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Reciprocal recombination products of V_K-J_K joining reactions in human lymphoid cell lines

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ABSTRACT

The recombination process that joins a V_K to a J_K segment of an immunoglobulin gene generates a second, reciprocal recombination product called f fragment. In this second product the regions flanking the V_K and J_K segments in the germline are joined in a head to head fashion. We now analysed f fragments in the human lymphoid cell lines Daudi, JI and IARC/BL41. All three f fragments contain J_K1 flanks; the V_K derived moiety of f Daudi and f41 could be traced back to known germline V_K genes. There is a precise head to head joining of the heptanucleotide signal sequences in f Daudi and fJI while in f41 six nucleotides are present between the signal sequences. In contrast to the V_K-J_K recombination products, the f fragments were found to lack somatic mutations. The structures of the f fragments are discussed in the context of the V_K-J_K rearrangement mechanism.

INTRODUCTION

The variable regions of K light chain genes are assembled by somatic recombination of variable (V) and joining (J) gene segments (reviews 3,4). The DNA between the V and the J gene segments is not simply deleted but is sometimes retained in an altered genomic context. From the DNA of a variety of mouse B cell lines and tumors recombination products of flanking sequences, called f fragments, were isolated in which the 3' flank of a V gene segment is linked in a head-to-head fashion to the 5' flank of a J gene segment (5-11). Recently a f fragment was isolated also from a human B cell line (12). In the f fragments the heptanucleotide recognition sequences of the V and J gene segments are usually precisely joined. The f fragments are reciprocal recombination products of V-J joinings but, as it was shown already in the first isolated f fragment (5), they are not reciprocal to the particular V-J joints present in the cell lines

from which they are isolated. They must have been formed in the cell lines in V-J joining reactions which had taken place previous to the ones which gave rise to the current V-J joints. These findings together with the observation that not all B cell lines harbor f fragments led to the current models for V_K to J_K rearrangements, namely the sister chromatid exchange (7,8) and an inversion-deletion model (9). Although the sister chromatid exchange model has not been formally ruled out, the finding of truly reciprocal recombination products in a mouse myeloma (13), the demonstration of inversional recombination in a retroviral system (14,15) and the finding of inversions in the T cell receptor ß chain locus (16-18) are compatible with an inversion-deletion model of kappa gene recombination.

The inversion-deletion model predicts secondary rearrangements to occur involving either preexisting f fragments or further V-J recombinations. Data obtained from our work on rearranged V_K genes of human lymphoid cell lines (19-21) and the availability of numerous cosmid clones of the human V_K locus (22-25) prompted us to extend the investigation of the human f fragments. With this background it seems feasible to establish, on a molecular level, the relationship between the V_K gene segments which underwent V-J joining and those V_K gene segments which participated in f fragment formation. We started this investigation by isolation and characterization of f fragments from three human lymphoid cell lines.

MATERIALS AND METHODS

The rearranged K alleles and the origin of the Burkitt lymphoma derived cell lines Daudi, IARC/BL41 and JI are described in refs. 19-21, respectively. High molecular weight DNA from cultured cells was prepared according to ref. 26. Conditions for blot hybridization were as described (19). The λ phage EMBL 3 (ref. 27) was used to construct partial genomic libraries of the cell line DNAs as in ref. 19.

DNA fragments were subcloned in M13mp10 and 11 (ref. 28) and sequenced by the dideoxy chain termination method (29). DNA sequences were aligned for maximal homology using the computer

program DNMAMO; homologies were calculated by the program DNPERC. Both programs are described in ref. 22.

RESULTS AND DISCUSSION

Cloning of f fragments of human lymphoid cell lines

Roughly half of the mouse lymphoid cell lines harbor recombination products of V_K and J_K flanks (10). We screened a panel of 16 human lymphoid cell lines (21) for the presence of such

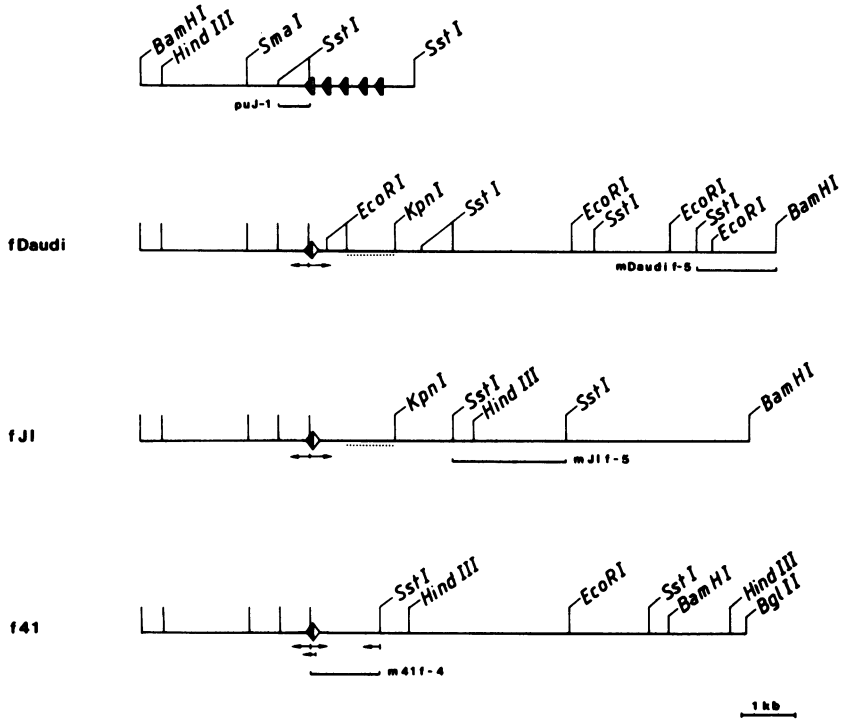

Figure 1. Restriction maps of the f fragments of the cell lines JI, Daudi, und IARC/BL41. Part of the human J_K region is shown for comparison; the location of probe puJ-1 (ref. 19) is indicated. J_K segments are depicted as open boxes, the recombination signal sequences as triangles (not to scale). Within the 5' J_K1 region the restriction sites of the f fragments are identical with the ones in the germline J_K map as indicated by the vertical lines. The fusion sites of the 5' J_K1 and the 3' V_K regions are indicated by the half-filled rhombus. Direction and extent of sequencing are symbolized by arrows underneath the maps. Regions homologous to L sequences are indicated by dotted lines (f Daudi and fJI). The subclones used for hybridization studies are shown underneath the maps.

f fragments: Southern blots of restriction nuclease digested genomic DNAs were hybridized with a probe from upstream of the J_K cluster (puJ-1 in ref. 19; Fig. 1). Eight of the 16 cell lines were found to contain f fragments. The f fragment positive cell lines Daudi, JI and BL41 were chosen for further investigation because the productive V-J joints in these cell lines had been characterized previously (19-21), and therefore a possible relation between the rearranged genes and the f fragments could be clearly defined.

Restriction fragments of the cell line DNAs which hybridized with the upstream J_K probe and presumably contained the longest 3' V_K flanks were chosen for molecular cloning (11.75 kb BamHI, 11.25 kb BamHI, and 11.2 kb BglII for Daudi, JI and BL41, respectively). Genomic libraries of size selected DNA of the cell lines were constructed in λ phages. The libraries were screened with the probe from upstream of J_K1 and hybridizing plaques were further purified. The restriction maps of the three clones obtained are shown in Fig. 1.

f fragments contain the 5' flanking sequences of J_K1

One general feature of mouse f fragments is that exclusively 5' J_K1 flanks are fused to the 3' V_K sequences (8,10,13). We determined the J_K flanks involved in the formation of the cloned f fragments by blot hybridization of restriction nuclease digests with probes from the human J_K region. No hybridization with J_K sequences was detected. Since the f fragments were isolated using a cloned fragment from the 5' J_K1 region which contains the nonanucleotide signal sequence of J_K1 but no J_K1 sequence we concluded that only 5' J_K1 flanks were present in the three f fragments (Fig. 1).

Restriction mapping and hybridization studies with probes which are derived from further upstream of the J_K1 segment confirmed that the cloned f fragments contain 3.2 kb of the germline J_K1 flank. Beyond the SstI site next to J_K1, however, the maps diverge from the human germline J_K-C_K map (30,19). The restriction maps 3' of this SstI site also differ considerably from one another (Fig. 1), suggesting that 5' J_K1 flanks were recombined with different genomic regions in each case.

The involvement of J_K1 flanks in the formation of f frag-

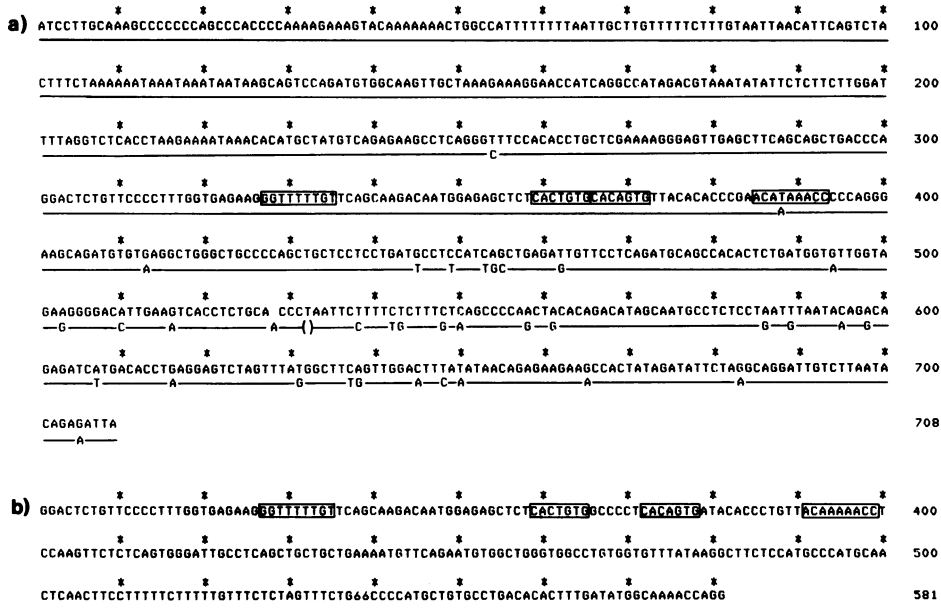

Figure 2. Nucleotide sequences of three human f fragments. a) Nucleotide sequences of fJI and f Daudi. The sequence of fJI is presented in full; in the f Daudi sequence (lower line) only those nucleotides are shown which differ from the ones of fJI . Sequencing only one strand was sufficient since the films were unequivocally interpretable in the reported regions. Nona- and heptanucleotide signal sequences are boxed. In order to achieve maximum homology upon alignment gaps were introduced into the sequences (pos. 528 of fJI and pos. 532 of f Daudi). The sequence 5' of the recombination site extends the 5' J_K1 sequence of Hieter et al. (30) by 110 bp. Several differences to the sequence of (30) were encountered but our three sequences were found to be identical to each other. b) Nucleotide sequence of f41. The sequence 5' of pos. 300 which is identical to the one of f Daudi has been omitted from the figure. Two nucleotides (pos. 538,539) marked 66 could not be determined unambiguously (G or T). The sequence of the 3' side of m41f-4 is not shown here but is being transmitted to the EMBL Data Library, Heidelberg, as "f41 outside flanking sequence".

ments seems to be a general phenomenon, since also the other f-like structures which we observed in human lymphoid cell lines did not hybridize with a J_K probe (data not shown). The probe pJ-1 (ref. 19) used for this purpose contains J_K1 to J_K5 with only 15 bp of the 5' J_K1 flank.

Closely related V_KI flanks are precisely joined with J_K1 flanks in fJI and f Daudi

To characterize further the genomic regions joined with J_K1 flanks in our cloned f fragments we hybridized them with a number of probes including V_KI-IV gene probes (20,21,31,32,) and the low repetitive L sequence (33). According to these studies no V_K gene segments are present in our clones. The L sequence probe (clone 20-16 of ref. 33), however, detected homologous sequences in fJI and f Daudi (Fig. 1). These sequences, although present also in other regions of the genome, seem to be characteristic for the 3' flanks of a group of V_KI gene segments located in the socalled L region (22). This result suggested that V_KI segments of this region participated in the formation of fJI and f Daudi.

We extended our analysis by sequencing the regions where the restriction maps of the cloned f fragments begin to diverge from the germline J_K region (Fig. 1). A region of 708 bp spanning the recombination site was sequenced from both, f Daudi and fJI (Fig. 2a). The recombination event had resulted in a precise fusion of the heptanucleotide signal sequence of J_K1 (CACTGTG) with the corresponding signal sequence of V_K gene segments (CACAGTG). Two nonanucleotide signal sequences separated from the respective heptamer boxes by 23 bp on the 5' and by 12 bp on the 3' side were also found in the sequences. With these features the f fragments of Daudi and JI cells have a structure similar to the ones previously described for mouse f fragments (e.g. 8,10).

The V gene flanks of f Daudi and fJI differ by 11.3% within the sequenced regions of 343 bp (Fig. 2A). A computer aided comparison with the sequenced 3' flanks of 30 human V_K gene segments (12,21-25,31,34-36 and E. Huber, E. Lötscher, R. Thiebe, unpublished) revealed that f Daudi and fJI contain V_KI flanks. As expected from the presence of the low repetitive L sequences the closest homologies were encountered with V_KI flanks from the L region (22,23,31). fJI is 94.2% homologous with the 3' flank of V1 (31; HK102 in ref. 35), and the 3' V_KI flank of f Daudi is identical with the flank of Ve'' (23,31).

In order to determine the V_K gene segment which was involved in the formation of fJI, a hybridization probe from fJI was prepared (mJIf-5 in Fig. 1) which did not contain highly

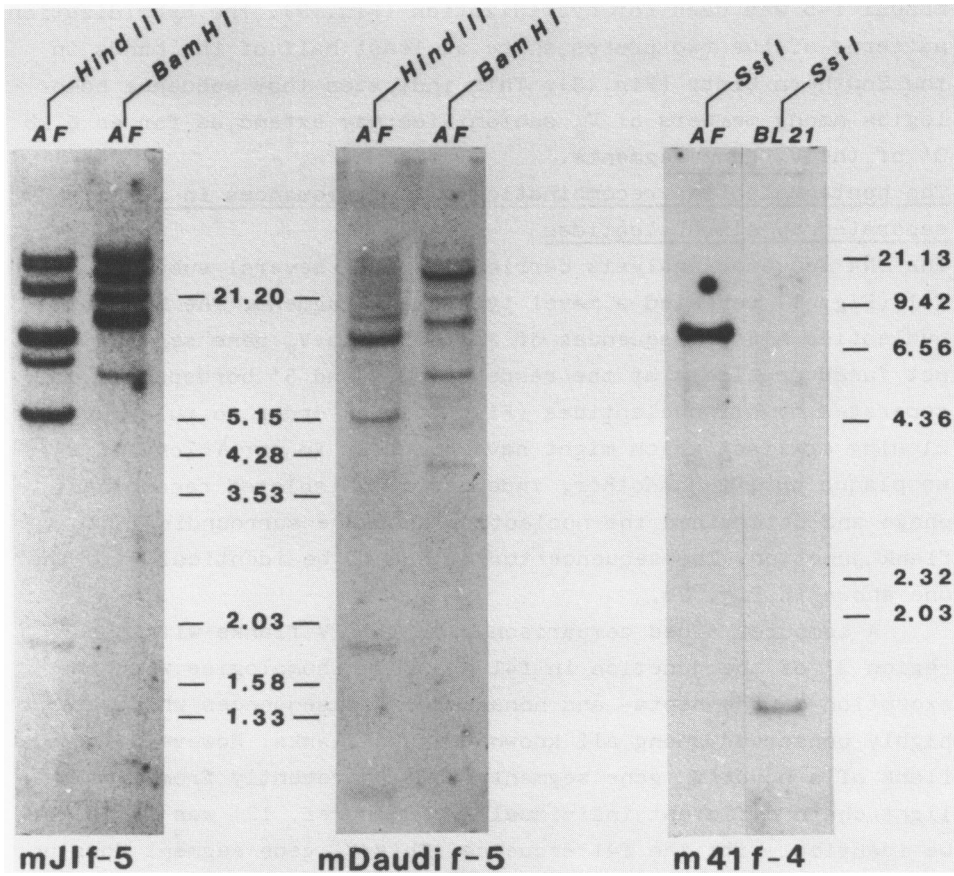

Figure 3. Blot hybridization of genomic DNA with probes from the V_K flanks of f fragments. Southern blots prepared from restriction nuclease digests of a human placenta DNA (AF) and IARC/BL21 DNA (38) were hybridized with the radioactively labeled probes as indicated. The restriction nucleases used and the DNA loaded onto each slot are indicated.

repetitive sequences. Unfortunately this probe hybridized with most of the V_KI flanks of the cosmid clones from the L region as well as with f Daudi (data not shown). Therefore we could not identify unambiguously the germline V_K gene segment which was involved in the formation of fJI.

The probe mJIf-5 (Fig. 1) recognized a set of homologous fragments in restriction nuclease digests of placenta DNA as illustrated in Fig. 3. A similar pattern showed up when the probe

mDaudi f-5 was used for hybridization (Fig. 3). The hybridization patterns of the two probes share at least half of the bands in the Southern blots (Fig. 3). This indicates that sequence homologies among members of V_K subfamilies may extend as far as 6 kb 3' of the V_K gene segments.

The heptanucleotide recombination signal sequences in f41 are separated by six nucleotides

The DNA sequence analysis carried out with several subclones of f41 (Fig. 1) revealed a novel type of f fragment. The heptanucleotide signal sequences of J_K1 and of a V_K gene segment are not fused precisely at the respective 3' and 5' borders but are separated by six nucleotides (Fig. 2b). In order to rule out a cloning artifact which might have occurred in our λ clone of f41, we plaque purified another, independently isolated recombinant phage and determined the nucleotide sequence surrounding the flank junction. The sequence turned out to be identical with the one shown in Fig. 2B.

A computer aided comparison of V_KI-V_KIV flanks with the region 3' of the junction in f41 showed no homologies with the exception of the hepta- and nonanucleotide sequences which are highly conserved among all known V_K gene flanks. However, the 3' flank of a novel V_K gene segment isolated recently from a kappa light chain deficient individual (EV15 in ref. 12) was found to be identical with the f41 sequence. This V_K gene segment does not fit into one of the four subgroups of human kappa proteins (37). Moreover Stavnezer et al. showed that this V_K gene segment is a single copy gene (12).

Five of the six nucleotides which separate the heptanucleotide signal sequences in f41 can be attributed to the J_K1 and the V_K (EV15) sequences (Fig. 4). One nucleotide stems from the J_K1 region and four are found adjacent to the heptanucleotide box of EV15. The exchange of T in the EV15 flank to a C in f41 (marked by asterisks in Fig. 4) may reflect an allelic difference between EV15 (ref. 12) and the gene segment of the BL41 line which was involved in the recombination or may be the result of a somatic mutation. The latter possibility is unlikely since there seem to be no somatic mutations in the f fragments. The J_K1 flanks of f Daudi and f41 were found to be identical within the sequenced

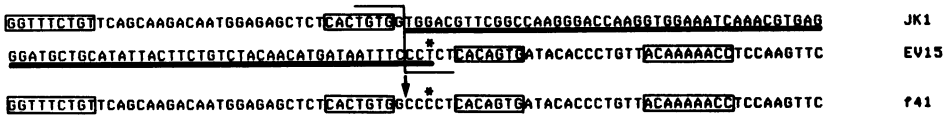

Figure 4. Nucleotide sequence of the joining region of f41. The recombination between J_K1 (ref. 30) and EV15 (ref. 12) is shown together with the resulting recombined f41 sequence. The hepta- and nonanucleotide signal sequences are boxed, potentially coding regions of J_K1 and EV15 are underlined. Note that the T marked by an asterisk in EV15 is changed to a C in f41 (see text).

region of 365 bp (Fig. 2), the J_K1 flank of fJI differs from f Daudi and f41 only by a single nucleotide (pos. 254 in Fig.2a). Also the 3' V_K flanks of f Daudi and f41 are identical to the respective germline sequences of Le'' (31) and EV15 (ref. 12). Inspection of the published sequences of mouse f fragments (e.g. refs. 8,13) shows that also those f fragments do not contain somatic mutations.

A f structure which has features similar to the ones of f41 was recently found by Malissen et al. (16) in a reciprocal joint of the T cell receptor ß chain locus. The authors found that the heptanucleotide signal sequences were separated by three nucleotides which could be attributed to the $V_ß$ and $D_ß$ elements. Their results together with our data on f41 suggest that, in contrast to previous findings (8,10,11,15), the recognition sequences need not be precisely joined during recombination. However, the "intervening nucleotides" in f41 could also be a characteristic feature of EV15-J_K f fragments, perhaps because of structural peculiarities of EV15. The question will be answered when further examples of intervening sequences in f fragments in general or in f fragments created from EV15 flanks are found.

The unique structure of the EV15 gene segment and its 3' flank permitted us to investigate whether the EV15 segment was involved in the formation of a f fragment also in other cell lines. The clone m41f-4 comprises a 1.35 kb SstI fragment which is derived mainly from the EV15 gene flank and contains only 15 bp of the J_K1 flank (Figs. 1 and 2b). The germline SstI fragment of EV15 homologous to our clone is 7 kb in length (12). Therefore

a f fragment which is formed upon SstI digestion from the EV15 flank can easily be distinguished from the germline EV15 fragment. SstI digests of human lymphoid cell lines were electrophoretically separated, transferred to nitrocellulose filters and hybridized with m41f-4. One of the cell lines (IARC/BL21; ref. 38; Fig. 3) was found to harbor a homologous 1.35 kb SstI fragment and therefore contains a f fragment similar to the one of IARC/BL41.

The cloned f fragments are not the reciprocal products of existing V-J rearrangements in the cell lines

The molecular analysis of eight f fragments isolated from mouse B cells (8,10) clearly showed that they do not represent reciprocal structures to the V-J recombination(s) in the respective cell lines. We examined our data on the f fragments and the V-J rearrangement(s) of the respective cells in the light of this observation.

The cell line Daudi has a productive $V_K I$-$J_K 4$ rearrangement (19), whereas the allelic J_K-C_K region has been deleted presumably during a rearrangement of the kappa deleting element with a V_K gene segment (39). The germline counterpart of the rearranged $V_K I$ gene segment could be localized in the Oa or Ob regions (A. Meindl and H.G. Klobeck, unpublished), two of the V_K gene regions established by genomic cloning procedures (genes O2 or O12 in ref. 24). The 3' V_K flank of f Daudi, however, is not derived from one of these V_K gene segments. It was found to stem from the V_K gene Ve'' which belongs to the V_K gene region Lb (23,31; called cluster b in ref. 23). Thus f Daudi is not the reciprocal product of the productive V-J joining of the cell line. It cannot be excluded, however, that f Daudi represents a reciprocal product of a V-J rearrangement of the allelic chromosome, since the extent of the deletion mediated by the kappa deleting element is unknown (39).

The relation of fJI to V-J rearrangements is complicated by the fact that cell line JI harbors both the productive $V_K IV$-$J_K 4$ rearrangement (21) and an aberrant recombination of a $V_K I$ gene segment with $J_K 4$ (H.G. Klobeck, G. Combriato, and H.G. Zachau, manuscript in preparation). fJI is clearly not related to the productive $V_K IV$-$J_K 4$ rearrangement, but a relation to the aberrant

V_KI-J_K4 joint is difficult to assess without knowing the germline sequence of the V_KI gene involved. Fortunately the cell line JI harbors the chromosomal translocation t (2;8) (ref. 40) and somatic cell hybrids were prepared from a fusion of JI with the rodent cell line NP3 (ref. 41). The cell hybrids which were kindly made available to us by Dr. J. Erikson allowed a chromosomal assignment of fJI. Hybridization of DNA from the cell hybrids containing the JI derived chromosomes 8, $2p^-$, $8q^+$ and 2, respectively, with the probe puJ-1 (Fig. 1) gave a signal only with the chromosome 2 containing DNA. This led to the assignment of fJI to the intact chromosome 2. Chromosome 2 contains also the productive V_KIV-J_K4 rearrangement (41,21), which excludes a direct relationship between the aberrant V-J rearrangement and fJI.

The cell line IARC/BL41 has also two V-J rearrangements (K^+, K^-) (20,21). The productive recombination (K^+) involves a V_KIII gene segment and the J_K2 segment, the K^- rearrangement consists of a V_KIV gene segment and an unknown J_K segment (21). Therefore neither the K^+ nor the K^- rearrangement are directly related to f41.

Concluding remarks

fJI, f Daudi, and f41 represent rearrangements of V_K and J_K1 flanks which are not reciprocal to the V_K and the J_K segments involved in the V-J joints of the respective cell lines. We could not detect in our cell lines structures like the "double recombination products" described by Feddersen and van Ness (11) and recently found also in a B cell line isolated from a kappa deficient individual (12). The existence of double recombination products in some cell lines demonstrates the possibility of secondary rearrangements and deletions in the process of V_K-J_K recombination and may account also for the observation that all f fragments isolated up to now contain J_K1 flanks.

The absence of somatic mutations in the f fragments in contrast to their abundance in V_K-J_K joints is interesting from a mechanistic point of view. The observation supports the notion that such mutations occur after and not during the recombination process (e.g. ref. 42).

The inversion-deletion model of V_K-J_K rearrangements is com-

patible with the results from a retroviral V_K-J_K system (14,15), the reports on chromosomal inversions in human chromosome 14 (refs. 43-45), and with the finding of inversions in the T cell receptor ß chain locus (16-18). The previous (5-12) and the new data on f fragments are not in contradiction to the inversion-deletion model. In order to define fully the mechanism of the V_K-J_K rearrangement more information is required on the structure of the V_K locus in the germline and the rearranged form, including information on the orientation of the V_K gene segments relative to the one of the J_K-C_K gene segment.

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