

THE κ -DELETING ELEMENT

Germline and Rearranged, Duplicated and Dispersed Forms

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Humans express one of two available Ig light chain classes on the surface of B cells at nearly equivalent percentages (κ 60%; λ 40%). Despite the frequent expression of each class there is an ordered sequence to L chain rearrangement in humans in which κ generally rearranges before λ (1–3). This hierarchy includes an unexpected deletion of the κ locus that precedes λ rearrangement during pre-B cell development. We previously cloned a κ -deleting element (Kde)¹ that uniformly mediates this elimination of the κ locus (4). Klobeck and Zachau (5) mapped the Kde to a position 24 kb 3' to C_{κ} . In the majority of instances the Kde rearranged into the J_{κ} - C_{κ} intron at a conserved heptamer (CACAGTG) to eliminate the C_{κ} and enhancer (E_{κ}) regions (4, 5). Moreover, the loss of κ genes in λ -producing B cells is also observed in the mouse (6, 7). The murine counterpart of the Kde, the recombining sequence (RS) has been characterized by Durdick et al. (8) and Moore et al. (9).

In this study, we address remaining questions concerning the role of the human Kde. In up to 40% of instances the Kde rearranges upstream to the J_{κ} region and eliminates J_{κ} as well as E_{κ} and C_{κ} . We wished to determine the identity of this upstream target site and in particular to ask if it might be a V_{κ} region. Moreover, when the Kde rearranges into the J_{κ} - C_{κ} intron (J_{κ} -Kde) it possesses an additional rearrangement at the 5' end of J_{κ} . We wished to know if these were aberrant attempts at V/J rearrangement that perhaps preceded the introduction of the Kde; or, whether the Kde was nondiscriminatory and destroyed κ alleles with valid V/J rearrangements. Furthermore, we searched the sequence of the Kde within its germline form to determine if it might encode a protein that could be postulated to perform a negative regulatory role in preventing λ rearrangement. Alternatively, rearrangements of the Kde always place it in the vicinity of a V_{κ} promoter with its octamer enhancer sequence. This could conceivably induce the production of a positive *trans*-acting factor from the Kde that would

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¹ Abbreviations used in this paper: E, enhancer region; Kde, κ -deleting element; RS, recombining sequence.

facilitate λ gene rearrangement. In addition to comparing the DNA sequence of germline Kde, V_{κ}/Kde , and V/J -Kde forms, we also searched for corresponding mRNA transcripts. Finally, we noted an additional genomic fragment that crosshybridized to the Kde and demonstrated that this conserved and duplicated locus was present at another chromosomal site.

Materials and Methods

Southern Blot Analysis. High molecular weight genomic DNA or isolated plasmid or phage DNA was digested to completion with restriction endonucleases, electrophoresed in agarose gels, and transferred to nitrocellulose filters (10). Purified, cloned DNA fragments were radiolabeled with ^{32}P by random hexanucleotide priming to specific activities of $1-5 \times 10^8$ cpm/ μ g for use as probes (11). Blots were hybridized in 10% dextran sulphate, 30–50% formamide, $4\times$ SSC, $1\times$ Denhardt's solution, and 10 μ g/ml salmon sperm DNA. Blots were washed three times in $2\times$ SSC, 0.1% SDS at room temperature and twice in 0.1% SDS with varying SSC and temperature conditions to control for stringency.

Northern Blots Analysis. Oligo(dT) column-purified poly(A)⁺ RNA was selected from guanidine thiocyanate-prepared total RNA of cell lines. 5 μ g was denatured in formamide, electrophoresed on agarose-formaldehyde gels, and transferred to nitrocellulose paper (12). A γ -actin probe guaranteed that intact, hybridizable RNA was present in each lane (13).

Genomic and cDNA Cloning. A genomic library of SU-DHL-6 was constructed by digesting DNA to completion with Bam HI and inserting into charon 28 phage vector and packaging in vitro (12). This library, an oligo(dT)-primed λ g10 cDNA library (14) of SU-DHL-6 and a germline genomic library of human peripheral blood in EMBL 3 were screened by the Benton and Davis technique (12). Plasmid subclones of isolates were restriction mapped and sequenced.

DNA Sequencing. DNA fragments were subcloned into M13 phage vectors and their sequences were determined by dideoxy-chain termination (15).

Chromosomal in Situ Hybridization. DNA fragments subcloned into plasmids were nick translated with [3H]dNTPs and used in a chromosome in situ hybridization of normal metaphases from PHA-stimulated lymphocytes from several normal males and one female (16).

Somatic Cell Hybrid Analysis. Genomic DNA from a previously characterized panel of hamster \times human and mouse \times human somatic cell hybrids were examined with human probes to map their location (17, 18).

Results

Aberrant V/J Rearrangements on J_{κ} -Kde Alleles. We noted that κ alleles that had rearranged the Kde into the J_{κ} - C_{κ} intron also possessed an additional rearrangement 5' to J_{κ} (Fig. 1). To determine the nature of such rearrangements, we mapped and sequenced the 5' rearrangements on both κ alleles of the pre-B cell stage acute lymphoblastic leukemia line, Nalm-6. We wished to determine whether these were attempted V/J rearrangements and whether they were valid recombinations or aberrant. Comparison of the two Nalm-6 alleles (Fig. 1, B and C) with the germline κ locus (Fig. 1A) revealed the rearrangements to be a V_{κ}/J_3 and a V_{κ}/J_5 . Upon closer inspection of the sequence the 11.5-kb allele was a V_{κ} subgroup I juncture with $J_{\kappa}3$ that was aberrant in nature (Fig. 2). 8 bp of $J_{\kappa}3$ information had been lost and 4 bp (GGGG) that were apparently extranucleotides had been added. These changes resulted in a frame shift and the prediction of a nonfunctional peptide product. The 8.8-kb Nalm-6 allele had introduced a

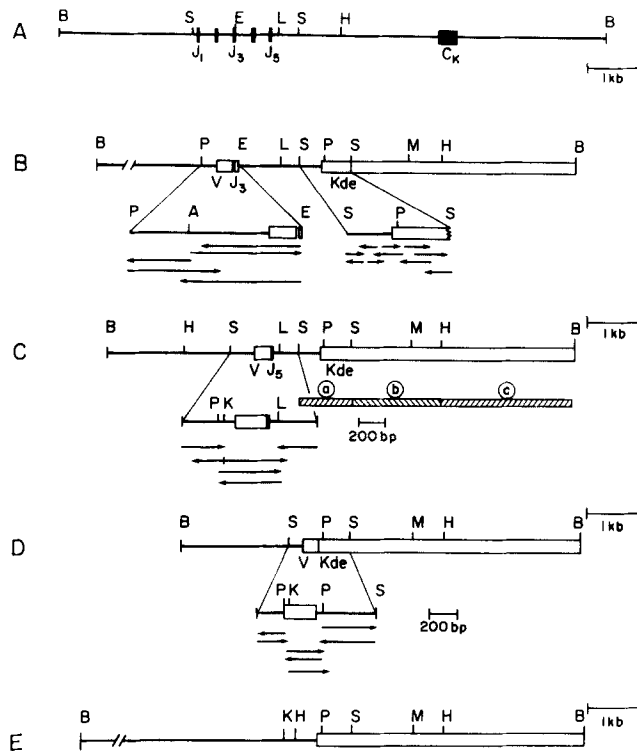


FIGURE 1. Schematic presentation of (A) the human germline J_{κ} - C_{κ} locus: (B and C) restriction maps and sequencing strategies for both the 11.5-kb and 8.8-kb κ alleles of the Nalm-6 pre-B cell line. (D) The V_{κ} / Kde rearranged allele of the SU-DHL-6 cell line. (E) The germline Kde . Sa cl (S), Eco RI (E), $Hind$ III (H), Pst I (P), Bam HI (B), Sma I (M).

V_{κ} III region into $J_{\kappa}5$ with the loss of 2 bp of J_{κ} information and the presence of 8 bp of uncertain origin (Fig. 3). Once again the frame shift resulted in an aberrant product.

The Upstream Target of the Kde is a V_{κ} Segment. In ~40% of instances when the κ gene is deleted the J_{κ} regions are eliminated along with the C_{κ} and E_{κ} . In this situation the Kde on the allele is always rearranged. We sought to characterize the target site of the Kde rearrangement that deleted J_{κ} , E_{κ} , and C_{κ} in the SU-DHL-6 cell line. Salient features of this cell included the fact that it was a κ chain-producing mature B cell line and that the Kde had eliminated the excluded κ allele (4). Moreover, it represented the rare example of a κ producer that possessed two rearranged λ gene alleles. A genomic library was prepared from SU-DHL-6 and its rearranged Kde allele was cloned (Fig. 1D) and sequenced (Fig. 4). The site of rearrangement within the Kde was the exact same area that also mediated its rearrangement with the conserved heptamer (CACAGTG) within the J_{κ} - C_{κ} intron. In this instance, the Kde was rearranging site specifically with a V_{κ} region. The site of recombination was cleanly focused at the 3' end of a V_{κ} III region implying that the heptamer-spacer-nonamer helped mediate this recombination.

Structural Analysis of the Rearranged and Germline Kde. The restriction map of the germline Kde (Fig. 1E) and rearranged forms of the Kde (Fig. 1, B, C, and D) suggested that this unique element repeatedly rearranged at the same site. The nucleic acid sequence of the Kde in its rearranged form was determined on a V_{κ} / J_{κ} - Kde allele (Fig. 1, B) and a V_{κ} / Kde allele (Fig. 1D), which is presented

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          -320      -310      -300      -290      -280
          *         *         *         *         *
          C TGC AGC TGT GCT CAG CCT GCC CCA TGC CCT GCT GAT TGA TTT
-270      -260      -250      -240      -230      -220
          *         *         *         *         *
GCA TGT TCA GAG CAC AGC CCC CTG CCC TGA AGA CTT TTT TAT GGG CTG GTC GCA CCC TGT
-210      -200      -190      -180      -170      -160
          *         *         *         *         *
CGA GGA GTC AGT CTC ACT CAG GAC ACA GCATG GAC ATG AGG GTC CCC GCT CAG CTC CTG G
          Leader
          Met Asp Met Arg Val Pro Ala Gln Leu Leu G
-150      -140      -130      -120      -110      -100
          *         *         *         *         *
GG CTC CTG CTA CTC TGG CTC CGA GGTA AGG ATG GAG AAC ACT AGG AAT TTA CTC AGC CAG
ly Leu Leu Leu Trp Leu Arg A
-90      -80      -70      -60      -50      -40
          *         *         *         *         *
TGT GCT CAG TAC TGA CTG GAA CTT CAG GGA AGT TCT CTG ATA ACA TGA TTA ATA GTA AGA
-30      -20
          *         *
ATA TTT GTT TTT ATG TTT CCA ATC TCA GGT GCC AGA TGT GAC ATC CAG ATG ACC CAG TCT
          L'-----FR1-----
          rg Ala Arg Cys Asp Ile Gln Met Thr Gln Ser
          30          40          50          60          70          80
          -----CDR1-----
CCA TCC TCC CTG TCT GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC CGG GCA AGT CAG
Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln
          90          100          110          120          130          140
          -----FR2-----
AGC ATT AGC AGC TAT TTA AAT TGG TAT CAG CAG AAA CCA GGG AAA GCC CCT AAG CTC CTG
Ser Ile Ser Ser Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu
          150          160          170          180          190          200
          -----CDR2-----FR3-----
ATC TAT GCT GCA TCC AGT TTG CAA AGT GGG GTC CCA TCA AGG TTC AGT GGC AGT GGA TCT
Ile Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser
          210          220          230          240          250          260
          -----
GGG ACA GAT TTC ACT CTC ACC ATC AGC AGT CTG CAA CCT GAA GAT TTT GCA ACT TAC TAC
Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr
          270          280          290          300
          -----CDR3-----Vk I-----Jk 3-----
TGT CAA CAG AGT TAC AGT ACC CCT GGG GCG GCC CTG GGA CCA AAG TGG AT
Cys Gln Gln Ser Tyr Ser Thr Pro Gly Ala Ala Leu Gly Pro Lys Trp
Germline Jk 3          CACTGTGA TTC ACT TTC GGC CCT GGG ACC AAA GTG GAT ATC AAA CGT
AA sequence FR4          Phe Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys Arg

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FIGURE 2. DNA sequence of 11.5-kb Nalm-6 κ allele at site of J_{κ} rearrangement reveals an aberrant $V_{\kappa}1/J_{\kappa}3$ juncture. These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number Y00646.

in Fig. 4. The juxtaposition of the Kde with a V_{κ} region and its promoter suggested the possibility of a fusion transcript and the generation of a potential fusion peptide. However, sequence analysis of this rearrangement indicates that only eight amino acids of Kde origin would be added to the V_{κ} region before a stop codon was encountered (Fig. 4). The remaining sequenced portion of the rearranged Kde also possessed numerous stop codons in all three potential reading frames. Thus, no attractive protein product was predicted from this portion of the rearranged Kde when introduced into either a V_{κ} or the J_{κ} - C_{κ} intron.

To further analyze the mechanism of recombination and to structurally characterize the native form of the Kde we obtained germline clones of the Kde from an EMBL3 genomic library prepared from human peripheral blood cells. A

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-260          -250          -240          -230          -220          -210
*            *            LEADER-----
CAG TTA GGA CCC AGA GGA ACC ATG GAA ACC CCA GCG CAG CTT CTC TTC CTC CTG CTA CTC
Met Glu Thr Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu

-200          -190          -180          -170          -160          -150
-----*-----*-----*-----*-----*-----*-----
TGG CTC CCA GGT GAG GGG AAC ATG GGA TGG TTT TGC ATG TCA GTG AAA ACC CTC TCA AGT
Trp Leu Pro A

-140          -130          -120          -110          -100          -90
*            *            *            *            *            *
CCT GTT ACC TGG CAC TCT GCT CAG TCA ATA CAA TTA AAG CTC AAT ATA AAG CAA TAA TTC

-80           -70           -60           -50           -40           -30
*            *            *            *            *            *
TGG CTC TTC TGG GAA GAC AAT GGG TTT GAT TTA GAT TAC ATG GGT GAC TTT TCT GTT TTA

-20           -10           1           10           20           30
*            L'-----FR1-----
TTT CCA ATC TCA GAT ACC ACC GGA GAA ATT GTG TTG ACG CAG TCT CCA GGC ACC CTG TCT
sp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser

40           50           60           70           80           90
-----CDR1-----
TTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC AGG GCC AGT CAG AGT GTT AGC AGC AGC
Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser

100          110          120          130          140          150
-----FR2-----CDR2-----
TAC TTA GCC TGG TAC CAG CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TAT GGT GCA
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Gly Ala

160          170          180          190          200          210
-----FR3-----
TCC AGC AGG GCC ACT GGC ATC CCA GAC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC
Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe

220          230          240          250          260          270
-----CDR3-----
ACT CTC ACC ATC AGC AGA CTG GAG CCT GAA GAT TTT GCA GTG TAT TAC TGT CAG CAG TAT
Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr

280          290          300          310          320          330
VkIII-----Jk5.....IVS
GGT AGC TCA CCT CCA TGT ACA CCT TCG GCC AAG GGA CAC GAC TGG AGA TTA AAC GTA.....
Gly Ser Ser Pro Pro Cys Thr Pro Ser Ala Lys Gly His Asp Trp Arg Leu Asn Val

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GERMLINE Jk5....CTCTGTG ATC ACC TTC GGC CAA GGG ACA CGA CTG GAG ATT AAA CGT
AA FR4 Ile Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg

FIGURE 3. DNA sequence of 8.8-kb Nalm-6 κ allele at site of J_κ rearrangement reveals an aberrant V_κIII/J_κ5 juncture. These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number Y00646.

restriction map of the germline Kde is shown in Fig. 1E. DNA sequence of the Kde surrounding the breakpoint site has been reported (5, 19) and will not be represented here in detail other than to note that the most highly conserved areas with the mouse RS (9) are the heptamer (CACTGTG), a 23-bp spacer, a nonamer (AGTTTCTGC), and an adjacent 3' region (Fig. 5).

Search for a Transcriptional Unit. We wished to determine if any portion of the Kde was transcriptionally active within either its germline or rearranged form. Probes representing the 1.0-kb Sac I (a), 1.8-kb Sac I-Hind III (b), and 2.5-kb Bam HI-Hind III (c) were derived from the cloned Kde (Fig. 1C). Probes were hybridized with Northern blots possessing 5 μ g of pA-RNA from one pre-B cell with germline Kde, three pre-B cells with rearranged Kdes, four κ -producing B cells with germline Kde, one κ -producing B cell with rearranged Kde, six λ -producing B cells with rearranged Kde, three T cells with germline

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-280      -270      -260      -250      -240
GAG CTC TGG AGA AGA GCT GCT CAG TTA GGA CCC AGA GGA ACC ATG GAA ACC CCA GCG
Leader-----
-230      -220      -210      -200      -190      -180
CAG CTT CTC TTC CTC CTG CTA CTC TGG CTC CGA GGT GAG GGG AAC ATG GGA TGG TTT
Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro G
-170      -160      -150      -140      -130      -120
TGC ATG TCA GTG AAA ACC CTC TCA AGT CCT GTT ACC TGG CAC TCT GCT CAG TCA ATA
-110      -100      -90      -80      -70
CAA TAA TTA AAG CTC AAT ATA AAG CAA TAA TTC TGG CTC TTC TGG GAA GAC AAT GGG
-60      -50      -40      -30      -20      -10
TTT GTT TTA GAT TAC ATG GGT GAC TTT TCT GTT TTA TTT CCA ATC TCA GAT ACC ACC
sp Thr Thr
1          10          20          30          40          50
FR1-----
GGA GAA ATT GTG TTG ACG CAG TCT CCA GGC ACC CTG TCT TTG TCT CCA GGG GAA AGA
Gly Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg
60          70          80          90          100         110
CDR1-----FR2-----
GCC ACC CTC TCC TGC AGG GCC AGT CAG AGT GTT AGC AGC ACC TAC TTA GCC TGG TAC
Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Thr Tyr Leu Ala Trp Tyr
120         130         140         150         160
CDR2-----
CAG CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TAT GGT GCA TCC AGC AGG GCC
Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Gly Ala Ser Ser Arg Ala
170         180         190         200         210         220
FR3-----
ACT GGC ATC CCA GAC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC ACT CTC ACC
Thr Gly Ile Pro Asp Arg Phe Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
230         240         250         260         270         280
CDR3-----
ATC AGC AGA CTG GAG CCT GAA GAT TTT GCA GTG TAT TAC TGT CAG CAG TAT GGT AGC
Ile Ser Arg Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser
290         300         310         320         330
TCA CTG GCA GCC CAG GGC GAC TCC TCA TGA GTC TGC AGC TGC ATT TTT GCC ATA TCC
Ser Leu Ala Ala Gln Gly Asp Ser Ser STOP
KDE-----
340         350         360         370         380         390
ACT ATT TGG AGT CTG ACC TCC CTA GGA AGC CTC CCT GCT CCC TAG GAC AAC CTG CTC
400         410         420         430         440         450
TGA CCT CTG AGG ACC TGT CTG TAA ACG TCC AGA GAA AAG CAT CTG CCT GAA GGG TCT
460         470         480         490         500         510
ATG AAG GGG CTT GAG GCA AGT AGG GAG CCC AGC CCA GCT AAC ATT TGC AGC CAT GGG
520         530         540         550         560
ATG GCT TTG TGT ACC TAG AAA AGC AAA GAT GAG GAC TAG GCG AGC ACA GGC CCC AAA
570         580         590         600         610         620
CGT TCA CGA TAC ACA TCT CAC TGC AAA AAG ATG TCC TCT GGC CAC TTT ATA ATG CAG
630         640         650         660         670         680
CTC TAA CTA TAA CTG GTG CTT TGC TGG TTT GTG GCC TGG GCT GGT CTC CCA GAG TCA
690         700         710         720         730
GTG GCT TTG GGT GAG ATG GCT CCA GGA GAC AGC AGA AAC TCT CAT ATA TGA AGC CTT
740         750         760         770         780         790
GCT TGC AGC GAT TTG AGG CTT ACT AGG GAA AAG CCA TGA TGG GTT TTA TAG AGC ATT
800         810         820
AAC TGT GAT ACG CCA TCA TGG GCT GAG AGC TC

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FIGURE 4. DNA sequence of the V_H/Kde juncture of the SU-DHL-6 cell line. These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number Y00646.

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Recombination points
          9 mer          7 mer
HUMAN  AGCTCTTACCCTAGAGTTTCTGCACGGGCAGCAGGTTGGCAGCGCACACTGTGGGAGCCCTAGTGGCAGCCCAGGGCGACTCCTCATGAGTCTGCAGC
MOUSE  ACTGCTCTTGAACCCAGTTTCTGCACGGGCAGTCAGTTAGCAGCACTCACTGTGAGGACCCCTAGTGGCAGCCCAGGGTGGATCTCCCTAGGACTGCAGT
          ↓ ↓ ↓

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FIGURE 5. Comparison of the human Kde and mouse RS sequence (9) at its region of highest conservation and localization of sequence breakpoints (arrows).

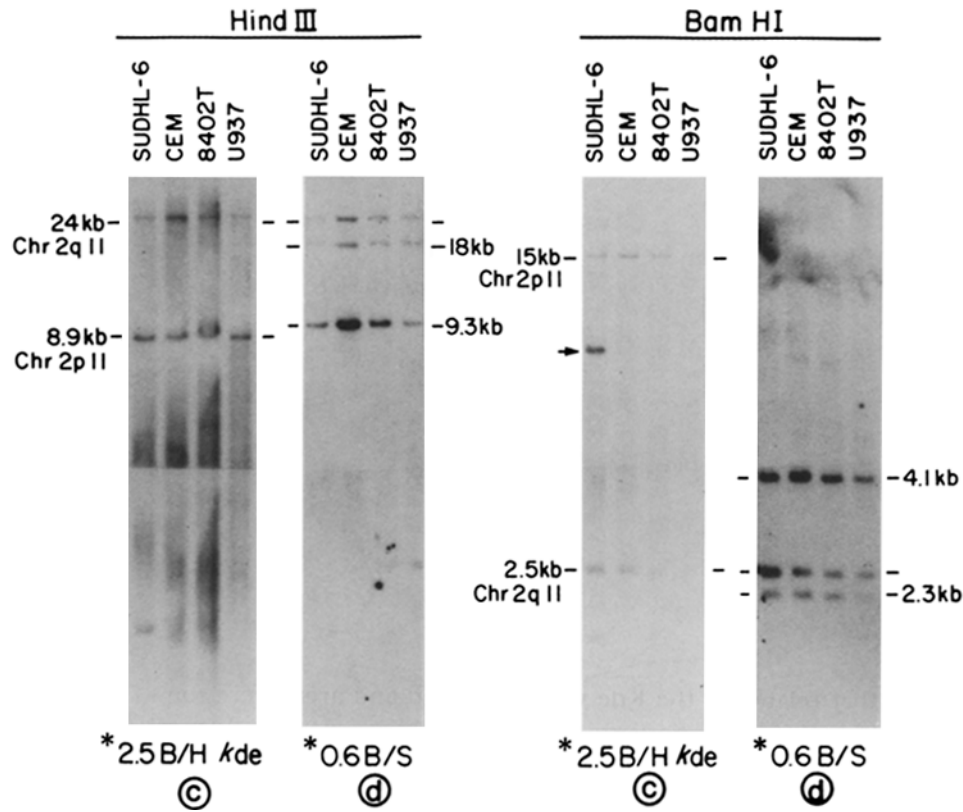


FIGURE 6. Southern blot of Hind III-digested and Bam HI-digested human genomic DNA from SU-DHL-6 (κ used producing B cell line with a rearranged Kde allele), CEM and 8402 (T cells), and U937 (monocyte). Probes utilized were the 2.5 Kb Bam HI-Hind III "c" and 0.6 Kb Bam HI-Sac I "d".

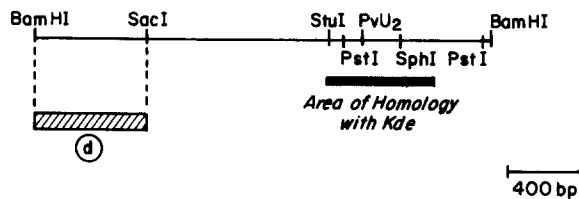


FIGURE 7. Schematic presentation of the 2.5 Kb Bam HI fragment cross-hybridizing with the Kde. The area of homology with the Kde is indicated while the 0.6 Kb Bam HI-Sac I fragment utilized as probe "d" did not share homology.

Kde, and two nonlymphoid cells with germline Kde. All examinations failed to reveal unique Kde transcripts while a γ -actin probe confirmed that intact, hybridizable RNA was present (data not shown). Moreover, a cDNA library was prepared in λ gt10 from SU-DHL-6 which possessed a V_{κ}/Kde rearrangement (Fig. 1D, Fig. 4). 75×10^4 plaques were screened with a V_{κ} III probe as well as a Kde probe and no unique V_{κ}/Kde fusion or Kde cDNAs were identified.

Duplication and Dispersion of the Kde. Southern analysis using the 2.5-kb Hind III-Bam HI region of the Kde (probe c in Fig. 1C) recognized its native 15-kb genomic fragment, but also routinely crosshybridized to a 2.5-kb Bam HI fragment (Fig. 6). When this same probe c was used upon Hind III-digested

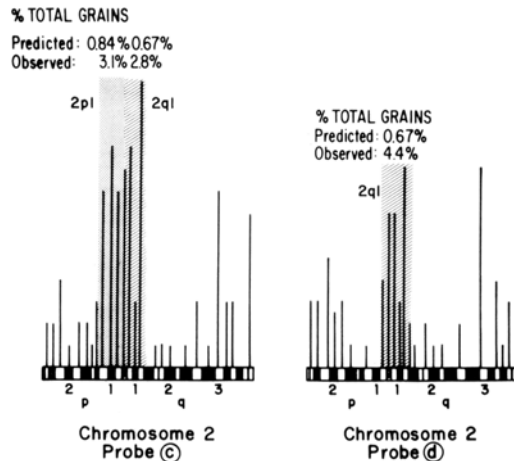


FIGURE 8. Histogram of chromosomal in situ hybridization of probe c and d to chromosome 2. Probe c recognized segment 2p1 where 3.1% of all grains localized, while on a size-calculated basis only 0.84% would be predicted by random distribution; and 2q1 where 2.8% was observed and 0.67% expected. In contrast, probe d was present at 2q1, 4.4% observed, 0.67% expected. A secondary site may be present at 2q3.

DNA it recognized its native 8.9-kb fragment as well as an additional 24-kb crosshybridizing fragment (Fig. 6). These data suggested that this additional crosshybridizing region was not simply a tandemly linked duplication of the Kde. To prove that this extra band represented a duplicated and dispersed region we cloned the 2.5-kb Bam HI crosshybridizing genomic fragment. Portions of this region that related to the Kde were identified and areas were found that were unique (Fig. 7). A 0.6-kb Bam HI–Sac I probe d was prepared from this area (Fig. 7) that recognized its native 2.5-kb Bam HI genomic fragment, but not the original Kde. However, probe d recognized two additional Bam HI fragments of 2.3 and 4.1 kb. Examinations of Hind III–digested DNA also revealed two additional crosshybridizing bands (Fig. 6). This implied that the unique portion of the 2.5-kb Bam HI genomic region (Fig. 7) had also been duplicated and dispersed.

To determine the chromosomal location of these genetically related regions we performed a series of chromosomal in situ hybridizations. The 2.5-kb Hind III–Bam HI Kde probe c was nick translated with [³H]dNTPs and hybridized to metaphase chromosomes from PHA-stimulated lymphocytes from normal subjects. Analysis of 314 metaphases revealed primary peaks at 2p11–13 and 2q11–13 (Fig. 8). When the 0.6-kb Bam HI–Sac I probe d was used it recognized its native location of 2q11–13 as a primary site, but only a potential secondary site at 2q3 (Fig. 8). The same 0.6 kb Bam HI–Sac I probe d was hybridized to Hind III and Bam HI–digested genomic DNA from a well-characterized panel of somatic cell hybrids (Fig. 9). This panel confirmed the assignment of the 2.5-kb Bam HI fragment to chromosome 2. The 2.3-kb Bam HI fragment and 4.1-kb Bam HI fragment were located on chromosome 2 as well (Fig. 9). These data indicate that the original 15-kb Bam HI Kde is localized to 2p11–13 as would be expected. The duplicated 2.5-kb Bam HI region resides at 2q11–13. The duplicated but perhaps not contiguous derivatives of the 2q11 region also reside on chromosome 2.

Chr:	1	2	M D H I	I D H I	A C P I	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Hybridization					
																											2.5	4.1	2.3			
70M1C*E	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	P	-	-	-	-	-	-	-	+	-	+	-	-	-	-		
70M2C*E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	
70M3C*D	-	-	-	-	-	-	M	-	M	-	-	-	-	-	-	P	-	-	M	-	-	-	-	+	-	+	-	-	-	-		
70M4C	+	+	+	+	M	+	+	-	+	+	+	+	+	+	+	P	+	-	P	+	+	+	M	P	P	+	+	+	+	+		
70M5C	-	-	-	-	+	-	-	-	M	+	-	-	-	-	-	-	-	-	-	-	-	-	P	-	P	-	+	-	-	-		
70M6C	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	U	U	U		
70M7C*D	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
70M8C*D	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	+	-	+	-	+	-	+	-	+	+	+	+	+	+	+	+	
70M9C*D	-	-	-	-	-	-	+	-	+	+	+	+	M	+	-	-	+	-	-	+	-	+	M	+	M	+	-	-	-	-	-	
70M10C	-	-	-	-	+	-	M	-	+	P	-	-	+	-	M	M	+	-	-	M	+	+	-	M	M	+	-	-	-	-		
70M11C	-	-	-	-	-	+	-	-	M	+	-	M	-	-	-	-	+	M	-	-	+	P	+	+	-	+	-	-	-	-		
70M12C	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-		
70M13C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	P	P	P		
70M14C	-	-	-	-	-	-	+	+	-	+	+	+	+	+	-	M	M	-	-	M	M	-	-	+	M	+	-	-	-	-		
70M15C*D	-	-	-	-	-	-	-	-	M	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	+	P	+	-	-	-		
70M16C*D	+	+	+	+	+	+	+	+	+	+	+	+	+	+	P	P	P	+	M	M	M	+	-	-	-	-	+	+	+	+		
80H1D	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	P	-	-	-	M	M	-	-	-	-	-	-		
80H2C*D	-	-	-	-	-	-	-	-	+	-	+	+	-	-	P	+	+	+	-	-	M	M	+	-	+	+	-	-	-	-		
80H3C*D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	M	-	M	M	+	-	-	-		
80H4D*E	+	M	-	-	-	-	-	-	P	-	-	+	+	+	-	+	+	-	-	+	+	-	-	-	-	+	P	M	P	P		
80H5D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	M	-	-	+	-	-	-		
80H6D	-	-	-	-	-	-	-	-	P	+	-	-	-	-	-	P	P	-	+	-	-	-	P	P	M	P	-	-	-	-		
80H7E	-	M	+	+	+	-	-	+	-	+	-	-	-	+	+	-	-	+	+	-	+	-	-	+	-	+	+	+	+	+		
80H8D*C	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	+	-	+	P	P	P	P		
80H9C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-		
80H10C*E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-		
80H11D	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-		
80H12D*F	-	-	-	+	-	-	+	-	+	-	P	M	-	-	-	-	M	+	-	-	+	-	-	-	-	-	+	P	P	-		
81P1E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
81P2E *D	-	+	+	+	+	+	+	-	+	+	-	+	-	+	+	+	M	+	+	-	+	+	-	+	+	+	+	+	+	+		
81P3F	-	-	-	-	P	+	+	-	-	-	-	-	P	+	-	-	+	+	-	-	+	-	-	-	-	+	+	-	-	-		
81P4F	+	-	-	-	P	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-		
81P5C*D	-	-	-	-	-	-	M	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	+	-	-	-		
81P6D	-	-	-	-	-	-	+	-	+	P	-	-	-	-	+	-	-	-	-	-	-	-	+	-	+	P	M	+	-	-		
81P7C*D	P	-	-	-	-	-	M	-	-	P	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	-	-		
81P8E *G	-	P	+	-	M	-	+	-	-	-	-	-	-	+	+	-	P	+	M	-	-	-	-	+	-	+	+	+	+	+		
81P10E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-		
81P11E*D	-	-	-	-	U	-	+	-	-	-	-	M	-	-	-	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-		
81P12E	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	+	+	-	-	-		
81P13E*G	-	-	-	M	-	+	-	U	U	-	+	+	-	+	U	U	+	+	-	U	U	+	+	-	-	+	P	+	+			
81P14E*F	-	-	-	-	+	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-		
81P15E*D	-	-	-	-	-	-	M	-	-	-	-	-	-	-	+	-	-	-	-	-	-	M	-	-	-	-	+	-	-	-		
81P16D	-	-	-	-	U	+	+	-	-	-	+	-	P	+	-	-	+	+	-	-	+	-	+	-	+	+	-	-	-	-		
81P17E	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	-	-	+	-	+	-	M	M	+	+	-	-		
81P18C	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	+	-	+	M	-	-	-	+	-	-	-		
% Discordancy 2.5 Kb																																
BamHI	17	7	7	7	17	23	32	17	30	23	27	21	23	30	19	26	41	24	23	17	30	17	42	33	34	70						
% Discordancy 4.1 Kb																																
BamHI	20	10	9	7	19	26	35	17	32	25	29	22	25	28	20	28	43	24	25	17	32	18	42	37	31	69						
% Discordancy 2.3 Kb																																
BamHI	16	7	7	10	16	21	36	14	33	23	28	21	23	27	17	24	40	27	23	15	33	14	39	33	33	72						

Discussion

We analyzed the structure of germline and rearranged Kde alleles to gain potential insights into the functional role of the Kde. We observed that whenever the Kde rearranges into the J_k - C_k intron there is also a rearrangement present 5' to J_k . We examined two such alleles and found both to be aberrant attempts at V_k/J_k junctions in which nucleotides were lost and unexpected extranucleotides were present. While we cannot exclude the possibility that unusual bases existed immediately 3' to these particular V_k regions in their germline form; the composition of these extranucleotides suggests that they may be "N" segment additions (20). While the addition of N segments is typical of V_H/D_H and D_H/J_H junctions it is atypical of light chain assembly. All (5/5) upstream V/J rearrangements on alleles with rearranged human Kde and murine RS loci (8, 9) have been aberrant. The presence of extranucleotides suggests that the initial V/J rearrangements were abortive rather than altered by secondary somatic mutation. While the number of V/J rearrangements analyzed in detail is small, these results raise the possibility that the Kde may selectively eliminate preexisting aberrant V/J attempts. This may reflect a proof-reading mechanism. Alternatively, such an association could be probabilistic if attempted V/J rearrangements occurred at a much faster rate than Kde rearrangements.

We noted that the Kde could also rearrange to upstream sites resulting in the elimination of J_k as well as E_k and C_k regions. We showed here that this target site was a V_k region, and the murine RS also uses V_k regions at times (9). The heptamer-11 bp spacer-nonamer flanking V_k regions is a more highly matched target site for the heptamer-23 bp spacer-nonamer that flanks the germline Kde. However, the lone heptamer within the J_k - C_k intron would presumably be more proximal to the Kde than V_k regions. If we compare the site of Kde rearrangement in the 18 alleles we assessed and the 11 assessed by Klobeck and Zachau (5) there is a slight preference for the J_k - C_k intron (63%) versus V_k regions (37%) (Fig. 10). However, either V_k /Kde or V/J-Kde rearrangements can be found in λ light chain-producing B cells. This indicates that if Kde rearrangement generates a positive signal for λ rearrangement either Kde form would be effective. Examples exist in which only a single Kde allele is rearranged and the other is germline (4); this observation argues against a negative regulatory role for the germline Kde in preventing λ rearrangement. To date, all λ -producing

FIGURE 9. Chromosomal phenotype of Chinese hamster \times human (80 + 81) series and mouse \times human (70 series) somatic cell hybrids. Chromosome scores indicate consensus results of G-banding and isoenzyme assessment (17, 18). Data for chromosome 2 isoenzymes malate dehydrogenase 1 (MDH1), isocitrate dehydrogenase 1 (IDH-1), and acid phosphatase-1 (ACP1) are shown. (P) present at low frequency; (M) uncertain negative due to broken chromosome or presence in 1/20 spreads; (U) not performed. Percent discordancy values indicate that all three Bam HI (2.5 kb, 4.1 kb, and 2.3 kb) fragments recognized by probe d mapped to chromosome 2. In addition, data for any fragment (2.5, 4.1, or 2.3 kb) were highly concordant with the other two in a range of 2-5% discordancy. The three discordancies (70M13c, 80H8DC, 80H12DF) all displayed hybridizable bands that were very submolar. This may represent a difference in sensitivity between G-binding and isoenzyme markers versus DNA hybridization.

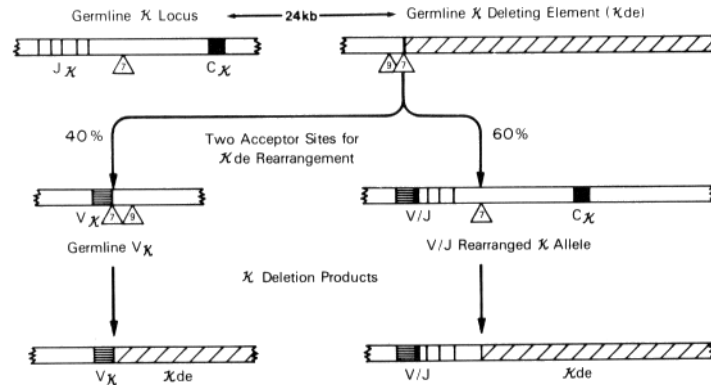
THE κ -DELETING ELEMENT

FIGURE 10. Schematic presentation of the germline Kde, the frequency and sites of its rearrangement, and rearrangement products. 24-kb distance from C_{κ} -Kde determined by Klobeck and Zachau (5).

cells have had at least one rearranged Kde. Identical observations have been made for the mouse RS (21). We also characterized a rare example of a κ -producing cell line (SU-DHL-6) with two λ gene rearrangements. Of note, this cell possessed one rearranged Kde in a V_{κ}/Kde configuration, further suggesting a positive role in progression to λ rearrangement.

These rearrangement findings prompted a detailed sequence analysis of the rearranged Kde of both V_{κ}/Kde and V/J -Kde varieties as well as the germline Kde near the breakpoint region. No attractive long open reading frames common to both V_{κ}/Kde and V/J -Kde were found. Furthermore, the longest open reading frame of the germline Kde was limited to 300 bp spanning the breakpoint region but lacked an ATG initiation codon and obvious promoter elements. When compared with the murine RS, this open reading frame region approached 50% DNA homology. However, the amino acid homology between RS and Kde for any reading frame comparison was much less (<30%). A dot matrix comparison of Kde and RS germline DNA sequences revealed that the most homologous regions were the rearrangement signals and an immediately 3' region (Fig. 5). Consistent with this, the only highly conserved amino acid stretch was within this signal region. Moreover, we found no significant homology of the Kde with Ig V regions, arguing against its being a vestigial V_{κ} region. These data argue that the open reading frame surrounding the rearrangement signals does not initiate or encode a complete protein, although it could represent a conserved exon. Of note, the majority (4/6) of determined breakpoints in mouse and man fell within the conserved region located 3' to the heptamer (Fig. 5). This may relate to this region or simply reflect exonuclease activity at the time of recombination.

To search for a Kde transcriptional product that might serve a putative *trans*-acting effect upon the λ locus we used the cloned Kde to search for a specific mRNA within pre-B cell, κ B cell, λ B cell, T cell, and nonlymphoid cell types. None displayed evidence of transcripts off of germline or rearranged Kde loci. Furthermore, no unique isolates were found when we screened a cDNA library from the unusual κ -producing cell that possessed a V_{κ}/Kde with λ gene rearrangements. This search included a relatively wide variety of cell types, however, it is

conceivable that a transiently expressed product might exist only at the time of Ig gene joining.

We noted a 2.5-kb Bam HI fragment that consistently crosshybridized with the Kde. We cloned, mapped, and localized this region to 2q11, indicating that this duplicated region was also dispersed. A comparative analysis of high resolution chromosomes from orangutan, gorilla, chimpanzee, and man suggested that a pericentric inversion occurred at the evolutionary emergence of the chimpanzee (22). The apparent chromosome segments involved would correspond to the current human 2p1 and 2q1. This raises the possibility that an ancestral portion of the Kde may have moved and been duplicated by a pericentric inversion event. None of the antigen receptor genes of B or T cells isolated to date map to 2q11 and this region was not rearranged in B or T cell lines (Fig. 6 and data not shown). However, the fact that this region is duplicated and retained in man suggests it serves a functional role.

Summary

Human light chain genes are used in a κ before λ order. Accompanying this hierarchy is the rearrangement of a κ -deleting element (Kde) which eliminates the κ locus before λ gene rearrangement. In ~60% of rearrangements the Kde recombines at a conserved heptamer within the J_{κ} - C_{κ} intron. We demonstrated that aberrant V/J rearrangements possessing apparent "N" nucleotides existed 5' to the J_{κ} -Kde rearrangements. This suggests that the Kde may selectively eliminate nonfunctional V/J alleles. A κ -producing cell that displayed the unusual finding of λ gene rearrangement demonstrated a rearranged Kde. This rearrangement was a V_{κ} /Kde recombination and the heptamer-11 bp spacer-nonamer flanking the V_{κ} is the target site of the Kde 40% of the time. The mouse possesses a counterpart to the Kde (recombining sequence [RS]) and the highly conserved regions surround the heptamer-spacer-nonamer signals. No complete protein product was predicted from the germline Kde near its breakpoint and no consistent fusion product was predicted from either the V/Kde or V/J-Kde rearrangements. A distal portion of the Kde is duplicated and is present at 2q11 as well as 2p11. The evolutionary conservation of the κ -elimination event, the duplication and maintenance of the Kde indicates that it has a function. A portion of the Kde may still prove to encode a *trans*-acting factor that directly affects λ rearrangement. A certain role for the Kde is its site-specific rearrangement, which destroys ineffective κ genes and sets the stage for λ gene utilization.

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