9 | 4 | 9/26 | 98.6-99.3* | Germline, RBC and | 65, 66, 67 |
| FL-16 | | | | (MOPC41, S2-14.2, H220-23)‡ | T cells, hemagglutinin | |
| FL-12 | | | | | | |
| FL-25 | | | | | | |
| FL-26 | | | | | | |
| FL-13 | | | | | | |
| FL-4 | | | | | | |
| FL-32 | 10 | 1 | 6/26 | 99.6-100 | Germline, $V_\kappa$Ars§, | 68, 67 |
| FL-21 | | | | (V10.1b, L2-10C1) | hemagglutinin | |
| FL-18 | | | | | | |
| FL-11 | | | | | | |
| FL-29 | | | | | | |
| FL-6 | 4,5 | 5 | 3/26 | 99.6-100 | Germline, $V_\kappa$Ox‖, | 54, 49 |
| FL-5 | | | | (H4, T6-416) | hemagglutinin | |
| FL-3 | | | | | | |
| FL-30 | 4,5 | 5 | 3/26 | 99.6-100 | Germline, $V_\kappa$Ox, | 36 |
| FL-28 | | | | (261.CON, NQ2/6.1) | 2-phenyloxazolone | |
| FL-20¶ | 4,5 | 5 | 1/26 | 100 | Germline, $V_\kappa$Ox, | 36 |
| | | | | (261.CON, NQ2/6.1) | 2-phenyloxazolone) | |
| FL-14 | 1 | 1 | 1/26 | 99.7 | Germline, DNA, dextran, | 31, 59 |
| | | | | ($V_\kappa$1.6, DNA14, 42-4B-12, | Arsonate | |
| | | | | 1210.7) | | |
| FL-31 | 1 | 2 | 1/26 | 96.3 | Germline, DNA, dextran, | 31, 59 |
| | | | | ($V_\kappa$1.6, DNA14, 42-4B-12, | Arsonate | |
| | | | | 1270.7) | | |
| FL-2 | 8 | 1 | 1/26 | 99.6 | Hemagglutinin | 49 |
| | | | | (T5-626) | | |
| FL-27 | 8 | 2 | 1/26 | 99.7-100 | Hemagglutinin, | 55, 56, 25 |
| | | | | (220GL, NC12-H5-PC3609) | Plasmacytoma | |

* Percent homology through the coding regions of the variable gene.
‡ Germline designation or hybridoma or cell line from which the gene was sequenced and/or cloned.
§ Germline gene associated with the antiarsonate response.
‖ Germline gene associated with the anti-2-phenyl oxazolone response.
¶ Two substitutions in $J_\kappa$.

ports indicate that the microenvironment of the the fetal omentum supports the development of B cells (4, 38). More importantly, the omentum seems to contain precursors that exclusively give rise to $CD5^+$ (B1a) and $CD5^-$ (B1b) sister B cell subpopulations (4). Therefore, it was interesting to compare the $V_\kappa$ repertoire in omentum with that of the fetal liver. For this purpose, two 15-d-old omentum cDNA libraries were constructed from fetuses from two separate litters. Day 15 of gestation was used since this appears to be when the amount of B cell lymphopoietic omental tissue is maximal (Solvason, N., personal communication). There was some concern whether $\kappa^+$ cells would be found at this stage since


Vκ9
-10
10
M D M R A P A Q I F G F L L L L F P G T R C D I Q M T Q S P S S
FL-17 AGC ATG GAC ATG AGG GCT CCT GCA CAG ATT TTT GGC TTC TTG TTG CTC TTG TTT CCA GGT ACC AGA TGT GAC ATC CAG ATG ACC CAG TCT CCA TCC TCC
FL-15
OM-713
Vκ10
M I S S A Q F L G L L L L C F Q G T R C D I Q M T Q T T S S
FL-18 CTC AGC CTG GAC ATG ATA TCC TCT GCT CAG TTC CTT GGT CTC CTG TTG CTC TGT TTT CAA GGT ACC AGA TGT GAT ATC CAG ATG ACA CAG ACT ACA TCC TCC
OM-6
OM-1913
OM-9
Vκ4,5
M D F Q V Q I F S F L L I S A S V I M S R G Q I V L T Q S P A I
FL-29 GAC AGA ATG GAT TTT CAG GTG CAG ATT TTC AGC TTC CTG CTA ATC AGT GCC TCA GTC ATA ATG TCC AGA GGA CAA ATT GTT CTC ACC CAG TCT CCA GCA ATC
FL-3
FL-20
OM-7
OM-913
Vκ1
M M S P A Q F L F L L V L W I Q E T N G D V V M T Q T P L T
FL-31 TAT TTC CTC AAA ATG ATG AGT CCT GCC CAG TTC CTG TTT CTG TTA GTG CTC TGG ATT CAG GAA ACC AAC GGT GAT GTT GTG ATG ACC CAG ACT CCA CTC ACT
FL-14
OM-2213
Vκ8
M E S Q T Q V F L S L L L W V S G T C G N V M M T Q S P S S
FL-2 AGG GGG ATC AAG ATG GAA TCA CAG ACT CAG GTC TTC CTC TCC CTG CTG CTC TGG GTA TCT GGT ACC TGT GGG AAC GTT ATG ATG ACA CAG TCG CCA TCA TCT
FL-27
OM-1113
Vκ9
CDR1
20
27
L S A S L G E R V S L T C R A S Q D I G S S L N W L Q Q
FL-17 TTA TCT GCC TCT CTG GGA GAA AGA GTC AGT CTC ACT TGT CGG GCA AGT CAG GAC ATT GGT AGT AGC TTA AAC *** *** *** *** *** *** TGG CTT CAG CAG
FL-15
OM-713
Vκ10
L S A S L G D R V T I S C S A S Q G I S N Y L N W Y Q Q
FL-18 CTG TCT GCC TCT CTG GGA GAC AGA GTC ACC ATC AGT TGC AGT GCA AGT CAG GGC ATT AGC AAT TAT TTA AAC *** *** *** *** *** *** TGG TAT CAG CAG
OM-6
OM-1913
OM-9
Vκ4,5
M S A S P G E K V T I S C S A S S S V S Y M Y W Y Q Q
FL-29 ATG TCT GCA TCT CCA GGG GAG AAG GTC ACC ATA TCC TGC AGT GCC AGC TCA AGT GTA AGT TAC ATG TAC *** *** *** *** *** *** *** TGG TAC CAG CAG
FL-3
FL-20
OM-7
OM-913
Vκ1
L S V T I G Q P A S I S C K S S Q S L L Y S N G K T Y L N W L L Q
FL-31 TTG TCG GTT ACC ATT GGA CAA CCA GCC TCT ATC TCT TGC AAG TCA AGT CAG AGC CTC TTA TAT AGT AAT GGA AAA ACC TAT TTG AAT *** TGG TTA TTA CAG
FL-14
OM-2213
Vκ8
L A V S A G E K V T M S C K S S Q S V L Y G S N Q K N Y L A W Y Q Q
FL-2 CTG GCT GTG TCT GCA GGA GAA AAG GTC ACT ATG AGC TGT AAG TCC AGT CAA AGT GTT TTA TAC GGT TCA AAT CAG AAG AAC TAC TTG GCC TGG TAC CAG CAG
FL-27
OM-1113
Vκ9
CDR2
40
50
60
70
E P D G T I K R L I Y A T S S L D S G V P K R F S G S R S G S D Y S
FL-17 GAA CCA GAT GGA ACT ATT AAA CGC CTG ATC TAC GCC ACA TCC AGT TTA GAT TCT GGT GTC CCC AAA AGG TTC AGT GGC AGT AGG TCT GGG TCA GAT TAT TCT
FL-15
OM-713
Vκ10
K P D G T V K L L I Y Y T S S L H S G V P S R F S G S G S G T D Y S
FL-18 AAA CCA GAT GGA ACT GTT AAA CTC CTG ATC TAT TAC ACA TCA AGT TTA CAC TCA GGA GTC CCA TCA AGG TTC AGT GGC AGT GGG TCT GGG ACA GAT TAT TCT
OM-6
OM-1913
OM-9
Vκ4,5
R P G S S P K P W I Y R T S N L A S G V P A R F S G S G S G S G T S Y S
FL-29 AGG CCA GGA TCC TCC CCC AAA CCC TGG ATT TAT CGC ACA TCC AAC CTG GCT TCT GGA GTC CCT GCT CGC TTC AGT GGC AGT GGG TCT GGG ACC TCT TAC TCT
FL-3
FL-20
OM-7
OM-913
Vκ1
R P C Q S P K R L I Y L V S K L D S G V P D R F T G S G S G T D F T
FL-31 AGG CCA GGC CAG TCT CCA AAG CGC CTA ATC TAT CTG GTG TCT AAA CTG GAC TCT GGA GTC CCT GAC AGG TTC ACT GGC AGT GGA TCA GGA ACA GAT TTT ACA
FL-14
OM-2213
Vκ8
K P G Q S P K L L I Y W A S T R E S G V P D R F T G S G S G T D F T
FL-2 AAA CCA GGG CAG TCT CCT AAA CTG CTG ATC TAC TGG GCA TCC ACT AGG GAA TCT GGT GTC CCT GAT CGC TTC ACA GGC AGT GGA TCT GGG ACA GAT TTT ACT
FL-27
OM-1113

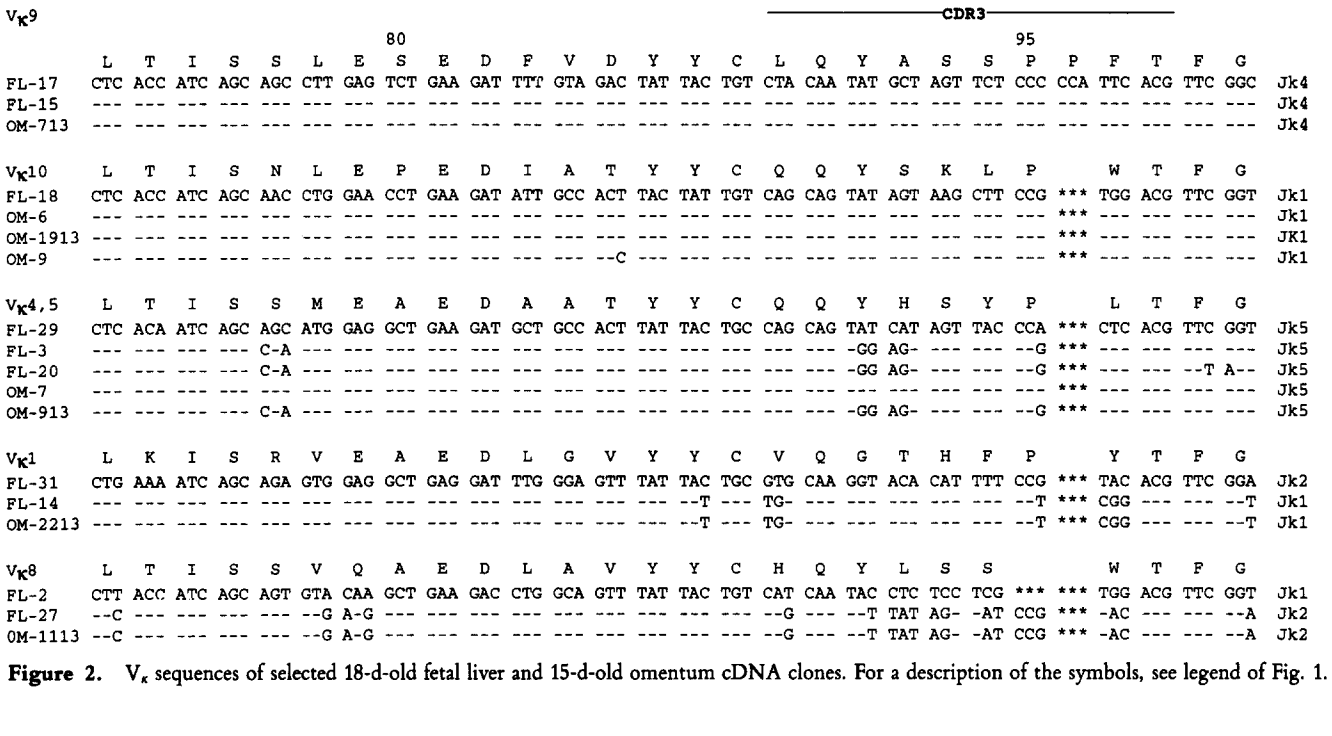



**Figure 2.** $V_\kappa$ sequences of selected 18-d-old fetal liver and 15-d-old omentum cDNA clones. For a description of the symbols, see legend of Fig. 1.

$sIg^+$ cells were absent at day 13 (4). However, $sIg^+$ cells were detected in human omentum at 8–12 wk of gestation (38). Moreover, a significant PCR reaction was obtained using the $C_\kappa$ primer for both libraries. Further amplification was not required for either library.

The data obtained from the omentum is shown in Table 4 and Fig. 2. Since the sequence obtained with both libraries were similar, the data were combined. The $V_\kappa$ families identified have been found previously in known $CD5^+$ cells including $V_\kappa 9$ and $V_\kappa 4,5$ which are predominant in the bromelain-RBC/phosphatidyl choline reactivity (39, 40). In general, a similar $V_\kappa$ repertoire was observed using the fetal omentum and fetal liver libraries. The same set of restricted $V_\kappa$ exons were repeatedly used. Moreover, identical $V_\kappa$-$J_\kappa$ combinations were identified with $V_\kappa 10$-$J_\kappa 1$, $V_\kappa 4,5$-$J_\kappa 5$, and $V_\kappa 9$-$J_\kappa 4$ recombinations dominating. $J_\kappa$ usage in the omentum library is different from that of the bone marrow library with increased use of $J_\kappa 4$ and $J_\kappa 5$ and decreased use of $J_\kappa 2$ (Table 2). The results of $V_\kappa$-$J_\kappa$ usage indicate significant overlap of the fetal omentum and fetal liver repertoires. Similarities in the B cell compartments of human omentum and fetal liver were also noted by Solvason and Kearney (38). This would be consistent with previous conclusions that B cells in early development are dominated by $CD5^+$ B cells including the fetal liver (15). However, we have also begun analyzing the $V_H$ repertoire of the omentum using the same omental RNA used here to generate cDNA $V_\kappa$ libraries. Initial results indicate that the omental $V_H$ library may be more restricted than the fetal liver library (Teale, J., and E. J. Morris, manuscript in preparation).

*Diversity at the $V_\kappa$-$J_\kappa$ Junction.* Diversity at the V-J junction is mainly the result of exonuclease activity with the removal of bases at either V or J. This introduces variability in the CDR3 even when identical $V_\kappa$-$J_\kappa$ combinations are used. Residues located 3′ of the $V_\kappa$ exon appear to be used sometimes during recombination to create a longer CDR3 (36). A probable example of this is BM-16 (Fig. 1) and the FL and OM sequences (Fig. 2) of $V_\kappa 9$ (MOPC41). This results in an extra Pro at position 96. An analysis of junctional diversity was used, in part, to establish independent rearrangements in the sequences shown here.

Milstein et al. (36) recently analyzed a genomic library of $V_\kappa 4,5$ rearrangements in adult spleen and found evidence for asymmetric trimming with more bases being removed from V than from J. In the sequences shown here where the germline $V_\kappa$ sequence could be clearly identified, some degree of asymmetry was found as well (Table 5).

Evidence has also been reported for the addition of bases at the $V_L$-$J_L$ junction through N or P residues (36, 41). BM-17N may represent one such example. The CDR3 of this clone appeared to lack the highly conserved Pro at position 95 and contained only eight amino acid residues. A close inspection of the V-J junction in this sequence showed two residues, TC, that do not seem to originate from the V or J genes. However, if this does represent nontemplated addition of nucleotides, it is rare for L chains as noted elsewhere (41).

Importantly, the H chain CDR3 of fetal/neonatal antibodies has been shown to be shorter than adult-derived antibodies. This is due to lack of N insertions as well as targeted rearrangements in early development involving homologous overlapping sequences (12–14). In the case of the fetal $\kappa$ sequences, there may be slightly less trimming of bases at J during V-J joining compared with the bone marrow (Table 5). However, the average lengths of the L chain CDR3s are

**Table 4.** *Omentum Clones*

| Clone | $V_\kappa$ | $J_\kappa$ | Frequency | Percent match | Specificity | Reference |
|---|---|---|---|---|---|---|
| OM-5 | | | | | | |
| OM-6 | 10 | 1 | 4/22 | 99.3-100* | Germline, $V_\kappa$Ars§ | 68, 67 |
| OM-3 | | | | (V10.1b, L2-10C1)‡ | Hemagglutinin | |
| OM-1313 | | | | | | |
| OM-513‖ | 10 | 1 | 2/22 | 99.3 | Germline, $V_\kappa$Ars, | 68, 67 |
| OM-1913‖ | | | | ($V_\kappa$10.1b, L2-10C1) | Hemagglutinin | |
| OM-9¶ | 10 | 1 | 1/22 | 98.9 | Germline, $V_\kappa$Ars, | 68, 67 |
| | | | | (V10.1b-L2-10C1) | Hemagglutinin | |
| OM-1613** | 10 | 1 | 1/22 | 99.3 | Germline, $V_\kappa$Ars, | 68, 67 |
| | | | | ($V_\kappa$10.1b, L2-10C1) | Hemagglutinin | |
| OM-13 | | | | | | |
| OM-2313 | 4,5 | 5 | 3/22 | 99.6-100 | Germline, $V_\kappa$Ox‡‡, | 54, 49 |
| OM-7 | | | | (H4, T6-416) | Hemagglutinin | |
| OM-46 | 4,5 | 5 | 4/22 | 99.6-100 | Germline, $V_\kappa$Ox, | 36 |
| OM-913 | | | | (261.CON, NQ2/6.1) | 2-phenyloxazolone | |
| OM-813 | | | | | | |
| OM-1713 | | | | | | |
| OM-1413§§ | 4,5 | 5 | 1/22 | 100 | Germline, $V_\kappa$Ox, | 36 |
| | | | | (261.CON, NQ2/6.1) | 2-phenyloxazolone | |
| OM-713 | | | | | | |
| OM-1513 | 9 | 4 | 4/22 | 98.6-99.3 | Germline, RBC and | 65, 66, 67 |
| OM-313 | | | | (MOPC41, S2-14.2, H220-23) | T cells, hemagglutinin | |
| OM-8 | | | | | | |
| OM-2213 | 1 | 1 | 1/22 | 99.3 | Germline, DNA, dextran, | 31, 59 |
| | | | | ($V_\kappa$1.6, DNA14, 42-4B-12 | arsonate | |
| | | | | 12.10.7) | | |
| OM-1113 | 8 | 2 | 1/22 | 99.7-100 | Hemagglutinin, | 55, 56, 25 |
| | | | | (220GL, NC12-H5, | Plasmacytoma | |
| | | | | PC3609) | | |

* Percent homology through the coding regions of the variable gene.
‡ Germline designation or hybridoma or plasmacytoma from which the gene was cloned and/or sequenced.
§ Germline gene associated with the antiarsonate response.
‖ One replacement in the CDR1; one silent mutation in the CDR2.
¶ One replacement in the FR1; two silent mutations in the FR1 and FR3.
** Designated as a nonproductive rearrangment.
‡‡ Germline gene associated with the anti-2-phenyloxazole response.
§§ Two substitutions in $J_\kappa$, same as FL-20.

essentially identical in all of the libraries analyzed arguing against major differences in junctional diversity. Selection may also contribute to these minor differences.

*κ Gene Expression in Early Ontogeny.* No sequences from the $V_\kappa$21 family, the most J-proximal family (18), were identified in the fetal libraries. This is consistent with previous results indicating relatively low or no $V_\kappa$21 family expression in LPS-stimulated fetal and neonatal B cells (29, 30). Instead, several families are expressed that appear to be spread throughout the κ locus. This argues against a position-dependent regulation of $V_\kappa$ gene expression as has been argued for $V_H$ gene usage (7). (It should be noted however, that constraints independent of mapping position appear likely in H chain gene rearrangement (11, 13, 42, 43]). The lack

**Table 5.** *Analysis of Junctional Diversity*

| | Bone marrow | Fetal liver | Omentum |
|---|---|---|---|
| Range of no. of nucleotides removed* | V:0-5 J:0-1 (17)‡ | V:0-5 J:0-1 (23) | V:0-5 J:0-1 (20) |
| Average no. of nucleotides removed/sequence analyzed | V:2.8 J:0.23 (17) | V:2.3 J:0.04 (23) | V:3.0 J:0.05 (20) |
| Average length of CDR3 | 8.7 (27) | 9.3 (26) | 9.2 (22) |

* For sequences when the germline $V_\kappa$ was known, the number of bases trimmed from either V or J was determined.
‡ Number of sequences analyzed.

of any evidence for position-dependent effects may relate to the fact that over 40% of the $V_\kappa$ genes appear to be in the opposite transcriptional orientation from $J_\kappa$ and rearrange by inversion (22). Importantly, with the exception of $V_\kappa 1$, all of the fetal sequences identified here are from $V_\kappa$ families known to rearrange by inversion (22). Moreover, in some cases the individual members used have been indicated to rearrange by inversion, i.e., $V_\kappa 9$–MOPC41 (24) and $V_\kappa 8$–PC3609 (25). Two of the three known functional $V_\kappa 10$ members rearrange by inversion (22). The mechanism of rearrangement for the third member is not known. Several members of the $V_\kappa 4,5$ family also rearrange by inversion (22, 26, 44). This suggests that the early $\kappa$ repertoire is dominated by inversion-type rearrangements.

Also likely to be biologically important is the apparent preference for particular $V_\kappa$-$J_\kappa$ combinations in the fetal repertoire. This is particularly striking since some of the same $V_\kappa$ exons are found in the bone marrow library with multiple $J_\kappa$ exons. Given their frequency in three different libraries, they presumably represent primary rearrangements. Interestingly, Kalled and Brodeur (44) recently reported on $\kappa$ rearrangements in A-MuLV-transformed pre-B cell lines in which 84% resulted in a $V_\kappa 4,5$-$J_\kappa 5$ recombination, one of the predominant rearrangements observed here. It has been suggested that primary inversion rearrangements may make 5′ $V_\kappa$ exons more accessible for secondary rearrangements (22, 44). However, recombination to $J_\kappa 5$ would prevent functional secondary rearrangements. Similarly, the $V_\kappa 9$-$J_\kappa 4$ would significantly limit secondary rearrangements particularly given the 5′ location of $V_\kappa 9$. Moreover, the repeated identification of $V_\kappa 10$-$J_\kappa 1$ rearrangement in plasmacytomas/cell lines (22) suggests that this may also represent a stable recombination event less prone to functional secondary rearrangements. Since secondary $\kappa$ rearrangements are thought to provide another mechanism for Ig diversity and increase chances for effective H and L chain pairing (27), a limitation on secondary rearrangements would result in a more restricted fetal repertoire.

Several lines of evidence indicate that the H chain fetal repertoire is significantly restricted compared with the adult repertoire. These include biased usage of particular $V_H$, D, and $J_H$ exons, a preferred D reading frame, and lack of junctional diversity (for a review see reference 1). The relative role of developmental, functional, and evolutionary pressures on the early repertoire remain unclear. It has been argued by many that a highly conserved early repertoire would effectively counteract ubiquitous bacterial pathogens (45). The propensity for self-reactivities also suggests an immunoregulatory role (46, 47). A conserved repertoire would necessitate restricted L as well as H chain diversity. L chains have recently been shown to contribute significantly to the fine specificity of antibodies. For example, the H9 heavy chain used in anti-DNA antibodies will also react with other antigens such as cardiolipin/RNA depending upon the L chain with which it pairs (48). Also of interest are recent molecular studies of the influenza hemagglutinin (HA) antigen system in which the antibody response to slight changes in a HA epitope were analyzed (49). It was shown that the altered epitope was accommodated in the antibody response, not by changes in the CDR3, but rather by changes in H and L chain pairing. Therefore, if there is evolutionary pressure to restrict the early repertoire, limitation at the L chain level is probably critical. Part of this limitation may be imposed at the genetic level. Fetal H chain expression is restricted and the lengths of the CDR3 significantly shorter. This may limit the number of L chains that can effectively pair with the fetal H chains. The ability to achieve H and L chain pairing may also underlie the observed $V_\kappa$-$J_\kappa$ preferences.

Results of a highly restricted fetal $\kappa$ repertoire also impact on theories of selection and clonal expansion. H chain rearrangement normally precedes L chain rearrangement (1–3), although exceptions have been reported (50). This appears to be true of all stages of development since pre-B cells antedate B cells. In bone marrow, it appears that pre-B cells are clonally expanded perhaps by selection through the H chain–surrogate L chain complex (51, 52). The expanded cells then appear to independently rearrange L chain genes resulting in a diverse set of B cells (51, 53). This is consistent with our bone marrow library where multiple $\kappa$ rearrangements were found. However, given the highly restricted, repeated sequences in the three fetal libraries shown here, and the likelihood that H chain rearrangement precedes L chain rearrange-

ment, it appears that clonal expansion/selection in the early repertoire occurs after L chain gene rearrangement. Alternatively, fetal B lineage cells may expand at both the pre-B cell and B cell stages. Expansion at the B cell level would further restrict the fetal repertoire by increasing the number of cells with identical H and L chain pairs. It also indicates a unique clonal selection/expansion mechanism for the early repertoire.

In summary, the early repertoire is restricted in terms of both H and L chain expression. The available data do not allow definitive conclusions to be drawn as to whether the underlying mechanisms involve primarily genetic forces, environmental forces, or a combination of both. Fetal pre-B cells develop in vitro with a fetal-like $V_H$ repertoire even when supported by adult bone marrow stromal cells (11). This, combined with lack of TdT and differences in CDR3s (1) suggest that at least some of the differences between fetal and adult repertoires are due to distinct progenitors and rearrangement strategies. The restriction in L chain expression shown here may also be due, in part, to genetic mechanisms. Certain $V_\kappa$-$J_\kappa$ recombinations may be preferred. In addition, L chain expression may be restricted on the basis of the ability to pair with a more limited fetal H chain repertoire. Selection mechanisms may also be operative. Once L chain rearrangement and expression occurs in fetal B cells, there appears to be considerable expansion, perhaps by selection through the intact Ig receptor. The end result is a highly conserved fetal repertoire.

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