Induced Rearrangement of κ Genes in the BLIN-1 Human Pre-B Cell Line Correlates with Germline J-C_κ and V_κ Transcription

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Summary

The human pre-B acute lymphoblastic leukemia cell line, BLIN-1, has been previously shown to undergo κ light chain rearrangement in vitro, making it a valuable resource for analyzing pre-B to B cell differentiation. We have examined the recombination potential of BLIN-1 by characterizing several independently derived κ-expressing subclones for DNA rearrangement and V_κ gene usage. Analysis of five κ-expressing subclones (all having the same heavy chain rearrangement) demonstrated independent κ light chain rearrangement events by DNA hybridization analysis. Northern blot analysis using probes recognizing the four different V_κ families revealed that two subclones used the most proximal V_κ (V_κIV), one subclone used a V_κI, and one subclone used a V_κII. By polymerase chain reaction analyses, we detected transcripts from rearranged V-J-C_κ genes as well as transcripts from germline J-C_κ and V_κ in BLIN-1 cells induced to rearrange the κ locus. κ germline transcripts were also detected in normal developing B cell populations in fetal liver and bone marrow. Our collective results indicate that: (*a*) BLIN-1 can be induced to rearrange the κ locus, and this correlates with the expression of germline κ locus transcripts that may play a role in activating or targeting gene rearrangement; and (*b*) active rearrangement and usage of V genes representing different κ families suggest that, like in the mouse, repertoire diversification in humans occurs in the presence of a fixed heavy chain rearrangement.

The recombination of the Ig locus has been extensively studied through characterization of murine cell lines, recombination substrates, transgenic mice, and, recently, recombinase activator genes (for review, see references 1 and 2). However, the mechanism and control of the ordered progression of rearrangement events are still not well defined. Early studies used tumor cell lines arrested at distinct developmental stages, which made it difficult to determine the timing and activation of Ig recombination events in these cells. Subsequently, analyses of Abelson MuLV-transformed cell lines, which rearrange Ig genes during in vitro cell culture, have proven extremely informative in examining B cell differentiation in the mouse (3, 4). In addition to demonstrating an ordered progression of H and L chain gene recombination (1–3), these cells have provided the opportunity to examine early biases in V and D gene selection (3). However, there are still uncertainties about the precise timing and activation of recombination events.

It has been proposed that transcriptional activation of germline gene segments may be required for, or at least facilitate, the Ig recombination events (1, 2). In the murine system, transcripts have been detected from unrearranged Cμ (5), J_H (2), D-J (6), V_H (7), and J-C_κ (8) regions of the Ig locus. The exact role, if any, of these transcripts is uncertain; some produce a translatable message, while others do not. It has been postulated that transcriptional activation of these segments may alter local chromatin structure to provide accessibility to recombinases (1, 2, 8). Alternatively, such RNA may be directly involved in recombinase complexes (9). The requirement for these germline transcripts seemed even more compelling when it was reported that in some mouse pre-B cell cultures increased expression of the germline genes correlated with an increase in recombination frequency (10). If germline expression is a requirement, evolutionary maintenance might be expected. It is noteworthy that both H and L chain germline expression have also been reported in human cells (11, 12). However, noticeably absent from the list of germline gene expression is V_κ; no reports have demonstrated V_κ germline expression in mouse or human, although some attempts at detection have been made (10, 13, 14).

The control and mechanism of human Ig recombination is likely very similar to that found in the mouse. However, until recently, no in vitro cell culture system, comparable to the A-MuLV-transformed cell system, was available to study

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pre-B to B cell development in humans. We have recently established a human leukemic pre-B cell line designated BLIN-1 (15). Analysis of Ig rearrangement and expression in the original BLIN-1 cell line revealed a functional H chain rearrangement, with κ and λ gene segments in germline configuration. When BLIN-1 cells were cultured in 10% FCS they maintained a pre-B phenotype, with little or no evidence of κ rearrangement or expression. However, depletion of FCS induced a significant population of BLIN-1 cells to rearrange the κ locus, leading to the differentiation of surface κ^- pre-B cells into surface κ^+ B cells. Although the induction of κ rearrangement and expression in BLIN-1 is not understood, this system offers a unique opportunity to examine events involved in human pre-B to B cell differentiation. In this report, we describe the recombination potential of BLIN-1 cells, by characterizing several individual κ-expressing subclones for DNA rearrangement and V_κ gene usage. In addition, we have characterized κ transcription products, including germline J-C_κ and V_κ transcripts that correlate with the induction of κ recombination in these cells, as well as primary fetal cells.

Materials and Methods

Cell Culture and Extract Preparation. Cells were grown in RPMI 1640 (Gibco Laboratories, Grand Island, NY) containing either 10% or 1% FCS (Bethesda Research Laboratories, Bethesda, MD) or HB101 Nutridoma (Ventrex, Portland, ME), 2 mM L-glutamine, 50 μM β-mercaptoethanol, 25 U/ml penicillin, and 25 μg/ml streptomycin.

DNA Isolation, Digestion, and Southern Blot Analysis. Cultured cells (10^7) were isolated by centrifugation and lysed in 0.5 M NaCl/0.1% SDS/10 mM EDTA, pH 7.5. Predigested proteinase K was added at 1–2 mg/ml, and the samples were incubated at 50°C overnight. The DNA was then extracted once with phenol, twice with phenol/chloroform (1:1), and once with chloroform/isoamyl alcohol (24:1), and precipitated with 2 vol ethanol. Appropriate quantities of resuspended DNAs (10–20 μg) were digested with restriction endonucleases according to manufacturer's specifications, plus 50 μg/ml RNase A. Samples were then subjected to electrophoresis through 0.8–1% agarose/TAE gels and blotted onto nylon membranes (Nytran; Schleicher & Scheull, Inc., Keene, NH). After baking, membrane prehybridization and hybridization was as suggested by the manufacturer. After hybridization, the membrane was washed as described (16) and prepared for autoradiography.

RNA Isolation and Northern Blot Analysis. Total RNA was isolated by the procedure of Chomczynski and Sacchi (17). Poly $(A)^+$ RNA was isolated by the procedure of Aviv and Leder (18). RNAs used for PCR were digested with 1 U DNase RQ1 (Promega Biotec, Madison, WI) for 10 min at 37°C as described by the manufacturer. RNAs were glyoxalated (19) and separated on 10 mM Na phosphate, 1% agarose gels, and blotted onto nylon membranes (Nytran; Schleicher & Scheull, Inc.). Sizes were determined using RNA markers (Bethesda Research Laboratories). After baking, membrane prehybridization and hybridization were as suggested by the manufacturer. After hybridization, the membrane was washed as described (16) and prepared for autoradiography. The membrane was reprobed after removal of the previous probe by washing the membrane three times in 100°C H_2O for 20 min each.

Probes. The Huκ1 plasmid (20) and the HuC_κ probe were kindly provided by Dr. Phillip Leder (Harvard Medical School). The J_κ (HuJ_κ) probe was a 1.9-kb SacI fragment isolated from the Huκ1 plasmid and subcloned into pGEM 2. The set of human V_κ family probes, m1-8 (V_κI) (21), m607V-4 (22) (V_κII), m41V-7 (23) (V_κIII), and mAF-1/7 (24) (V_κIV), were kindly provided by Dr. Hans Zachau (University of Munich). The probes were nick-translated with α-[^{32}P]dCTP to a specific activity of 0.8–1 $\times$ 10^9 cpm/μg and annealed with the nylon-bound RNA or DNA.

Primers. The primers used were synthesized by the Institute of Human Genetics Micro-Chemical Facility (University of Minnesota, Minneapolis, MN): consensus V_κ, 5'GGTACCCAGTCTCCATCCTCCCTGTCT 3'; HC_κRT, 5'GCAGGCACACAACAGAGGCAGTTCC 3'; H5'J_κSEN, 5'CAGCTGACCCAGGACTCTGTT 3'; HV_κIV3', 5'AAGACCTACTGGCCCAGCGTATGG 3'; 7-9V_κIV, 5'CACAGTGCTTCAGCCTCGAACACAAA 3'. Primers were end-labeled using γ-[^{32}P]ATP and T4 polynucleotide kinase.

RNA PCR. RNA amplification was as described by Perkin-Elmer Cetus (25). For these experiments, 2 μg of total RNA was reverse transcribed and amplified using 20–30 pmol of the primers indicated in the text and subjected to cycling conditions of 30-s denaturation at 94°C, 1-min annealing at 42°C, and 1-min polymerization at 72°C repeated for five cycles. Then, for the next 25 cycles, annealing was done at 55°C for 1 min. Products were sized on a 2% Tris-borate-EDTA (TBE) agarose gel and subjected to subsequent DNA blot hybridization.

FACS® Analysis. Immunofluorescent detection of cell surface κ L chains was conducted on a FACScan® (Becton Dickinson & Co., Mountain View, CA) as previously described (15). Surface κ L chains were detected with a mAb recognizing the constant region of all κ L chains (hybridoma clone HB61; American Type Culture Collection, Rockville, MD), or the V_κIIIb-specific mAb, JGB1 (26), kindly provided by Dr. George Abraham, University of Rochester, Rochester, NY.

Results

Induced κ Gene Rearrangement in the Human BLIN-1 pre-B Cell Line. As previously reported (15), the BLIN-1 cell line maintains a stable pre-B phenotype when cultured in RPMI 1640/10% FCS, expressing cytoplasmic μ but no cytoplasmic or surface κ. Although there is some variation in frequency, we have repeatedly observed that when BLIN-1 cells are switched from serum-containing medium to serum-free medium, 4–20% of the cells become surface κ–positive after 10–21 d in culture. Randomly selected κ-positive subclones have been shown to be karyotypically identical to the original leukemic clone (15), and do not demonstrate any distinct growth advantage over κ-negative subclones. In this study, five independent κ-positive subclones were derived from BLIN-1 cells grown in serum-free medium (see Materials and Methods), and designated F4, B22, B31, 1E8, and 2E6. Each of these subclones demonstrated surface μ/κ expression as determined by immunofluorescent staining (data not shown). DNA and RNA were extracted and analyzed for κ gene rearrangement and V gene usage, respectively. Fig. 1 shows that by DNA blot hybridization with a C_κ probe, each of the subclones contains a unique pattern of κ gene rearrangement, confirming the individuality of each subclone and the recombination potential of the BLIN-1 cell line. It further appears that in two of the subclones (1E8 and 2E6) one κ allele has been deleted, presumably by recombination involving the κ-deleting element (κde) (27).

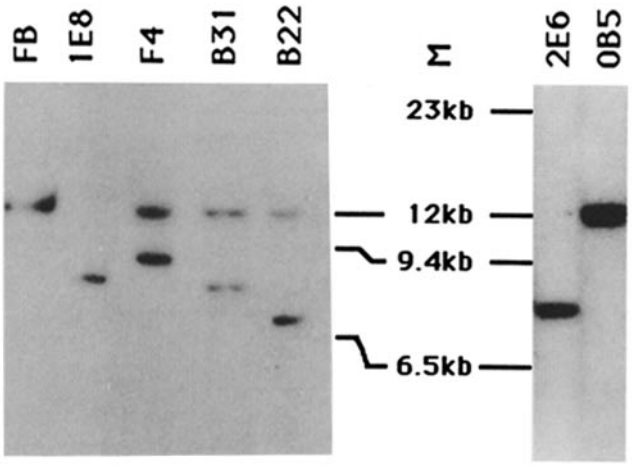

Figure 1. DNA blot hybridization analysis of the BLIN-1 subclones. Genomic DNA (10 μg) was digested with BamHI restriction nuclease. Digests were run through a 0.8% TAE/agarose gel and blotted onto Nytran membrane. The membrane was subsequently hybridized to HuC$_\kappa$. Fibroblast genomic DNA (FB) was also analyzed as a marker for the germline (unrearranged) κ alleles. Sizes were determined with a λ/HindIII (Bethesda Research Laboratories) ladder (M).

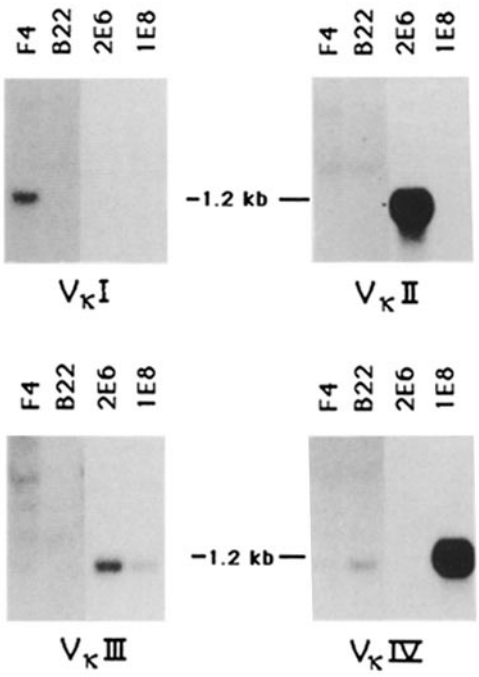

Figure 2. Analysis of BLIN-1 V$_\kappa$ usage by sequential hybridization of a Northern blot. 20 μg of RNA (F4 and B22) or 3 μg poly(A)$^+$ RNA (2E6 and 1E8) were subjected to electrophoresis through a 1% phosphate agarose gel and blotted onto Nytran membrane. The blot was probed, stripped, and reprobed with V$_\kappa$ family–specific probes (see Materials and Methods).

RNA extracted from four of the κ-positive subclones was analyzed by Northern blot hybridization (RNA from the B31 subclone was unavailable). The blot was subjected to sequential hybridization with probes representing the four different human V$_\kappa$ families (V$_\kappa$I, V$_\kappa$II, V$_\kappa$III, V$_\kappa$IV) under conditions that can distinguish family usage (Fig. 2). The V$_\kappa$I- and V$_\kappa$II-specific probes uniquely hybridized with the 1.2-kb mature κ mRNA species from subclones F4 and 2E6, respectively. Both the 1E8 and B22 RNA samples strongly hybridized to the V$_\kappa$IV probe. Based on these results, we concluded that both 1E8 and B22 express V$_\kappa$IV genes. Some minor crosshybridization to the V$_\kappa$III probe was noted in poly(A)$^+$-selected RNA from 2E6 and 1E8. Consistent with this conclusion was the fact that none of the subclones reacted with the V$_\kappa$IIIb-specific mAb JGB1 (data not shown). Although 1E8 and B22 express genes encoded by the V$_\kappa$IV family, they apparently arose from independent recombination events, as revealed by distinct κ recombination patterns on the DNA blot hybridization (Fig. 1). Since V$_\kappa$IV is a single-member gene family, the difference in the size of the productively rearranged alleles likely reflects different J segment utilization. While the number of clones examined is small, analysis of five randomly selected κ-positive subclones demonstrated independent recombination events of the starting BLIN-1 population, two of which appeared to result in expression of the same V$_\kappa$ gene.

Detection of V-J-C$_\kappa$ Transcripts in BLIN-1 Cells Grown in Serum-free Medium. Because BLIN-1 cells appear to actively rearrange Ig κ genes during serum-free culturing, we were interested in correlating transcriptional events with growth conditions that induce the recombination events. A PCR assay was used to increase our detection sensitivity for V-J-C$_\kappa$ transcripts in BLIN-1 populations. The BLIN-1 pre-B clone, OB5, was grown under different serum concentrations (10, 1, and 0%), and RNA was extracted from aliquots of cells after 1 and 3 wk. RNA was reverse transcribed with a C$_\kappa$ oligonucleotide primer (HC$_\kappa$RT), and the resultant single-stranded cDNA was amplified by PCR for 25 cycles, using the C$_\kappa$ primer paired with a consensus V$_\kappa$ primer (see Materials and Methods). The amplified products were subjected to electrophoresis on an agarose gel, blotted, and hybridized with a J$_\kappa$-specific probe (HuJ$_\kappa$) that only detects sequences internal to the two primers. As shown in Fig. 3 *A*, no detectable V-J-C$_\kappa$ transcripts were visable in PCR-amplified RNA from OB5 cells grown in either 10 or 1% serum, while a strong signal of the predicted 420 bp was present in PCR-amplified RNA from cells grown for 1 and 3 wk in serum-free medium. In this experiment, flow cytometric analysis demonstrated that at 3 wk, 4% of the BLIN-1 population grown in serum-free medium were surface κ positive, while surface κ–positive cells in serum-supplemented medium were below levels of detection (data not shown). Thus, V-J-C$_\kappa$ transcripts were easily detected under conditions that led to a significant frequency of surface κ–positive cells.

Detection of Transcripts from the Unrearranged J-C$_\kappa$ Locus in BLIN-1 Cells. Because germline transcription of unrearranged Ig genes has been proposed to be involved in their recombination (1, 2, 8), we analyzed BLIN-1 cells for the presence of the J-C$_\kappa$ germline transcript, which we recently demonstrated could be detected in human B cells (12). The 8.5-kb primary transcript from the unrearranged J-C$_\kappa$ locus

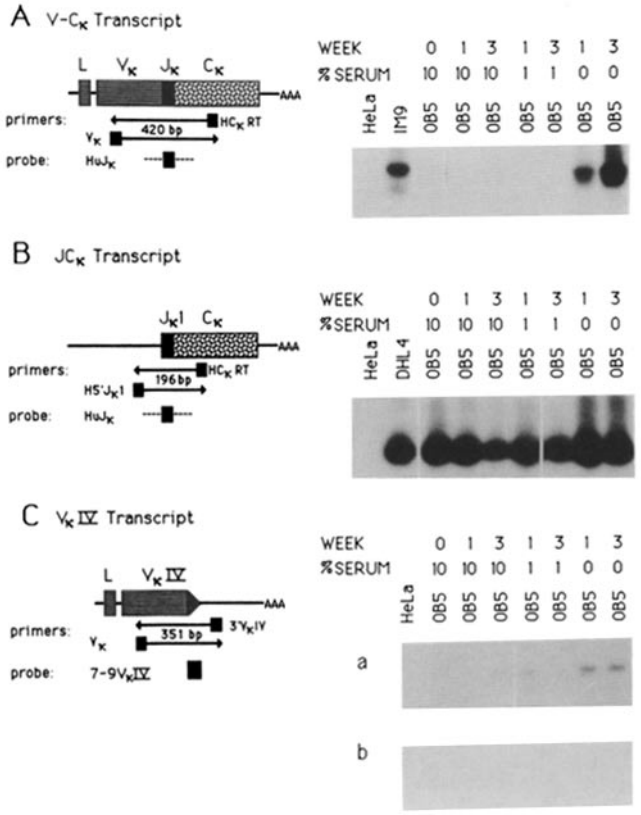

Figure 3. Identification of VJC_κ and κ germline transcripts in BLIN-1 cells. For each reaction, 2 μg of OB5 RNA was reverse transcribed and amplified. (*A*) Schematic diagrams of the V-J-C_κ ~420-bp RNA/PCR product (size depends upon which V_κ is used). RNA (2 μg) from control cell lines, HeLa (a negative control), and IM9 (VJC_κ^+) were also amplified and analyzed. Inserted panel shows the DNA blot hybridization autoradiograph of the PCR products. (*B*) Schematic diagrams of the JC_κ 196-bp RNA/PCR product. RNA (2 μg) from control cell lines, HeLa, and SU-DHL 4 (VJC_κ^+ and JC_κ^+) were also amplified and analyzed. Inserted panel shows the DNA blot hybridization autoradiograph of the PCR products. (*C*) Schematic diagram of the V_κIV 351-bp RNA/PCR product. Inserted panel shows the DNA blot hybridization autoradiograph of the PCR products for the reaction diagrammed. (*a*) The RNA/PCR that included reverse transcriptase before amplification; (*b*) an identical PCR using the same samples but without the reverse transcriptase step. For all PCR analyses, the primers and probes used for amplification and hybridization, respectively, are indicated.

initiates 4.1 kb 5' of $J_\kappa 1$, and is processed by two splicing events to generate a 1.5-kb mature mRNA; one of which results in a $J_\kappa 1$ splice to the C_κ coding region (D. J. Martin, unpublished results). We previously demonstrated by Northern blot studies that the BLIN-1 pre-B cell clone, OB5, constitutively expresses this germline J-C_κ transcript, and is not significantly induced by mitogenic activators, such as LPS (12; and D. J. Martin, unpublished results). RNA from the BLIN-1 cells grown as described above was analyzed by PCR for J-C_κ expression. RNA was reverse transcribed with the C_κ primer, followed by amplification using H5'$J_\kappa$sen, as a second primer specific to the 5' portion of the germline transcript (see Materials and Methods). Fig. 3 *B* shows that J_κ probe hybridization of the PCR products generated from RNA isolated in all growth conditions resulted in significant detection of the predicted 291-bp PCR product. Although the method is not strictly quantitative, expression of the germline J-C_κ appears constitutive, and does not appear to significantly change under conditions that induce V-J recombination.

Detection of Transcripts from the Unrearranged V_κIV Gene in BLIN-1 Cells. To test for the possibility of germline V_κ transcripts in BLIN-1 cells, a PCR assay was developed to detect transcripts specifically from the unrearranged V_κIV gene. The V_κIV gene was chosen for several reasons: (*a*) V_κIV is a single-member gene family that is most proximal to the J_κ locus (24, 28), and if similar to observed V(H) and D segment usage (29), may have an increased frequency of recombination; (*b*) sequence information for the 3' flanking region is available (24) for designing a specific PCR primer; and (*c*) two of the four κ-positive subclones demonstrated V_κIV gene usage, suggesting it may represent a frequent V gene targeted for recombination in the BLIN-1 cells. RNA from BLIN-1 cells grown in different serum concentrations was treated twice with DNAse and reverse transcribed with a primer immediately 3' of the V_κIV flanking heptamer-nonamer recognition sequences. This sequence is deleted upon V_κIV recombination, and thus would only be reverse transcribed if expressed from the unrearranged gene. PCR amplification was performed on RNA from the 1- and 3-wk BLIN-1 cultures using the V_κIV 3' flanking primer, paired with the consensus V_κ primer. The products were subjected to electrophoresis on a 2% agarose gel, blotted, and hybridized with an internal oligonucleotide specific to the V_κIV heptamer-spacer-nonamer sequence (see Fig. 3 *C*). A hybridizing product of the predicted 351 bp was easily detected when RNA from BLIN-1 cells grown in serum-free medium was used (Fig. 3 *C*, panel *a*), with minor hybridization detectable in some of the other samples. Because DNA contamination could result in the same PCR product, the RNA was subjected to identical amplification with the same primers, but without the inclusion of the reverse transcription step. Under these conditions, no amplification of V_κIV was detected (Fig. 3 *C*, panel *b*), indicating the V_κIV detected by PCR was derived from mRNA, not DNA. In agreement with previous reports demonstrating transient expression of V(H) germline genes, germline V_κIV transcripts were not detected in RNA from the BLIN-1 κ-expressing subclones (data not shown). Since subclones 1E8 and B22 express the rearranged V_κIV, this serves to also confirm that the amplification procedure was specific for the germline gene. We have not determined initiation and termination sites for these transcripts.

Detection of κ Germline Transcripts in Fetal Bone Marrow and Liver. To determine if the κ germline transcripts we detected in BLIN-1 cells are present in normal lymphoid tissue, a limited sample of lymphoid cells from fetal bone marrow or liver was assayed for the presence of κ germline transcripts. Previous reports indicated that the first detectable pre-B cells appear in fetal liver at 8–10 wk of gestation (30). B cells expressing surface μ can be detected by 13 wk (30, 31). Between 16 and

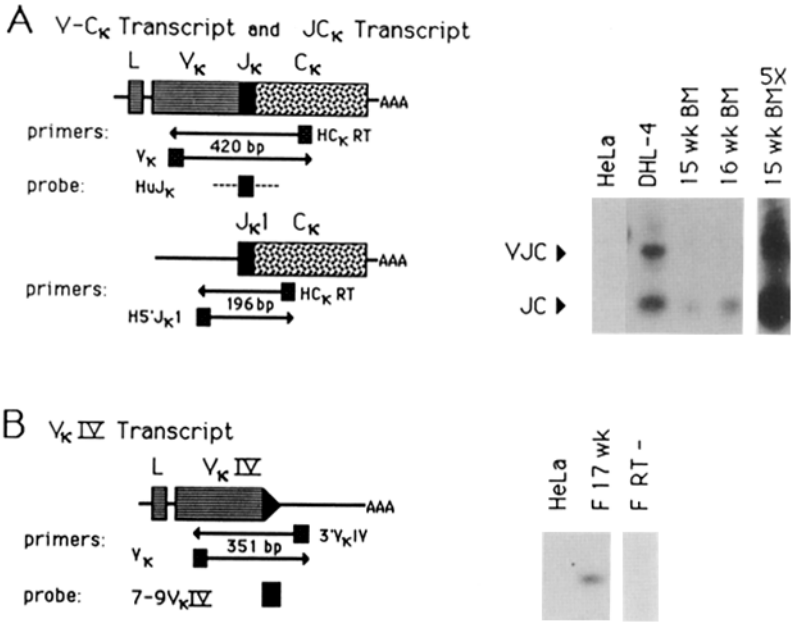

Figure 4. Identification of VJC_κ, JC_κ, and $V_\kappa IV$ transcripts in human fetal tissues. (*A*) Schematic diagrams and autoradiographs of the VJC_κ 420-bp RNA/PCR product and the JC_κ 196-bp RNA/PCR product. RNA (2 μg) from 15- and 16-wk fetal bone marrow (BM) and control cell lines, HeLa (a negative control), and SU-DHL 4 (a positive control for both VJC_κ and JC_κ transcripts) were reverse transcribed and amplified. (*B*) Schematic diagram and autoradiograph of the $V_\kappa IV$ 351-bp RNA/PCR product. 17-wk fetal liver (F) and HeLa RNA (2 μg) were reverse transcribed and amplified. A control PCR reaction using the same samples but without the reverse transcriptase step is shown (F RT–). Primers and probes used for amplification and hybridization, respectively, are indicated.

18 wk, a rapid wave of B cell expansion is observed; thus, the period between 13 and 18 wk appears to represent a critical interval in B cell development (30, 31). Lymphoid cells from a 15- and 16-wk fetal marrow were isolated by Ficoll-Hypaque gradient centrifugation, lysed, and the RNA was extracted. Bone marrow RNA was reverse transcribed with the $HC_\kappa RT$ primer, and subsequently amplified by PCR after adding both the consensus V_κ and $H5'J_\kappa sen$ primers. In this reaction, both the germline J-C_κ and any rearranged V-J-C_κ transcripts should be co-amplified (see Fig. 4 *A*). As a positive control, RNA from the cell line SU-DHL 4 was also amplified in the same manner. This cell line contains both a functionally rearranged V-J-C_κ allele and a germline allele, which are both transcribed (12). As described for the BLIN-1 analysis, PCR products were run on an agarose gel, blotted, and probed with a J_κ-specific probe. Fig. 4 *A* shows that after amplification of SU-DHL 4 RNA, both the rearranged and germline transcripts are easily detectable and are at comparable levels. The germline J-C_κ PCR product is also easily detectable in the fetal RNA samples, and V-J-C_κ products were only visible after longer exposure (five times). Although we have not attempted accurate quantitation, these results suggest germline transcription of the J-C_κ locus is significant in the early developing B cells of these fetal samples. Fetal RNA was also amplified using the primers that specifically detected $V_\kappa IV$ germline transcripts in the BLIN-1 cells (Fig. 4 *B*). Reverse transcription, PCR amplification, and DNA blot hybridization were performed as described above, and resulted in a detectable signal of the expected size. A control reaction to ensure there was no DNA contamination (amplification without reverse transcription) was run simultaneously.

In summary, we detected germline transcripts from three independent fetal samples, and conclude from the representative results presented in Fig. 4 that germline transcription is active in human fetal bone marrow and liver during a critical period of early B cell ontogeny.

Discussion

Our initial characterization of the BLIN-1 pre-B leukemic cell line (15) suggested that this cell line could provide a unique opportunity to analyze a critical developmental stage in human B cell differentiation. In the current study, we have examined independent κ-expressing subclones derived from BLIN-1 cells grown in serum-depleted medium. Each subclone represents an independent κ recombination pattern, confirmed by the DNA blot hybridization results presented in Fig. 1. The V_κ gene family usage determined by Northern analysis showed that two of the clones expressed $V_\kappa IV$ (a single-member gene family), one subclone expressed $V_\kappa I$, and one subclone expressed $V_\kappa II$. Although the current study is obviously a limited clonal analysis, it convincingly demonstrates the recombination potential of the BLIN-1 cell line. It is noteworthy that $V_\kappa IV$ has been mapped as the most proximal to the J_κ cluster (28) (23 kb upstream); and, in a survey of lymphoid cell lines, the $V_\kappa IV$ gene was found to be frequently rearranged (24). Moreover, in a recent analysis of fetal κ expression, the $V_\kappa IV$ gene was highly overexpressed compared with other V_κ genes (33). Preferential rearrangement of the most proximal V_κ gene would be analogous to reports demonstrating preferential recombinations of the most proximal V and D gene segments in the H chain locus (29). However, the active rearrangement and usage of V genes representing different κ families suggest that in humans, as seen for the murine system (4), repertoire diversification at this stage of B cell development can occur in the presence of a single, functional H chain rearrangement.

The analysis of germline transcription strongly correlates with the recombination potential of the BLIN-1 cell line.

Because the frequency of cells that appear to undergo κ rearrangement is small, we made use of PCR amplification to monitor expression. In each case the specificity was ensured by annealing requirements of the two primers, as well as the use of internal sequences as hybridization probes. We have not rigorously established the quantitative correlation of PCR products; however, in some cases significant differences in the detection of PCR products were reproducibly observed. Compared with serum-supplemented growth conditions, a significant frequency of recombination is observed in serum-depleted medium; and this is clearly reflected in the amount of V-J-C_κ PCR product obtained (Fig. 3). The germline J-C_κ transcript appears to be constitutively expressed under all growth conditions (13, and Fig. 3 *B*). Furthermore, we have obtained evidence strongly suggesting that the germline V_κIV gene can be transcribed in BLIN-1 cells (Fig. 3 *C*). Although germline V_κIV transcripts could be detected by PCR in cells grown in 1% serum, a significant increase was consistently found in cells grown in 0% serum. Longer exposure of Fig. 3 *A* also revealed small but detectable levels of PCR products from rearranged V-J-Cκ transcripts in cells grown in serum, although the frequency of κ-producing cells was below the sensitivity of FACS® analysis. The increased sensitivity afforded by our PCR analysis suggests that while a low frequency of κ rearrangement may occur in BLIN-1 cells grown in low serum, induction of κ rearrangement is greatly enhanced in cells grown in 0% serum, concomitant with a significant increase in VκIV germline transcription. This appears to be a transient event, since no germline V_κIV expression was detected in any of the κ-producing subclones. Despite several different approaches, others have not detected transcripts from unrearranged κ V regions in the mouse (10, 13, 14). It is possible that germline V_κ expression is very transient, and uniquely prolonged in BLIN-1 cells. To determine if germline transcripts could be detected in normal lymphoid cells, we assayed 15–17-wk human fetal liver and marrow samples. The results presented in Fig. 4 demonstrate that PCR products derived from both germline J-C_κ and V_κIV could be detected, and germline expression appeared to be greater than rearranged gene expression at early fetal time points (Fig. 4 *A*). Recently, a developmental time course of RNA transcribed from germline and rearranged Ig genes in murine fetal liver was reported (14). The order of expression was consistent with the idea that transcriptional activation of germline loci is a prerequisite for Ig gene rearrangement. A similar analysis in humans might be accomplished by FACS®-purifying B cell precursors and B cells from fetal tissue of different gestational ages. However, our current study is qualitatively consistent with a model in which germline transcription precedes gene rearrangement. It also correlates with the high levels of V_κIV expression observed in early fetal bone marrow (33).

In summary, our results demonstrate the recombination potential of the BLIN-1 pre-B cell line, and add to the growing body of evidence that suggests transcriptional activation of the germline gene segments is important in B cell development. Activation of Ig κ gene rearrangement has been correlated with the induction of κ germline transcription in the mouse (10). Because these transcripts cannot be translated (34, and D. J. Martin, unpublished results), it appears unlikely that they code for critical protein products. It has been suggested that transcriptional activation of germline gene segments may serve to make the recognition signal sequences that flank Ig gene segments accessible to recombinases (1, 2, 8). Alternatively, it is possible that the RNA transcripts participate in the recombinase complex directly (9).

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