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Subgroup IV of human immunoglobulin K light chains is encoded by a single germline gene

H.-Gustav Klobeck, Georg W.Bornkamm+, Gabriele Combriato, Ralph Mocikat, Hans-Dieter Pohlenz and Hans G.Zachau

Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie der Universität München, and +Institut für Virologie, Zentrum für Hygiene, Universität Freiburg, FRG

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ABSTRACT

The series of studies on the human K light chain genes of the various subgroups is concluded by this report on the isolation and nucleotide sequence determination of a functional V_KIV gene (abbreviations ref. 1) and its germline counterpart. The rearranged gene which stems from a lymphoid cell line and the germline gene differ in four nucleotides which can be attributed to somatic mutations; three of the mutations are clustered in CDR3. The germline gene regions of two unrelated individuals were identical over a stretch of 1267 bp. By hybridization experiments it is shown that the human K locus contains only one V_KIV gene. In 16 lymphoid cell lines studied here, the V_KIV gene is frequently deleted or aberrantly rearranged which may be a consequence of peculiarities of its function and/or its structural organization.

INTRODUCTION

The discovery of subgroups of the immunoglobulin K light chains in the late 1960s, was an important advance in the discussions of germline and somatic mutation theories of antibody diversity (review ref. 2). All K light chain sequences available at the time could be assigned to the three subgroups V_KI - V_KIII. In 1973 a fourth subgroup of K light chains was found in the K light chains of the so-called cold agglutinins (3). One of the KIV proteins has been fully sequenced (4) while the others are known in their amino terminal sections only (5).

In our attempts to elucidate the organization of the individual genes within the human K locus (review ref. 6) we had to isolate genes of the four subgroups which can be used as hybridization probes for screening genomic libraries and for identifying individual genes. This work which led to the

isolation of KI, KII and KIII genes (7-9) included also the productively rearranged K gene of the cell line JI. The JI line had been isolated from a German case of Burkitt's lymphoma and was found to have a translocation involving chromosomes 2 and 8 (10). In the studies reported here the rearranged gene was shown by sequence analysis to belong to subgroup IV. The assignment to subgroup IV was confirmed by immunochemical experiments (9). A subclone derived from the rearranged gene served as a probe for isolating the corresponding germline gene and for studying the state of the V_KIV gene in a number of lymphoid cell lines. In these studies the V_KIV gene turned out to be one of the more interesting members of the V_K gene family.

MATERIALS AND METHODS

Cell lines and cell culture

The cell lines Raji (11) and Ramos (12) were obtained from H. Wolf, München, and from E. Wecker, Würzburg, respectively. The origin and some characteristics of the other cell lines have been described in the accompanying paper (9). Conditions for cell culture and isolation of DNA were as described (7).

Genomic libraries

A phage λ library was constructed (H.-G. K.) from DNA of placenta AF by partial digestion with Sau3A and ligation of size selected DNA fragments to BamHI digested purified arms of the phage EMBL3 (ref.13). Another phage λ library was constructed (G.W. B.) in the same way from DNA of the cell line JI using the phage EMBL2 (ref.13) as a vector; this library was screened with the cDNA clone NG9C_K donated by T.H. Rabbitts; this clone contains J_K and C_K sequences. In vitro packaging, plating and screening of the phage libraries were as described (7). A cosmid library from DNA of the placenta St (the same placenta DNA as in ref. 14; cosmid library IIIb of H.-D. P.) was prepared by partial digestion with Sau3A, size selection and ligation to the vector pJB8 (ref. 15).

Nucleotide sequences were determined with the dideoxy chain termination method (16). Computer programs and conditions for blot hybridization are described in ref. 9.

RESULTS

The productively rearranged V_K gene of the cell line JI is a member of subgroup IV

A rearranged K gene from DNA of the cell line JI was originally cloned by one of us (G.W. B.) because of a possible relation between a K gene rearrangement and the variant chromosomal translocation t(2;8) which had been found in this cell line (10). The DNA clone which was studied to a certain extent by subcloning a 7.5 kb BamHI and a 6.2 kb EcoRI fragment in pACYC 184 (ref. 17) and by restriction mapping did not contain the breakpoint of the chromosomal translocation. A V_K gene, however, was found on the clone; the gene appeared to be interesting in the context of our search for genes of the various subgroups and was therefore further studied. Detailed restriction mapping (Fig. 1A) and the comparatively weak hybridization signal withV_KI and V_KII probes indicated that the gene did not belong to those subgroups. M13 subclones were prepared from the pACYC 184 clones and sequenced. Fig. 2 shows the nucleotide sequence of a 1.309 kb region. A comparison of the deduced amino acid sequence of VJI with sequences of K light chains (5) revealed it to be a member of subgroup IV. In FR1 and CDR1 of the JI sequence all invariant residues of subgroup IV (ref. 5) are present; the homology to protein LEN (4) which is the only complete V_KIV sequence presently available, is 96% (differences at positions 9, 29, 92 and 93). Like the LEN protein the JI protein has an insertion of 6 amino acids (27 A-F) in CDR1, as compared to K chains of subgroup I. An examination of CDR3-FR4 shows that the JI protein is shorter than the LEN protein by one amino acid. This feature can be explained by a difference at the V-J joining site (see below).

The DNA sequence analysis suggested that the rearranged gene we are studying is expressed in the cell line JI, since no stop codons or sequence abnormalities were found. In addition, immunochemical analysis of the cell culture fluid clearly demonstrated that the cell line JI secretes a KIV protein (A. Solomon, cf. ref. 9). Finally, a recent paper on JI - rodent cell hybrids (21) reported that the chromosome 2 which is not

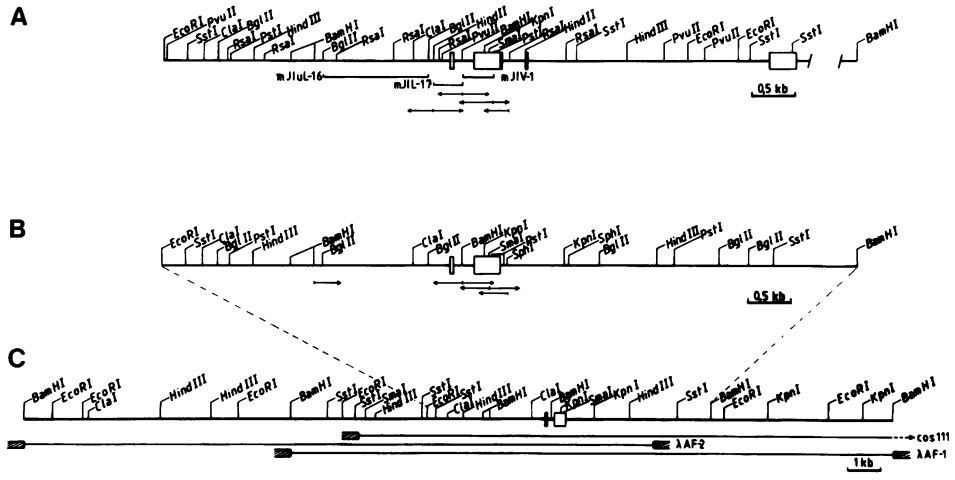

Figure 1. Restriction maps of the productively rearranged V_KIV gene of the cell line JI (VJI) and the germline V_KIV gene region. Gene segments are shown as open boxes, vector sequences as hatched bars. Extent and direction of sequencing (A,B) are symbolized by horizontal arrows.
A. The productively rearranged V_KIV region of JI. The map was constructed from two overlapping clones (6.1 kb EcoRI and 7.5 kb BamHI). RsaI sites were not determined (with one exception) downstream of the gene region. Subclones prepared for hybridization experiments are shown underneath the restriction map. The clones mJIuL-16, mJIL-17, and mJIV-1 contain a 1.26 kb BglII, a 0.34 kb HindII-BamHI and a 0.4 kb BamHI-PstI fragment, respectiveley.
B. Restriction map of the germline V_KIV gene region. All restriction nuclease sites shown were determined on the DNA of both placentae. RsaI and PvuII sites were not determined.
C. This map was constructed from the two overlapping phage clones λAF-1 and λAF-2 from placenta AF and a cosmid clone (cos 111) from placenta St. The insert of cos 111 extends further to the 3' side of the phage clone λAF-1 (--►).

involved in the t(2;8) translocation gives rise to the above mentioned 7.5 kb BamHI fragment and shows expression of a K chain.

Only one V_KIV gene is present in the human genome

In order to assess the number of V_KIV genes in the human genome, subclones were prepared from the VJI region and used in blot hybridization experiments with placenta DNA (Fig. 3). For a description of the subclones see the legend of Fig. 1. Under non-stringent hybridization conditions the V gene probe mJIV-1 recognizes a number of fragments. The pattern resembles the one

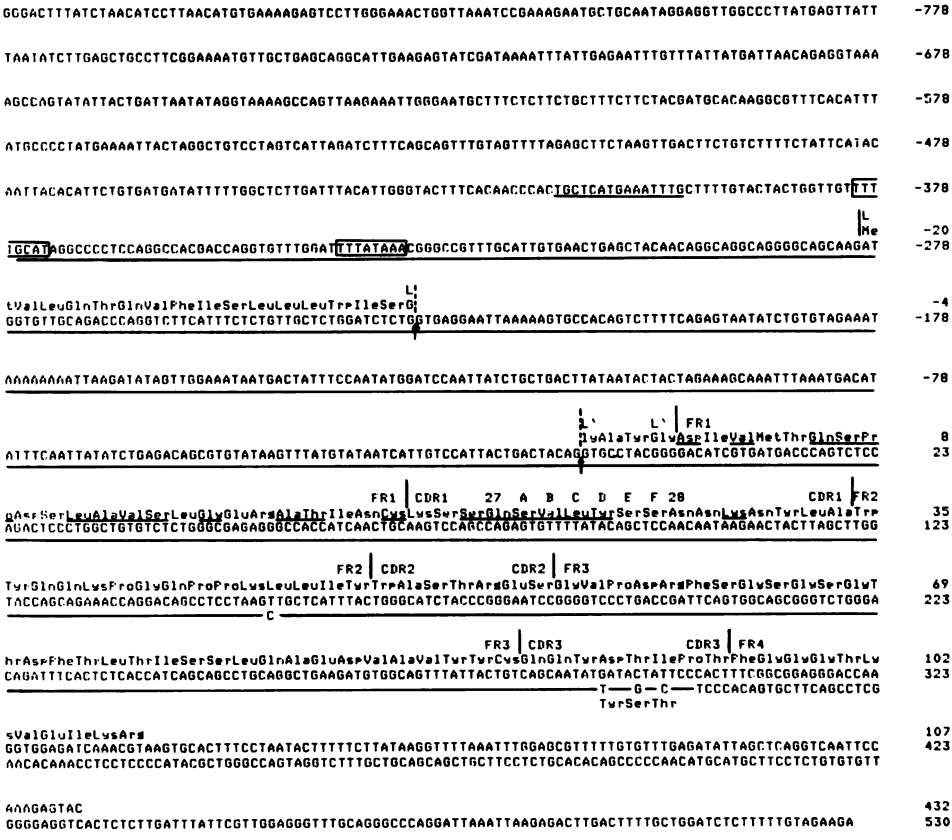

Figure 2. Nucleotide sequences of the rearranged V_KIV gene region of the cell line JI (VJI) and its germline counterpart. The sequences were determined following the strategy shown in Fig. 1A and B. The VJI sequence is presented completely (pos. -877 to +432) together with the deduced amino acid sequence of the coding regions. For the germline V_KIV region (pos. -376 to +530) only those nucleotides/amino acids which differ from the VJI sequence are shown. The putative regulatory sequence dc (18) and a TATA related sequence (19) are boxed; a pd (18) related sequence is underlined. Vertical arrows designate the putative splice sites (19). Invariant amino acid residues (5) are underlined (FR1 to CDR2). Downstream of the J_K4 segment the following differences between the VJI sequence and the published germline sequence (20) were found: our position 357, A instead of G; pos. 371, GGTTTT instead of GTTT; pos. 384, GGA instead of GA.

obtained with a V_KI gene probe (8). Using stringent conditions, however, only one prominent band is left on the autoradiogram. Note that this band does not correspond to one of the most prominent bands seen under non-stringent conditions. The strong

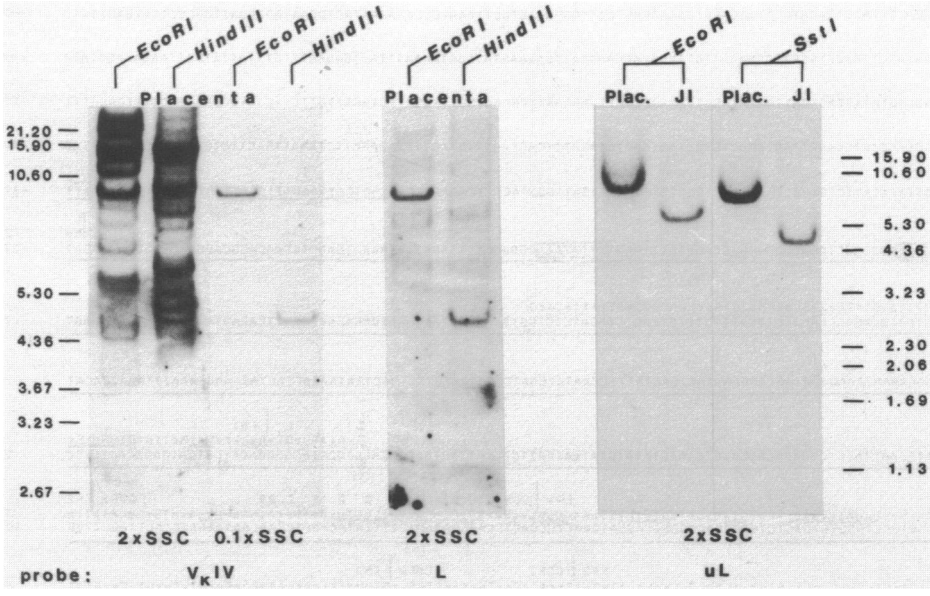

Figure 3. Blot hybridization of DNA from placenta and JI with V_KIV region probes. Southern blots prepared from restriction nuclease digests of placenta AF and JI DNA were hybridized with the V_KIV probe mJIV-1, the leader probe mJIL-17 and a probe from upstream of the leader segment (mJIuL-16), respectively. The restriction nucleases used, the DNA loaded onto each slot and the stringencies of hybridization are indicated.

hybridization under the non-stringent conditions is due to the fact that within one band several genes of other V_K subgroups are present which are fairly homologous to the VJI gene probe (e.g. 72.5% to the V_KI gene V1/HK102, ref. 22,23; 69.3% to the V_KII gene V607, ref. 8; 73.2% to the V_KIII gene V41, ref.9).

The evidence for the existence of only one V_KIV gene in germline DNA is strengthened by hybridization experiments with probes from the V_KIV leader region and from the region upstream of the leader segment (Fig. 3). With these probes only one band is seen under both, stringent and non-stringent conditions. The homology between the V_KIV leader sequence and leader sequences of V_KI-V_KIII genes is below 57% which accounts for the fact that the V_KIV leader probe is practically subgroup specific even under non-stringent conditions. Two representative digests which were hybridized with the probe from upstream of the leader

sequence are shown in Fig. 3; also in digests with five other restriction nucleases only one hybridizing band is detected (BamHI, 1.85 kb; BglII, 1.26 kb; EcoRI, 10.9 kb; HindIII, 4.95 kb; SstI, 7.1 kb; SstI-KpnI, 3.64 kb; PstI, 3.1 kb).

The region upstream of the V_KIV gene (clone mJIuL-16; Fig. 1A) contains sequences which in blot hybridization experiments with the V_KIV gene probe (mJIV-1) give a weak signal under non-stringent conditions. However, in sequencing no V gene related sequences or minigenes (14) were detected.

The germline V_KIV gene differs only slightly from the rearranged one and is identical among two unrelated individuals

The existence of only one germline V_KIV gene offers the possibility to establish unambiguously the relation between a rearranged gene and its germline counterpart. A similar assignment of rearranged V_K genes of subgroups I, II, and III (7-9) to their germline counterpart is more difficult because of the crosshybridization among the members of the subgroups and because of the possibility of extensive somatic mutations of the rearranged genes (24-26).

The germline V_KIV gene region was cloned from the DNA of two unrelated individuals (placenta AF and St) using the probe upstream of the leader region (mJIuL-16). From a phage λ library of AF-DNA two overlapping recombinants were isolated. The restriction map of a region of 25.8 kb is shown in Fig. 1C. From a cosmid library of placenta St a clone (cos 111) was obtained which starts 5.8 kb upstream of the V_KIV gene segment and extends on the 3' side beyond the end of the phage map of Fig. 1C (not shown). Restriction mapping of λAF-1 and cos 111 with 7 different nucleases revealed no differences within the overlapping region of 15.9 kb.

Analogous M13 subclones of the V_KIV gene regions were prepared from both placenta DNA clones. Fig. 2 shows the nucleotide sequence in comparison with the VJI sequence. It was surprising to find no differences between the two V_KIV gene regions within the 907 bp sequenced. Also the nucleotide sequence of a region located 1.9 kb upstream of the V gene segment (Fig. 1B) proved to be identical within 360 bp sequenced (sequence not shown here, but included into the EMBL Data

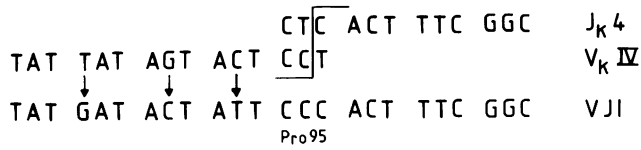

Figure 4. The sequence around the joining region (codon 95) of VJI is aligned with the germline sequences of the V_KIV and J_K4 gene segments. Somatic point mutations are marked by arrows. The V-J joining site is indicated.

Library, Heidelberg). The sequence elements which are thought to mediate the V-J rearrangement are present 3' of the V gene segment in the expected positions and show no deviation from the canonical hepta- and nonamer sequences.

A comparison of the VJI sequence with the V_KIV germline sequence revealed four nucleotide differences (Fig. 2) which are due to somatic mutations. One of the differences does not lead to an altered amino acid sequence (pos. 154, CTG→TTG, Leu). The other three differences are located in CDR3 (codons 92-95), resulting in a replacement of three amino acids (Fig. 2). Differences between our sequence downstream of the J4 gene segment and the published sequence of this region (20) are mentioned in the legend of Fig. 2.

Codon 95 of VJI arose in the V-J rearrangement process and is a composite structure of codon 95 of the germline V_KIV gene segment and codon 1 of the J_K4 gene segment (Fig.4). This mode of V-J joining leads to a deduced amino acid sequence for the JI protein which is shorter by one amino acid than the LEN protein (4). In the latter protein codon 95 of the germline V_KIV gene segment seems to be joined to codon 1 of the J_K2 gene segment without loss of nucleotides. Two somatic mutations which lead to amino acid changes (positions 9 and 25) must have taken place when the LEN gene was formed from the V_KIV and the J_K2 gene segments.

An aberrantly rearranged V_KIV gene which was recently published (27) is very similar to our V_KIV germline gene. The only differences are at positions 72/73 (TG instead of GT) and 302/303 (TC instead of CT). Our V_KIV germline and VJI sequences were also compared with the sequence of a cDNA clone (28) from

the lymphoid cell line B17X2 which produces a KIV protein. The B17X2 sequence differs from our germline sequence in one position in L and in 8 positions in the CDRs; one (possibly allelic) difference in FR2 (pos. 154) is identical to the one observed in the VJI sequence. Also in the B17X2 gene codon 95 is a composite structure; it is formed from two nucleotides of the germline V_KIV gene and the nucleotide adjacent to the heptanucleotide box of J_K1. Apparently in the B17X2 line more (and different) somatic mutations have occurred than in the JI line.

On the basis of partial amino acid sequence data a K protein was recently assigned tentatively to subgroup IV (29). If this assignment proves to be correct an even larger number of somatic mutations must have occurred during or after the V_KIV-J_K rearrangement.

The V_KIV gene segment is frequently deleted or aberrantly rearranged in human lymphoid cell lines

Hybridization experiments with a probe upstream of the leader segment (mJIuL-16) showed that in the cell line JI only the rearranged V_KIV gene segment is present (Fig. 3), the other V_KIV allele was obviously deleted. This finding prompted us to investigate the configuration of V_KIV alleles in another 15 human lymphoid cell lines. 14 K chain producing cell lines (including the JI line) which have been characterized with respect to the subgroups of the expressed V_K chains (9) and two λ light chain producers were included into this study. The DNAs were digested with EcoRI and Southern blots were prepared. The filters were hybridized with the upstream leader clone mJIuL-16 and the C_K probe pC-2 (ref. 7) which served as an internal control. Fig. 5 shows some representative examples. The experiments were carried out also with a number of other restriction nucleases which confirmed the results of the EcoRI experiments. The data are summarized in Table 1.

It was surprising to find that only in six cell lines the V_KIV allele(s) are present in germline configuration. The remaining 10 cell lines exhibited deletions and/or rearrangements of the V_KIV alleles. Out of the 32 alleles of the 16 cell lines 14 were found to be in the germline context, four

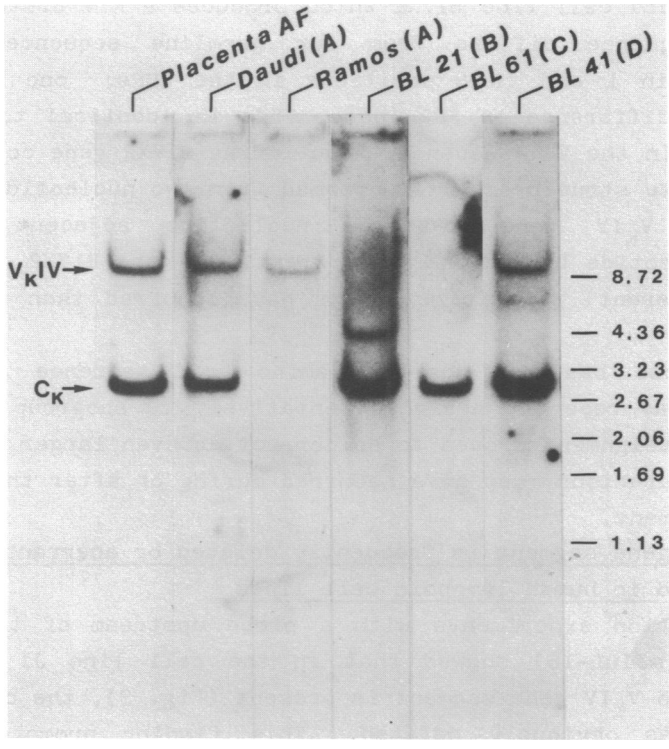

Figure 5. Configuration of V_KIV alleles in human lymphoid cell lines. DNA of the cell lines was digested with EcoRI, electrophoresed and blotted onto a nitrocellulose filter. The filter was hybridized with the C_K probe pC-2 (ref. 7) and the probe from region upstream of the leader segment (mJIuL-16; Fig. 1A). The 2.8 kb C_K and the 9 kb germline V_KIV fragments are indicated. Sizes of the marker fragments (digests of pBR322 and pBR clones) are given in kb. The letters in parenthesis behind the designations of cell line DNAs refer to the respective letters in Table 1 for the four types of configuration of V_KIV alleles.

showed rearrangements and 14 were deleted. The number for the deleted alleles may even be higher, since in our experiments we could not determine unambiguously whether in the group of cell lines which show germline configuration both alleles are present or if one is deleted (Table 1).

It is interesting to note that in all 10 cell lines which have both J_K-C_K alleles rearranged at least one of the V_KIV genes is deleted or rearranged; in six of the 10 cell lines even both V_KIV alleles are deleted (Table 1).

Cell Lines	Configuration of K Alleles	Configuration of V_KIV Alleles
Walker	K^o (10.6 BamHI/pC-2); K^+ (11.3 BamHI/pC-2)	germline/germline or germline/deleted (A)
Daudi	deleted; K^+ (11.7 BamHI/pC-2)	
IARC/BL36	K^o (10.6 BamHI/pC-2); K^+ (17.0 BamHI/pC-2)	
GM607	K^o (10.6 BamHI/pC-2); K^+ (8.2 BamHI/pC-2)	
RPMI6410	K^o (10.6 BamHI/pC-2); K^+ (8.2 BamHI/pC-2)	
Ramos	deleted; deleted	
JI	K^+ (7.0 BamHI/pC-2); K^- (14.8 BamHI/pC-2)	deleted/rearranged (B)
IARC/BL21	K^+, K^- (3.8, 4.4; EcoRI/pI-1)	
IARC/BL61	K^+, K^- (8.7, 9.8; BamHI/pC-2)	deleted/deleted (C)
GM1500	K^+ (9.2 BamHI/pC-2); K^- (5.7 BamHI/pC-2)	
U698M	K^+, K^- (4.4, 5.5; EcoRI/pI-1)	
IARC/BL31	K^+, K^- (2.6, 6.8; EcoRI/pI-1)	
Ly91	K^+ (8.0 BamHI); K^- (9.5 BamHI)	
Raji	deleted; K^- (10.2 BamHI/pC-2)	
WI-L2	K^+, K^- (6.8, 7.8; BamHI/pC-2)	germline/rearranged (D)
IARC/BL41	K^+ (19.0 BglII/pC-2); K^- (13.5 BglII/pC-2)	

Table 1. K gene rearrangements and the four types of configuration of V_KIV alleles of human lymphoid cell lines. The origin and characterization of the cell lines is described in ref. 9, for references on the lines Raji and Ramos see Materials and Methods. Configurations of K genes were determined by hybridizing Southern blots of DNA digests; fragment sizes in kb, restriction nucleases, and probes (7) are indicated. The configuration of the K alleles of Ly91 was determined by B. Henglein (unpublished). For a description of the rearranged K genes of the cell lines Daudi, Walker, GM607, RPMI 6410, and BL41 see refs. 7-9. The configuration of V_KIV alleles was determined by Southern blot hybridizations with the clone mJIuL-16 (see text).

The V_KIV gene is aberrantly rearranged at least in two of the four cell lines which contain V_KIV rearrangements: the lines Wi-L2 and BL41 express KII and KIII chains, respectively. Whether the rearranged V_KIV gene of the cell line BL21 is productively or aberrantly rearranged cannot be decided since no K chains could be detected in cell culture fluids (9). Only for the line JI a productive V_KIV rearrangement could be proven both by structural analysis of the gene and by immunochemical assays (9).

DISCUSSION

In a recent report from this laboratory it was shown that a segment of the human V_K locus of at least 40 kb but probably comprising 90 kb or more is duplicated (30). The degree of divergence between the two 40 kb segments and their 5 V_K genes each is very small. Also a single human V_KI gene unrelated to this gene cluster has been shown to be duplicated (31). In this context it was interesting to ascertain whether the V_KIV gene reported in the present paper occurs in one or two copies within the haploid human genome. All data from digestion experiments with numerous restriction nucleases point to the existence of only one V_KIV gene. This holds for experiments with both, placenta DNA and DNA from various lymphoid cell lines. It also fits into this picture that the V_KIV gene regions of two unrelated individuals are identical and that the sequence of the rearranged V_KIV gene VJI is very similar. One can, of course, not rule out that there exist within the genome two copies of the V_KIV gene region which are identical in all restriction nuclease cleavage sites studied. But this is rather unlikely. We therefore conclude at the present state of investigation that there is only one V_KIV gene per haploid genome. The existence of a single V_KIV gene facilitates the study of a number of questions of general interest.

Studies on somatic mutations are frequently made difficult by the high sequence homology among the members of a subgroup. In the one-member subgroup of the V_KIV gene it was, of course, easy to establish the relation between the rearranged gene and its germline counterpart. The extent of somatic mutations of VJI

is in the range observed for some other V_K genes (e.g. 24,25) but lower than found in some recent examples (e.g. 26). The observation that in the V_KIV gene three out of four somatic mutations lead to amino acid changes is in agreement with the general belief (e.g. 32) that in the variable regions of immunoglobulin genes those mutations are selected for which lead to an increased diversity.

The finding of 14 V_KIV deletions within the 32 alleles of the 16 lymphoid cell lines studied here can be interpreted in different ways. It is not clear whether the high percentage of deletions is a pecularity of the V_KIV gene or whether similar numbers would be found for genes of the other subgroups in which such studies are very difficult or impossible because of the high degree of crosshybridization of the genes within the subgroups. There exists also the possibility that the extent of deletions is higher in the established lymphoid cell lines than in normal antibody producing lymphoid cells. But still, considering the various mechanisms of V_K-J_K rearrangement which are being discussed one has to keep in mind the high deletion rate of the V_KIV gene.

The third and possibly most interesting observation is the finding that in four of the 16 cell lines the V_KIV gene is rearranged. The rearrangement is productive in one of the lines and aberrant in two others; in the fourth line it could not be clarified yet whether the rearrangement is productive or aberrant. By now sequences of 27 presumably non-allelic V_K genes and pseudogenes have been published (compiled in ref. 30) and the true number of V_K genes will certainly be higher. The fact that the V_KIV gene is rearranged in one fourth of the cell lines studied although it is only one out of 30 or probably more genes, is surprising. Within the mouse V_H locus certain gene(s) which have been mapped to be located in a J_H proximal position (33) have been found to be rearranged particularly often, at least in the early stages of lymphocyte development (34,35). Whether the human V_KIV gene is located in a J_K proximal position is not yet known. There may be other structural and also functional reasons for the high proportion of V_KIV rearrangements. In any case, the V_KIV gene is a particularly

interesting candidate for studying the V_K-J_K rearrangement mechanisms.

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