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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) that uniformly mediates this elimination of the κ locus (4). Klobeck and Zachau (5) mapped the Kde to a position 24 kb 3' to Cκ. In the majority of instances the Kde rearranged into the Jκ-Cκ intron at a conserved heptamer (CACAGTG) to eliminate the Cκ and enhancer (Eκ) 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 Jκ region and eliminates Jκ, as well as Eκ and Cκ. We wished to determine the identity of this upstream target site and in particular to ask if it might be a Vκ region. Moreover, when the Kde rearranges into the Jκ-Cκ intron (Jκ-Kde) it possesses an additional rearrangement at the 5' end of Jκ. 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 Vκ 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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† 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, Vκ/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 32P by random hexanucleotide priming to specific activities of 1–5 × 10⁶ cpm/μg for use as probes (11). Blots were hybridized in 10% dextran sulphate, 30–50% formamide, 4X SSC, 1X Denhardt’s solution, and 10 μg/ml salmon sperm DNA. Blots were washed three times in 2X 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 μg 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 λg10 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 × human and mouse × human somatic cell hybrids were examined with human probes to map their location (17, 18).

Results

Aberrant V/J Rearrangements on Jκ-Kde Alleles. We noted that κ alleles that had rearranged the Kde into the Jκ-Cκ intron also possessed an additional rearrangement 5' to Jκ (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 Vκ/Jκ and a Vκ/Jκ. Upon closer inspection of the sequence the 11.5-kb allele was a Vκ subgroup I juncture with Jκ3 that was aberrant in nature (Fig. 2). 8 bp of Jκ3 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
V, III region into J, 5 with the loss of 2 bp of J, 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, Segment. In ~40% of instances when the k gene is deleted the J, regions are eliminated along with the C, and E,. 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, E,, and C, in the SU-DHL-6 cell line. Salient features of this cell included the fact that it was a k chain–producing mature B cell line and that the Kde had eliminated the excluded k allele (4). Moreover, it represented the rare example of a k producer that possessed two rearranged a 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, C, intron. In this instance, the Kde was rearranging site specifically with a V, region. The site of recombination was cleanly focused at the 3' end of a V, 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/J-Kde allele (Fig. 1, B) and a V, Kde allele (Fig. 1D), which is presented.
in Fig. 4. The juxtaposition of the Kde with a V, 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, 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, or the J,-C, 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
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 SacI (a), 1.8-kb SacI-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 µg of pA-RNA from one pre-B cell with germline Kde, three pre-B cells with rearranged Kdes, four λ-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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FIGURE 4. DNA sequence of the V.\(111/\text{Kde}\) 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.

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 (e 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".

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 λgt10 from SU-DHL-6 which possessed a V./Kde rearrangement (Fig. 1D, Fig. 4). 75 × 10⁴ plaques were screened with a V.III probe as well as a Kde probe and no unique V./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 cross-hybridized to a 2.5-kb Bam HI fragment (Fig. 6). When this same probe c was used upon Hind III–digested
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.
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.-C. intron there is also a rearrangement present 5' to J. We examined two such alleles and found both to be aberrant attempts at V./J. junctures 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. 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./D. and D./J. junctures 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. as well as E. and C. regions. We showed here that this target site was a V. region, and the murine RS also uses V. regions at times (9). The heptamer-11 bp spacer-nonamer flanking V. 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.-C. intron would presumably be more proximal to the Kde than V. 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.-C. intron (63%) versus V. regions (37%) (Fig. 10). However, either V./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 × human (80 + 81) series and mouse × 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.
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 λ-producing cell line (SU-DHL-6) with two λ gene rearrangements. Of note, this cell possessed one rearranged Kde in a Vκ/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 Vκ/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 Vκ/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 Vκ 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, κB cell, λB 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 Vκ/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 \( \kappa \) before \( \lambda \) order. Accompanying this hierarchy is the rearrangement of a \( \kappa \)-deleting element (Kde) which eliminates the \( \kappa \) locus before \( \lambda \) gene rearrangement. In \( \approx 60\% \) of rearrangements the Kde recombines at a conserved heptamer within the \( J_C \) intron. We demonstrated that aberrant V/J rearrangements possessing apparent "N" nucleotides existed 5' to the \( J_K \) rearrangements. This suggests that the Kde may selectively eliminate nonfunctional V/J alleles. A \( \kappa \)-producing cell that displayed the unusual finding of \( \lambda \) gene rearrangement demonstrated a rearranged Kde. This rearrangement was a \( V_\kappa/Kde \) recombination and the heptamer-11 bp spacer-nonamer flanking the \( V_\kappa \) 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 \( \kappa \)-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 \( \lambda \) rearrangement. A certain role for the Kde is its site-specific rearrangement, which destroys ineffective \( \kappa \) genes and sets the stage for \( \lambda \) gene utilization.

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References