Restricted κ Chain Expression in Early Ontogeny: Biased Utilization of VK Exons and Preferential VK-JK 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 VK families. From the sequence analysis of 27 bone marrow clones, 10 different families and 20 unique VK genes were identified. In contrast, the VK expression in the fetus is highly restricted and clearly differs from the broader distribution seen in 3-wk-old bone marrow. Although several VK families were represented in the fetal library including VK9, VK10, VK4,5, VK8, and VK1, one or two members of individual families were observed repeatedly. The fetal liver and omentum libraries were found to be largely overlapping. Given the VK families/exons identified in the fetal sequences, the mechanism of K rearrangements in the early repertoire appears to occur predominantly by inversion. Importantly, the fetal repertoire was further restricted by dominant VK-JK combinations such as VK4,5-JK5, VK9-JK4, and VK10-JK1. Since in some cases independent rearrangements could be established, the results indicate a bias for particular VK-JK 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, 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 VK gene families with preference for D-proximal families 7183 (7, 8) and Q52 (9). This contrasts with the adult where VK family usage in bone marrow correlates with the complexity of the families in the germ-line (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 VK 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 VK groups according to amino acid similarities up to Cys 23 (17). More recently, 14–16 VK families have been defined based upon 80% nucleotide similarity and RFLP analysis (18, 19). VK32, VK33, and VK20 are among the new families described by several groups (20, 21). Importantly, up to 40% of the VK 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 VK gene rearrangements are common and take place even when the initial rearrangement is a productive one (27, 28). Therefore, VK replacements may play a critical role in increasing the diversity of VK gene usage.

Comparative analyses of adult and fetal/neonatal VK gene family usage have been done (29–31). No evidence was found for a bias in J-proximal families early in ontogeny. Among
the studies, some differences were noted between adult and fetal \( V_k \) 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 \( V_k \) family probes
containing the more conserved 3' portions of \( V_k \)
exons resulting in detection of more than one family (32, 33). In general, \( V_k \) families are more homologous in sequence
to each other than \( V_n \) families, making \( V_k \) family
analyses by probe hybridization less reliable (32).

To address more definitively \( V_k \) 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 \( V_k \) gene families. The results indicate a consistent
preference during fetal life for a small set of \( V_k \) exons from
multiple families. Diversity appeared to be further restricted
by the repeated use of particular \( V_k-J_k \) combinations.

Materials and Methods

**Mice** All experiments were performed with BALB/c mice ob-
tained 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 dis-
secting 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. Unincor-
porated nucleotides and primers were removed from the reaction
mixture by separation through columns (Elutip-d; Schleicher &
Schuell, Inc., Keene, NH). Instructions provided by the manufac-
turer were followed except that all the solutions were prepared using
potassium salts, as sodium is known to inhibit terminal deoxynucleo-
tide transferase (TdT) activity. RNA-DNA hybrids were dis-
rupted by alkaline hydrolysis with 0.2N KOH followed by neutral-
ization with 1 N HCI. 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 x 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 Wood-
lands, 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 precau-
tions were taken to prevent contamination. All reactions were car-
rried 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-CAFATC-GAA-TGTG-GAA-
CAG-CAG-3'. To facilitate cloning, the primer at the 5' end included
a SacII restriction site and the one at the 3' end (C\(_e\)) a ClaI site.
PCR bands were resolved in 1% agarose gels and the appro-
priate 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 restric-
tion sites. DH5αF' or DH11S competent cells (GIBCO BRL)
were transformed with the ligation reaction and plated onto nitrocel-
lulose filters.

To screen for positive clones, a consensus J\(_e\) and the C\(_e\) primer
used in the amplifications were endlabeled with [\(^{32}\)P]ATP using
T7 polynucleotide kinase (New England Biolabs, Inc., Beverly, MA).
Double positive (J\(_e\), C\(_e\)) clones were expanded, and single
stranded DNA obtained by superinfection with M13K07 helper
phage (GIBCO BRL). DNA sequencing was performed using the
dideoxynucleotide 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 deter-
mined using the FASTDB program (35).

Results and Discussion

**Multiple \( V_k \) Exons Are Expressed in 3-wk-old Bone Marrow.**
To evaluate the expressed repertoire of \( \kappa \) light chains, bone
marrow was pooled from three 3-wk-old BALB/c mice. Im-
munocytochemical staining with anti-\( \kappa \)g 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 antici-
pated from previous \( V_k \) 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 \( V_k \) fami-
lies. \( V_k-J_k \) rearrangements were identified after cloning and
sequencing of the amplified cDNA fragments. All of the rear-
rangements analyzed appeared to be productive ones. The
data are summarized in Table 1. Clones representing prob-
able independent rearrangements are listed separately. If two
or fewer substitutions were found between individual clones
they were grouped as identical since two substitutions ap-
proximated 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.

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1 Abbreviation used in this paper: TdT, deoxynucleotide transferase.
Table 1. 3-wk-old Bone Marrow Clones

| Clone  | V<sub>x</sub> | J<sub>x</sub> | Frequency | Percent match | Specificity | Reference |
|--------|--------------|--------------|-----------|---------------|-------------|-----------|
| BM-17N | 4, 5         | 1            | 1/27      | 99.6*         | Germline, V<sub>x</sub>Ox<sup>+</sup> | 36        |
| BM-12  | 4, 5         | 1            | 1/27      | 100           | Germline, V<sub>x</sub>Ox | 54        |
| BM-6   | 4, 5         | 1            | 1/27      | 98.9 (H3, CH5)| Germline, V<sub>x</sub>Ox | 54, 39    |
| BM-2n  | 4, 5         | 4            | 1/27      | 99.3 (76.CN)  | Germline, V<sub>x</sub>Ox | 36        |
| BM-10n | 4, 5         | 2            | 1/27      | 99 (R13)      | Germline, V<sub>x</sub>Ox | 54        |
| BM-10  | 4, 5         | 5            | 2/27      | 100           | Germline, V<sub>x</sub>Ox, Hemagglutinin | 54, 49    |
| BM-41  | 8            | 1            | 1/27      | 100           | Hemagglutinin | 55, 56, 25 |
| BM-5n  | 8            | 1            | 1/27      | 99.7-100 (T5-626) | Hemagglutinin, Plasmacytoma, Histone | 57        |
| BM-29  | 8            | 2            | 1/27      | 98.3 (H4, T6-416) | Hemagglutinin, Histone | 55, 56, 25 |
| BM-23n | 8            | 2            | 2/27      | 100           | Germline, DNA, dextran, Arsonate | 31, 59    |
| BM-15  | 8            | 5            | 1/27      | 100           | Plasmacytoma | 25        |
| BM-11  | 8            | 5            | 1/27      | 99.6 (33.28)  | Colon-carcinoma antigen | 60        |
| BM-46  | 1            | 1            | 1/27      | 99.7 (V<sub>x</sub>1.6, DNA14, 42-4B-12, 1210.7) | Germline, DNA, dextran, Arsonate | 31, 59    |
| BM-8n  | 23           | 2            | 1/27      | 99.3 (V<sub>x</sub>23.32) | Germline | 31        |
| BM-36  | 23           | 1            | 1/27      | 84.6 (V<sub>x</sub>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)  | DNA | 21        |
| BM-15  | 19           | 5            | 1/27      | 99.6 (PC7043) | Plasmacytoma | 25        |
| BM-21N | 21           | 5            | 1/27      | 99.0-99.7 (V<sub>x</sub>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  | 10           | 1            | 2/27      | 99.6-100 (KL2.21, CH12) | Germline, V<sub>x</sub>Arsl<sup>+</sup>, Multireactive | 63, 64, 39 |
| BM-37  |              |              |           |               | Germline, RBC and T cells, Hemagglutinin | 65, 66, 67 |
| 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   | 9            | 1            | 2/27      | 99.3-99.6 (MOPC41, S2-14.2, H220-23) | Germline, RBC and T cells, Hemagglutinin | 65, 66, 67 |
| BM-18  | 9            | 2            | 1/27      | 99.6-100 (MOPC41, S2-14.2, H220-23) | Germline, RBC and T cells, Hemagglutinin | 65, 66, 67 |
| BM-30  | 9            | 2            | 1/27      | 99.6-99.3 (MOPC41, S2-14.2, H220-23) | Germline, RBC and T cells, Hemagglutinin | 65, 66, 67 |
| BM-16a | 9            | 4            | 1/27      |               |             |           |

* 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.
Figure 1. Nucleotide sequences of bone marrow k clones. Sequences are grouped according to V 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 FL31 are available from EMBL under accession numbers Z17400 and Z17401, respectively.
The data in Table 1 reveals a pattern of considerable V, sequence diversity. In all, 10 different families and 20 unique V, 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, exons found. Some of the same V, 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, genes but different J, segments added to the diversity of the K chains.

The bone marrow sequences are shown in Fig. 1. Of particular interest is BM-36, a V,23, which only shows 83% homology to the closest gene, V,23.32. Therefore, it likely represents a new V,23 germline gene. As discussed previously (33), it is difficult to accurately assess the extent of V, 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,4,5 (>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,4,5 family (V,ox) confirms multiple, functional V, genes (36). Based upon use of the more specific 5' V, family probes used by Kalled and Brodeur (33), V,8 (4-7 members) and V,9 (5-10 members) are also relatively large families and are represented prominently in the library shown here. In contrast, V,21 (13 members) and V,19 (5 members) were found infrequently. However, the splenic cDNA libraries analyzed by Kalled and Brodeur (33) for V, gene family utilization also showed unexpectedly low frequencies of V,21 (3.8%). None of the smaller V, 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, families using the anchored PCR.

Nonrandom Features of the V, Bone Marrow Repertoire. Although multiple V, families and exons were found in the library analyzed, indicating considerable diversity, the utilization of V, genes was not random. One member of V,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, segments with J,1 preferentially used and J,4 underutilized (Table 2). Similar frequencies of the use of individual J, 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,1 was recently reported (37). The DNA binding protein KLP was shown to bind at a site 5' of the J,1 segment, an event that could target the recombinase to this region and increase the frequency of rearrangements involving J,1.

Restricted V, J, Gene Expression in Fetal Liver. V, J, 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,1.6 (11 substitutions in the coding region). Therefore, FL-31 may represent a new V,1 germline gene.

The results indicate a highly restricted V, 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,4,5, V,9, V,10, V,8, and V,1, and presumably a more extensive library would have revealed additional V, 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, J, combinations such as V,9-J,4, V,10-J,4, and V,4,5-J,5. This is unlikely to be artifact since the same combinations found in the fetal liver 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, J, joins are genetically favored or that B cells undergoing such rearrangements are selected.

Analysis of the V, J, Repertoire in Omentum. Recent re-

**Table 2. Summary of J, Utilization**

| cDNA library      | No. sequences analyzed | J,1 | J,2 | J,4 | J,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.
Table 3.  **Fetal Clones**

| Clone | $V_\alpha$ | $J_\alpha$ | Frequency | Percent match | Specificity | Reference |
|-------|-------------|-------------|-----------|---------------|-------------|-----------|
| FL-17 | 9           | 4           | 9/26      | 98.6-99.3*    | Germline, RBC and T cells, hemagglutinin | 65, 66, 67 |
| FL-23 |             |             |           |               |             |           |
| FL-1  |             |             |           |               |             |           |
| FL-15 |             |             |           |               |             |           |
| FL-7  | 9           | 4           | 9/26      | (MOPC41, S2-14.2, H220-23)† | Germline, RBC and T cells, hemagglutinin | 65, 66, 67 |
| FL-16 |             |             |           |               |             |           |
| FL-12 |             |             |           |               |             |           |
| FL-25 |             |             |           |               |             |           |
| FL-26 |             |             |           |               |             |           |
| FL-13 |             |             |           |               |             |           |
| FL-4  |             |             |           |               |             |           |
| FL-32 | 10          | 1           | 6/26      | 99.6-100      | Germline, $V_\alpha$Ars§, hemagglutinin | 68, 67 |
| FL-21 |             |             |           |               |             |           |
| FL-18 |             |             |           |               |             |           |
| FL-11 |             |             |           |               |             |           |
| FL-29 |             |             |           |               |             |           |
| FL-6  | 4,5         | 5           | 3/26      | 99.6-100      | Germline, $V_\alpha$Ox‖, hemagglutinin | 54, 49 |
| FL-5  |             |             |           |               |             |           |
| FL-3  |             |             |           |               |             |           |
| FL-30 | 4,5         | 5           | 3/26      | 99.6-100      | Germline, $V_\alpha$Ox, 2-phenyl oxazolone | 36 |
| FL-28 |             |             |           |               |             |           |
| FL-20§ | 4,5        | 5           | 1/26      | 100           | Germline, $V_\alpha$Ox, 2-phenyl oxazolone | 36 |
| FL-27 |             |             |           |               |             |           |
| FL-14 | 1           | 1           | 1/26      | 99.7          | Germline, DNA, dextran, Arsonate | 31, 59 |
| FL-31 | 1           | 2           | 1/26      | 96.3          | Germline, DNA, dextran, Arsonate | 31, 59 |
| FL-2  | 8           | 1           | 1/26      | 99.6          | Hemagglutinin | 49 |
| FL-27 | 8           | 2           | 1/26      | 99.7-100      | Hemagglutinin, Plasmacytoma | 55, 56, 25 |

* 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 antiaarsonate response.
‖ Germline gene associated with the anti-2-phenyl oxazolone response.
§ Two substitutions in $J_\alpha$.

Reports 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 $V_\alpha$ 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
\section*{Diversity at the \(V_{\kappa}^{-}\)J Junction.} Diversity at the \(V_{\kappa}-J_{\kappa}\) 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 \(V_{\kappa}\) (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 \(V_{\kappa},J_{\kappa}\) rearrangements in adult spleen and found evidence for asymmetric trimming in more bases being removed from \(V\) than from \(J\). In the sequences shown here where the germ-line \(V_{\kappa}\) 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 \(V_{\kappa}-J_{\kappa}\) 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

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|c|}
\hline
\(V_{\kappa}^-\) & L & T & I & S & S & L & S & D & V & Y & C & L & Q & Y & A & S & S & P & F & F & F & C \\
\hline
FL-17 & CTC & ACC & ATC & ACG & AGC & CTG & GAT & TTC & GAA & GAT & GAT & TTC & TAT & TAG & CTA & CA & TAA & GTG & TCC & CCC & GCA & TAC & TGG & GGC \\
\hline
FL-15 & CTC & ACC & ATC & ACG & AGC & CTG & GAT & TTC & GAA & GAT & GAT & TTC & TAT & TAG & CTA & CA & TAA & GTG & TCC & CCC & GCA & TAC & TGG & GGC \\
\hline
OM-73 & CTC & ACC & ATC & ACG & AGC & CTG & GAT & TTC & GAA & GAT & GAT & TTC & TAT & TAG & CTA & CA & TAA & GTG & TCC & CCC & GCA & TAC & TGG & GGC \\
\hline
\end{tabular}
\caption{\(V_{\kappa}^-\) 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.}
\end{table}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2}
\caption{\(V_{\kappa}\) 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.}
\end{figure}
| Clone  | V<sub>x</sub> | J<sub>x</sub> | Frequency | Percent match | Specificity | Reference |
|--------|---------------|---------------|------------|---------------|-------------|-----------|
| OM-5   | 10            | 1             | 4/22       | 99.3-100*     | Germline, V<sub>x</sub>Ars<sup>$</sup> | 68, 67    |
| OM-6   | 10            | 1             | 2/22       | 99.3          | Germline, V<sub>x</sub>Ars, Hemagglutinin | 68, 67    |
| OM-3   | 4, 5          | 5             | 3/22       | 99.6-100      | Germline, V<sub>x</sub>Ox<sup>**</sup>, 2-phenyloxazolone | 54, 49    |
| OM-13  | 9             | 4             | 4/22       | 98.6-99.3     | Germline, RBC and T cells, hemagglutinin | 65, 66, 67|
| OM-2313| 4, 5          | 5             | 3/22       | Germline, V<sub>x</sub>, 2-phenyloxazolone | 36        |
| OM-7   | 4, 5          | 5             | 4/22       | Germline, V<sub>x</sub>Ox<sup>**</sup>, 2-phenyloxazolone | 36        |
| OM-46  | 4, 5          | 5             | 3/22       | Germline, V<sub>x</sub>Ox, 2-phenyloxazolone | 36        |
| OM-913 | 4, 5          | 5             | 4/22       | Germline, V<sub>x</sub>, DNA, dextran, arsonate | 31, 59    |
| OM-813 | 8             | 2             | 1/22       | 99.7-100      | Hemagglutinin, Plasmacytoma | 55, 56, 25|

* 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 rearrangement.
⊗ Germline gene associated with the anti-2-phenyloxazole response.
$ Two substitutions in J<sub>x</sub>, 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.

**B**ien expression in Early Ontogeny**. No sequences from the V<sub>x</sub> 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<sub>x</sub> family expression in LPS-stimulated fetal and neonatal B cells (29, 30). Instead, several families are expressed that appear to be spread throughout the k locus. This argues against a position-dependent regulation of V<sub>x</sub> gene expression as has been argued for V<sub>k</sub> 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
of any evidence for position-dependent effects may relate to
the fact that over 40% of the \( V_e \) genes appear to be in the
opposite transcriptional orientation from \( J_e \) and rearrange by
inversion (22). Importantly, with the exception of \( V_eI \), all
of the fetal sequences identified here are from \( V_e \) families
known to rearrange by inversion (22). Moreover, in some
cases the individual members used have been indicated to
rearrange by inversion, i.e., \( V_e9-MOPC41 \) (24) and \( V_e8-
PC3609 \) (25). Two of the three known functional \( V_e10 \)
members rearrange by inversion (22). The mechanism of rear-
arrangement for the third member is not known. Several
members of the \( V_e4,5 \) family also rearrange by inversion (22).
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members of the \( V_e4,5 \) family also rearrange by inversion (22).
Interestingly, Kalled and Brodeur (44) recently reported on
\( \kappa \) rearrangements in A-MuLV-transformed pre-B cell lines in
which 84% resulted in a \( V_e4,5-J_e5 \) recombination, one of the
predominant rearrangements observed here. It has been
suggested that primary inversion rearrangements may make
\( 5' \) \( V_e \) exons more accessible for secondary rearrangements
(22, 44). However, recombination to \( J_e5 \) would prevent
functional secondary rearrangements. Similarly, the \( V_e9-J_e4 \)
would significantly limit secondary rearrangements particu-
larly given the \( 5' \) location of \( V_e9 \). Moreover, the repeated
identification of \( V_e10-J_e1 \) rearrangement in plasmacytomas/
cell lines (22) suggests that this may also represent a stable
recombination event less prone to functional secondary rear-
rangements. 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 reper-
toire is significantly restricted compared with the adult reperto-
toire. These include biased usage of particular \( V_n \), \( D \), and
\( J_n \) 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 coun-
teract 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 ac-
commodated in the antibody response, not by changes in the
CDR3, but rather by changes in H and L chain pairing. There-
fore, if there is evolutionary pressure to restrict the early reper-
toire, 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 ob-
served \( V_e-J_e \) preferences.

Results of a highly restricted fetal \( \kappa \) repertoire also impact
on theories of selection and clonal expansion. H chain rear-
arrangement 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 antede-
date 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 likeli-
hood that H chain rearrangement precedes L chain rearrange-

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**Table 5. Analysis of Junctional Diversity**

|                 | Bone marrow | Fetal liver | Omentum |
|-----------------|-------------|-------------|---------|
| Range of no. of nucleotides removed* | V:0-5 J:0-1 | V:0-5 J:0-1 | V:0-5 J:0-1 |
| Average no. of nucleotides removed/sequence analyzed | (17)* | (23) | (20) |
| Average length of CDR3 | 8.7 | 9.3 | 9.2 |

* For sequences when the germline \( V_e \) was known, the number of bases trimmed from either V or J was determined.

† Number of sequences analyzed.
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, 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, J, 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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