Volume 13 Number 18 1985 Nucleic Acids Research

Human immunoglobulin kappa light chain genes of subgroups II and III

H.-Gustav Klobeck, Alfons Meindl, Gabriele Combriato, Alan Solomon[1] and Hans G.Zachau

Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie der Universität München, FRG, and [1]Department of Medicine, University of Tennessee, College of Medicine/Knoxville Unit, Knoxville, TN 37920, USA

Received 5 July 1985; Accepted 6 September 1985

ABSTRACT

The first complete sequences of functionally rearranged V_K genes (abbreviations ref.1) of subgroups II and III are reported. The genes have been cloned from lymphoid cell lines synthesizing KII or KIII light chains as evidenced from immunochemical analyses with anti-V_K subgroup-specific antisera. These data, together with the sequence of a KIV gene (described in the accompanying paper) and those of previously published KI genes make possible a comparison of genes representative of the four known V region subgroups of human K light chains. The V_KII gene is distinguished from the V_KI, V_KIII, and V_KIV genes by a much longer intron within the leader sequence: 426 bp vs ca. 120-220 bp. Blot hybridization experiments with human DNA digests using probes from the KII and KIII genes and from the respective upstream regions help to define subgroup specific probes and hybridization conditions.

INTRODUCTION

Considerable information has become available over the past several years on the composition and chromosomal location of the human immunoglobulin genes (reviewed in refs. 2,3) but the chromosomal organization of the variable genes is still largely unknown. In this regard, we have focused our efforts on the genomic organization of the human K light chain locus (review ref. 4) with special attention directed towards isolating genes representative of the four known V_K subgroups I, II, III, and IV. V_K subclones derived from these genes seemed essential as probes for screening genomic libraries. The human V_K genes which had been found initially by crosshybridization with mouse V_K probes belonged to subgroup I (5,6). Additional clones obtained with these human V_KI genes as probes were all confined to subgroup I (6-8). To identify for gene cloning studies cells producing non-KI light chains, we examined with anti-V_K

subgroup-specific antisera (9), culture fluids from 11 different human lymphoblastoid cell lines. Our serological analyses resulted in the identification of the line GM607 as a KII producer and the subsequent isolation and sequence analysis of the rearranged KII gene (10). A subclone derived from the GM607 clone was helpful in localizing and identifying a V_KII pseudogene within a cluster of V_K genes of other subgroups (11). Although some V_KII and V_KIII genes could be localized on isolated clones by their weak crosshybridization with V_KI probes (11,12, and H.D. Pohlenz, B. Straubinger, E. Lötscher, H.G. Bauer, unpublished) it seemed desirable to isolate from lymphoid cell lines complete and functional K genes of all subgroups. In the course of this work two KI genes (13) and a KIV gene (14) were cloned and characterized.

Since the above mentioned KII gene clone lacks the leader sequence (10) and otherwise only germline V_KII pseudogene segments are known (11,15) the KII gene reported in this paper represents the first complete and functional gene of this subgroup. The same holds for the KIII gene since the known sequences stem from a cDNA clone (16), from germline V_KIII gene segments (12), and from an aberrantly rearranged V_KIII gene segment (17). Subclones derived from the new KII and KIII genes were used to study the multiplicity of the respective germline genes by blot hybridization.

MATERIALS AND METHODS

Immunodiffusion assays of human K light chains

Lymphoid cells were grown to a final density of $1.5\text{-}2.0\text{x}10^6$ cells/ml as previously described (13). Cells were harvested by centrifugation, the culture fluids were lyophilized and resuspended in water at a protein concentration of 200 mg/ml for immunodiffusion assays. The reconstituted cell supernatants were tested against specific anti-KI, KII, KIII, and KIV antisera and against anti-K antisera that are capable of distinguishing subsubgroups within KI, KII, KII, and KIV proteins (9). All antisera were absorbed with fetal calf serum prior to use. Immunodiffusion assays were carried out in 2% agar-3% polyethylene glycol 6000 as described (9).

Cell lines and cell culture

The human lymphoid cell lines GM1500 and GM607 were obtained from the Human Mutant Cell Repository, Camden, New Jersey; the lines IARC/BL36, 41 and 61 (ref. 18) and the lines IARC/BL21, 31 and Ly91 (ref.19) were provided by G.M. Lenoir, Lyon, WI-L2 (20) by G. Riethmüller, München, U698M (21) by E. Wecker, Würzburg, JI (22) by G.W. Bornkamm, Freiburg, RPMI 6410 (23) and Walker by H. Rodt, München, Daudi by H. Wolf, München; for the characterization of the Walker and Daudi cells and for the conditions of cell culture see ref. 13. High molecular weight DNA was prepared from the same placenta (AF) as previously (refs. 10,13).

Blot hybridizations

Gel electrophoresis and transfer of DNA digests (placenta AF) were as described (13). Hybridizations were carried out in 4xSSC, 1xDenhardt's solution, 50 µg/ml sheared and denatured salmon sperm DNA at 68°C; the filters were washed with 2xSSC, 0.5% SDS (non-stringent) or 0.1xSSC, 0.5% SDS (stringent conditions) at 68°C. Isolation of DNA fragments from agarose gels and cloning procedures were as described (13).

DNA sequencing and computer programs

DNA fragments were cloned in the M13 phages mp8-11 (24) or mp18,19 (25) and sequenced by the chain termination method (26). For sequencing a long restriction fragment a 1 m long sequencing gel (27) was used.

DNA sequences were aligned for maximum homology with the help of the program DNMAHO (DNa MAximize HOmology). The program DNPERC (DNa PERCentage) was used to calculate the percentage of homology within selected sequence sections. The programs which were written by P.S. Neumaier are described in ref. 11.

RESULTS

Immunochemical investigation of K light chains from human lymphoid cell lines

Culture fluids of 11 K light chain-producing cell lines were examined serologically to determine the V_K subgroup of the excreted protein. Immunodiffusion assays of cell culture fluids with V_K subgroup-specific antisera (9) led to the assignment to one of the four human K subgroups as summarized in Table 1.

Table 1.

V_K subgroup	Cell Line	Anti-KI	Anti-KII	Anti-KIII	Anti-KIV
I	Daudi	++	0	0	0
	Walker	++	0	0	0
	GM1500	++	0	0	0
	IARC/BL36	+	0	0	0
	IARC/BL61	++	0	0	0
II	RPMI 6410	0	++	0	0
	GM607	0	++	0	0
	Wi-L2	0	+	0	0
	U698M	0	++	0	0
III	IARC/BL41	0	0	++	0
IV	JI	0	0	0	++

Immunochemical reactivity of culture fluids from human lymphoid cell lines with anti-K subgroup specific antisera.
Immunodiffusion data obtained by screening culture fluids of human lymphoid cell lines with V_K subgroup specific antisera are shown (++, +, 0; strong, weak, and no precipitin reaction, respectively). In the culture fluids of the cell lines IARC/BL21,31 and Ly91 no K light chains were detected. This might be explained, at least for the cell line Ly91, with the finding that only a low percentage of cells were K positive in immunofluorescence assays (18). The serologic classification is in agreement with DNA sequence data for the Daudi and Walker cell lines (13), the GM607 line (10), and the JI line (14).

Certain of our anti-KI specific antisera were capable of differentiating KI proteins into three sub-subgroups (data not shown). The Daudi and Walker KI chains are members of the same sub-subgroup, which also comprise the KI proteins GAL, MEV, BI, HBJ4 and CAR (28). Furthermore, one antiserum (R226) distinguished K chains Walker and Daudi. The proteins BL36 and BL61 showed a somewhat similar pattern of reactivity as did protein Walker.

Among the cell lines which synthesize KII proteins, protein RPMI 6410 could be readily distinguished serologically from protein GM607 and from proteins TEW, CUM, BATES (28). With certain KII antisera, the reactivity of protein RPMI 6410 was more similar to protein CUM than to TEW.

The K light chain produced by the cell line BL41 was

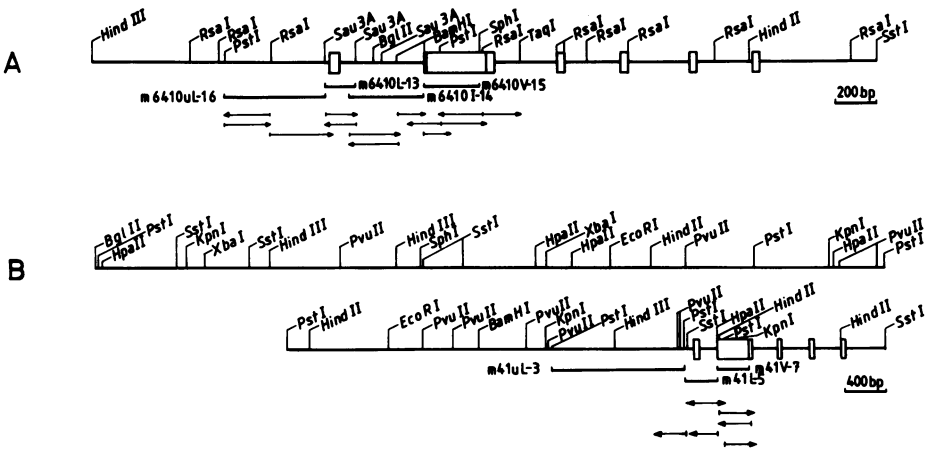

Figure 1. Restriction maps, sequencing strategies, and subclones of the productively rearranged V_K gene regions of the cell lines RPMI 6410 (A) and IARC/BL41 (B). Gene segments are shown as open boxes. Extent and direction of sequencing are symbolized by arrows underneath the restriction maps. Subclones prepared for blot hybridizations are delineated underneath the restriction map. The V6410 subclones m6410uL-16, m6410L-13, m6410I-14 and m6410V-15 contain a 0.51 kb PstI-Sau3A, a 0.17 kb Sau3A, a 0.35 kb HaeIII-BamHI, and a 0.3 kb BamHI-SphI fragment, respectively. The V41 subclones m41uL-3, m41L-5 and m41V-7 contain a 1.32 kb PstI, a 0.3 kb PstI-HpaII, and a 0.32 kb HpaII-Sau3A fragment, respectively. The restriction map of the V gene region from IARC/BL41 is shown in two parts, the upper one being an 8.2 kb extension of the 5' flanking region of the V gene.

classified serologically as KIII, and further could be assigned to sub-subgroup KIIIb (29).

The rearranged V_KII gene of the cell line RPMI 6410

The first rearranged V_KII gene, as it was cloned from the cell line GM607 (10), lacked the intron and leader sequences. In order to gain insight into the sequence organization of V_KII genes and to be able to compare not only the V_K gene segments of the different subgroups but also their flanking regions we cloned a rearranged V_KII region which extends to the region 5' of the leader segments. The cell line RPMI 6410 was selected since it contains only one rearranged K gene. In addition, we wanted to clarify a discrepancy: on the basis of limited sequence data of CDR3 (ref.30) the rearranged K gene from RPMI 6410 had been classified as a member of subgroup I (ref.28), which was in contrast to our immunochemical analyses.

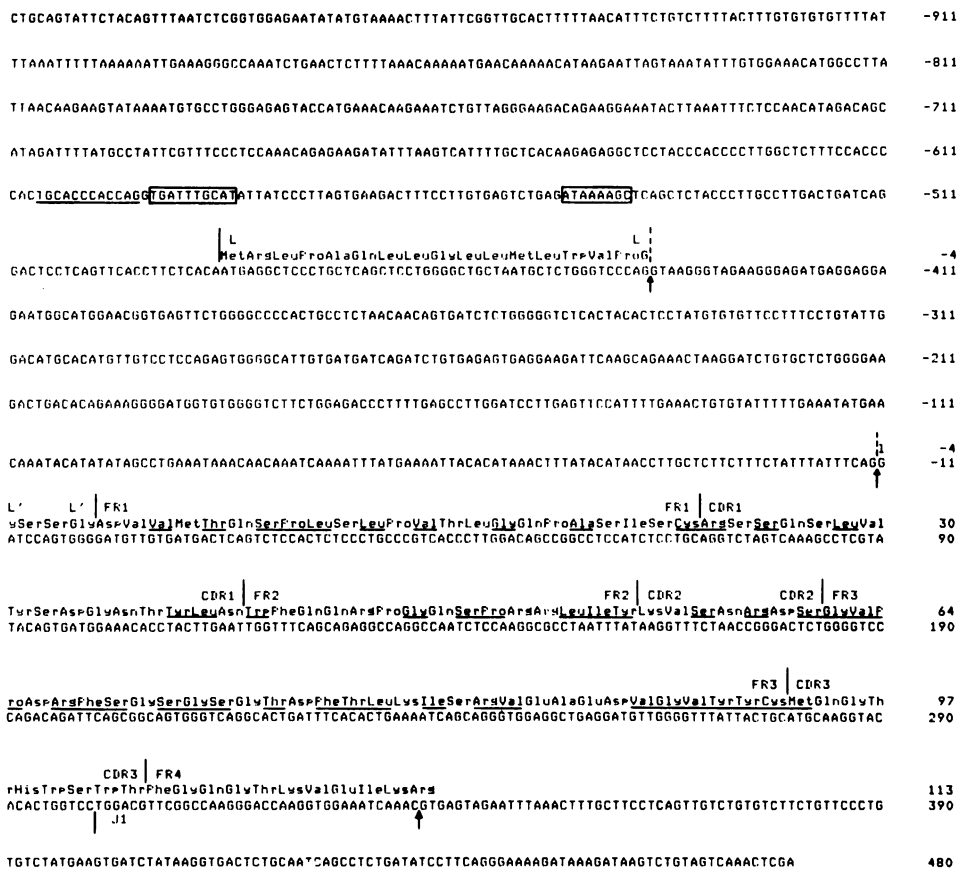

Figure 2. Nucleotide sequence of the V gene region of RPMI 6410 (V6410) and deduced amino acid sequence. The sequence was determined using the strategy shown in Fig. 1A. Vertical arrows designate the putative splice sites. V_KII specific residues (28) are underlined. The TATA sequence (32) and the putative regulatory sequence element dc (33,34) are boxed, a pd (33) related element is underlined.

HindIII fragments from genomic DNA of RPMI 6410 cells which were size-selected by agarose gel electrophoresis were ligated into the phage vector Charon 30 (ref.31) and screened with the intron probe pI-1 (ref.13). The restriction map of the cloned rearranged V_K gene region is shown in Fig. 1A. Subclones were prepared in M13 phages and sequenced with the dideoxy chain termination method. Fig. 2 presents the nucleotide sequence of the relevant parts of the clone.

A comparison of the deduced amino acid sequence of the cloned V_K gene with known protein sequences of the four human K chain subgroups (28) confirmed our immunochemical data. Within the FR1, CDR1, CDR2 and FR3 of protein RPMI 6410 all KII invariant amino acid residues (28) are present. Five residues within the FR2 and CDR3 (pos. 36, 37, 39, 46, and 95) have not as yet been found among the relatively few human KII proteins sequenced (28). The homology of the amino acid sequence deduced from V6410 to that deduced from the V_KII gene of GM607 (10) is 79.4%. As expected there is less homology of the KII protein RPMI 6410 to the deduced sequences of the KI protein V1 (ref.6; HK102 in ref.5), the KIII protein BL41 (see below) and the KIV chain JI (14), i.e. 49.5%, 54.7% and 64.7%, respectively. The corresponding nucleotide sequence homologies are 85.3%, 62.8%, 66.6% and 66.8% for GM607, HK102, Bl41 and JI, respectively. Thus the assignment of the expressed V gene of RPMI 6410 to subgroup II is unambiguous.

The intron between the leader segment and the L' region of V6410 is much larger (426 bp) than the introns of known V_KI (~125 bp; refs. 5-8,11), V_KIII (186 bp; ref. 12 and this paper) and V_KIV (219 bp; ref. 14) genes. According to the splice rules (32) the localization of the L segment is unequivocal. The TATA box and the putative regulatory sequence dc (33,34) appear in the expected positions. The unusually large intron seems to be characteristic for V_KII genes since it was also found in three other genes of this subgroup (B. Straubinger, E. Huber, H. Smola unpublished). The intron within the leader sequence of V6410 contains a number of direct and inverted repeats as well as sequences with a homology to the so-called enhancer core sequence of SV40 (review 35). It is not yet known whether some of these sequences are functionally important.

The V6410 region contains the J_K1 segment as was previously shown by Hieter et al. (30). A comparison with their sequence data which start at codon 86 revealed the following differences: A instead of C at pos. 300; A instead of G at pos. 318; GTG instead of GTTG at pos. 401-403; C instead of T at pos 436. The sequence we found in the latter two positions (which are located within the J_K1 region) was also found in the respective part of

Figure 3. Nucleotide and deduced amino acid sequence of the V_K region of the cell line IARC/BL41 (V41). The sequence was determined following the strategy shown in Fig. 1B. Vertical arrows designate the putative splice sites (32). V_KIII specific (28) amino acid residues are underlined. The TATA sequence (31) and the putative regulatory elements dc (33,34) and pd (33) are boxed.

the V607 gene (10).

The productively rearranged V_KIII gene of the cell line IARC/BL41

The cell line IARC/BL41 which excretes a KIII protein (Table 1) contains two rearranged K genes (K^+, K^-). Size-selected BglII fragments were ligated into the phage EMBL3 (ref.36). A recombinant DNA clone was then isolated by hybridizing with the C_K probe pC-2 (ref.13). Fig. 1B shows its restriction map. The V gene segment is rearranged to J_K2. No additional V gene is present on the V41 clone according to blot hybridizations with probes of the four subgroups. The DNA sequence analysis (Fig. 3) proved that we have cloned the expressed KIII gene, since no stop codons or indications of an aberrant rearrangement were found.

The assignment of the rearranged gene to subgroup III is

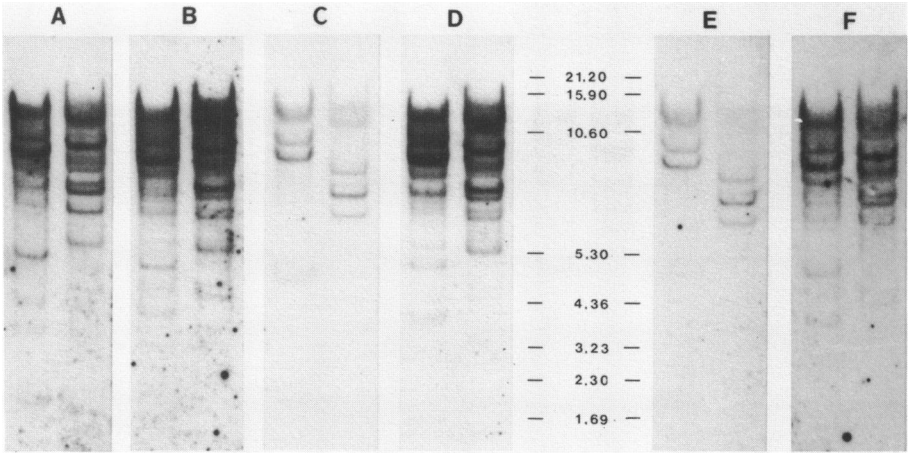

Figure 4. Blot hybridization of placenta DNA digests with probes from the V6410 gene region. Two Southern blots prepared from EcoRI and HindIII digests of placenta DNA were hybridized with probes from upstream of the leader segment (m6410uL-16; A), the leader segment (m6410L-13; B), the intron (m6410I-14; C and E) and the V_K gene (m6410V-15; D and F). Hybridization was under non-stringent (A-D) and stringent conditions (E,F). In each of the subpanels A-F the left slots contain EcoRI digests, the right ones HindIII digests. Sizes of marker fragments (digests of pBR322 and pBR clones) are given in kb.

unambiguous on the basis of the codons for invariant KIII associated amino acid residues (28). As expected there is a close homology to the nucleotide sequences of the germline V_KIII genes Vg and Vh (90.9% and 87.8%, respectively; ref. 12), the cDNA clone derived from the cell line NG9 (92.3%; ref. 16), and the aberrantly rearranged V_KIII gene of the EVJK 11 clone (93 %; ref. 17).

Blot hybridization of placenta DNA digests with probes from the V_KII and V_KIII gene regions

The availability of hybridization probes from the V_KII and V_KIII gene regions allows us to address the question of crosshybridization between the genes of the various subgroups and, with that, the problem of the relation between the number of bands in genomic blot hybridization experiments and the number of V_K genes in the human light chain gene repertoire.

Probes from the regions upstream of the leader segment (uL), the leader (L) and the V_K gene segments (V) of V6410 and V41

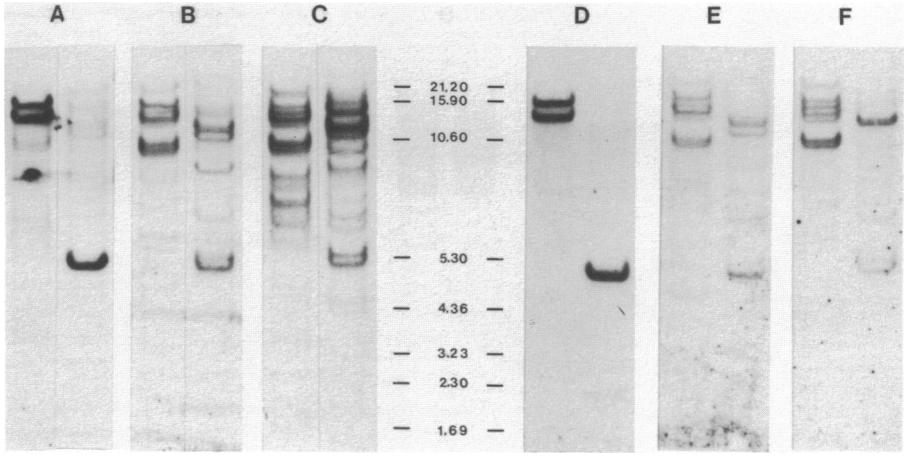

Figure 5. Blot hybridization of placenta DNA digests with probes from the V41 gene region. Two Southern blots prepared from EcoRI and BglII digests of placenta DNA were hybridized with probes from upstream of the leader segment (m41ul-3; A and D), the leader segment (m41L-5; B and E) and the V_K gene (m41V-7; C and F). The filters were washed under non-stringent (A-C) and stringent conditions (B-F). In each of the subpanels A-F the left slots contain BglI digests, the right ones EcoRI digests. Sizes of marker fragments (digests of pBR322 and pBR clones) are given in kb.

(Fig. 1) as well as Southern blots of placenta DNA digested with a number of restriction nucleases were prepared. The filters were hybridized with the probes under relaxed and stringent conditions.

The autoradiogram of an experiment carried out with the probes from the V6410 region is shown in Fig. 4. In addition to the probes described above a probe from the intron region (m6410I-14; Fig.1) was included into the studies. The restriction nucleases EcoRI and HindIII were chosen because they do not cut within the region spanned by the probes in V6410 and other known V_KII germline genes and pseudogenes (11,15 and H.D. Pohlenz, B. Straubinger, E. Huber, H. Smola, unpublished). This may be true for all or most V_KII genes since the band patterns obtained with the probes from upstream of the leader segment, the leader segment, and the V_K gene itself are indistinguishable under non-stringent hybridization conditions (Fig. 4A,B.D). The intron probe detects fewer bands (Fig. 4C). Apparently only in a few

V_KII gene regions the intron sequences are sufficiently similar to allow hybridization with our probe. The hybridization of the probes from the V6410 region appears to be V_KII specific even under non-stringent conditions because the same band pattern is detected under stringent hybridization conditions (Fig. 4E,F). Also with another V_KII gene probe (V607, ref. 10) which shares 85.3% homology with the V6410 probe the same patterns were found (data not shown).

The autoradiogram of an analogous experiment carried out with the probes from the V41 gene region is shown in Fig. 5. The V_KIII gene itself (m41V-7) hybridizes with a large number of fragments under relaxed conditions (Fig. 5C). The number of bands hybridizing with the leader probe (m41L-5; Fig. 5B) is roughly one third of those hybridizing with the V_KIII probe. Using the probe from upstream of the leader segment (m41uL-3) only one prominent band is left in an EcoRI digest (Fig. 5A) together with some faint bands, which correspond to bands seen also with the other two probes from the V_KIII region. Under stringent conditions the number of bands seen with the V_KIII probe is reduced dramatically (Fig. 5F); seven and five bands are left in the BglII and EcoRI digests, respectively. The same set of bands, although with different signal intensities shows up in the autoradiogram using the leader probe (Fig 5E). The probe from upstream of the leader segment detects only one band in an EcoRI digest and two bands in a BglII digest (Fig. 5D).

While the hybridization patterns obtained with three probes from the V_KII gene region are very similar to each other, even under non-stringent conditions, the patterns with the V_KIII region probes differ drastically. Possible reasons for that will be discussed below.

DISCUSSION

The four human V_K subgroups defined initially on the basis of protein sequence and serological data (reviewed in refs. 9,28, 37) can now be studied on the gene level. Up to now all known gene sequences (with one possible exception; ref. 17) fit into the subgroups. Our approach has been to define the conditions under which certain probes hybridize in a subgroup-specific

manner. It should be pointed out, that all hybridization data discussed in this paper, refer to hybridization between a probe and genomic DNA digests; hybridization between isolated gene fragments shows slightly different characteristics. We wanted to clarify whether more stringent hybridization conditions would help to overcome problems arising from crosshybridization between members of different V_K subgroups and whether probes from the leader regions or from regions upstream of the leader segment would yield subgroup specific hybridization signals in Southern blots of genomic DNA.

V_KI genes, which seem to comprise the major part of the human V_K gene repertoire, appear to crosshybridize with V_K genes of subgroups III and IV. This was deduced from the fact that the respective proteins are fairly homologous (7). With respect to subgroup III the contention was supported by blot hybridization experiments (16), and with respect to both, subgroups III and IV, by DNA sequence data which reveal a homology of 69-74% between V_KI genes and the V_KIII gene V41 and of 70-74% between V_KI genes and the single V_KIV gene (14). Further work is required to develop if possible probes and conditions which define the V_KI genes or groups of V_KI genes more precisely with respect to their crosshybridization to V_KIII and V_KIV genes. Considering the low homology between V_KI and V_KII genes (64%) and the quite different hybridization patterns (10) we conclude that no detectable crosshybridization occurs between these V_K subgroups even under non-stringent hybridization conditions.

The V_KII genes seem to be closely related to each other since the number of hybridizing bands is practically identical under stringent and non-stringent hybridization conditions. Furthermore, the probes from upstream of the leader segment and the leader probe detect the same bands as the V_KII gene probe itself. With the intron probe a subset of the V_KII genes is seen. A crosshybridization of the V_KII gene probe with subgroup III and IV genes is very unlikely, since the homologies of the V6410 gene to the known members of these subgroups are below 67%. The V_KII leader region probe apparently does not crosshybridize with leader regions of other genes since the homology for instance to the corresponding region of a subgroup I gene (V1) is restricted to the leader sequence itself (85%). The data demonstrate that

blot hybridization with V_KII gene probes is subgroup specific even under non-stringent conditions and that these probes probably recognize most or all members of the V_KII subgroup.

With a V_KIII gene probe a large number of bands is seen under non-stringent hybridization conditions, most of the bands being also present in experiments with a V_KI gene probe (7,10,16); but additional bands are present which are not detected by a V_KI gene probe. The crosshybridization can be avoided by using stringent conditions which reduce the number of bands to seven or less depending on the restriction nuclease digest studied. It seems unlikely that these bands represent all the V_KIII genes of the V_K locus considering the high proportion of sequenced V_KIII proteins in relation to all sequenced V_K chains (28). More likely only a subset of the V_KIII genes is detected. A relation between this subset and the immunochemically defined sub-subgroups (9,29) cannot be established at present. The observation that the 5' flanking regions of V_K genes (5-8,11-15) are well conserved within the subgroups but differ significantly between the subgroups offers the opportunity to use, under relaxed conditions, fragments from the 5' regions of the genes as subgroup specific probes. This approach seems to be successful: the V_KIII leader region probe (m41L-5) detects only about one third of the bands found with the V_KIII gene probe and those bands possibly represent most or all V_KIII genes.

In the case of V_KIV, only one germline gene has been detected using the subgroup specific probes. Specific hybridization conditions are described in the accompanying paper (14).

The present work is part of a continuing effort of our laboratory to establish the number and organization of the human V_K genes. Our studies have indicated the essentiality of performing (in parallel to the cloning and sequencing work, e.g. refs.11,15) hybridization experiments, such as reported here, and to correlate the results of the various methods employed in gene analysis. The V_KII and V_KIV gene containing fragments can be unequivocally identified in blot hybridization experiments (10,14). We have now achieved similar results with the V_KIII gene-containing fragments. However, our data indicate the

complexity of the V_KIII genes within the V_K locus; additional structural work on distantly related V_KIII genes is necessary. Similarly, the large group of V_KI genes requires further structural work and hybridization studies before the known patterns (7,10) can be interpreted in a reliable way with respect to crosshybridization between more or less distantly related genes. The results on the V_KII and V_KIII genes reported in this paper and on the V_KIV gene described in the accompanying paper (14) contribute to our knowledge of the properties of the V_K multigene family.

ACKNOWLEDGMENT

We are indebted to G.M. Lenoir, G. Riethmüller, E. Wecker, G.W. Bornkamm, H. Rodt, and H. Wolf for cell lines. A.S. thanks D. Weiss for assistance and acknowledges support by NCI grant CA 10056. Otherwise the work was supported by Bundesministerium für Forschung und Technologie and Fonds der Chemischen Industrie.

REFERENCES

1. Abbreviations: L,L', leader, V, variable, J, joining, C, constant segments of the immunoglobulin K light chain genes. V641o, V6o7, V41, VJI are the productively rearranged V gene segments of the respective cell lines. FR, CDR, framework and complementarity determining regions of the immunoglobulins. The designation of clones is the following: the letter m in front of the numbers refers to the vector phage M13; u, upstream (5'), e.g. m41uL-3 is a subclone from the region upstream of the leader segment of the rearranged V41 prepared in M13. kb, kilobase pairs; bp, base pairs.
2. Tonegawa, S. (1983) Nature 302, 575-581.
3. Honjo, T. (1983) Ann. Rev. Immunol. 1, 499-528.
4. Zachau, H.G., Pech, M., Klobeck, H.G., Pohlenz, H.D., Straubinger, B., and Falkner, F.G. (1984) Hoppe-Seyler's Z. Physiol. Chem. 365, 1363-1373.
5. Bentley, D.L. and Rabbitts, T.H. (1980) Nature 288, 730-733.
6. Jaenichen, H.R., Pech, M., Lindenmaier, W., Wildgruber, N., and Zachau, H.G. (1984) Nucl. Acids Res. 12, 5249-5263.
7. Bentley, D.L. and Rabbitts, T.H. (1981) Cell 24, 613-623.
8. Bentley, D.L. and Rabbitts, T.H. (1983) Cell 32, 181-189.
9. Solomon, A. (1985) in Methods in Enzymology, in press.
10. Klobeck, H.G., Solomon, A., and Zachau, H.G. (1984) Nature 309, 73-76.
11. Pech, M., Jaenichen, H.R., Pohlenz, H.D., Neumaier, P.S., Klobeck, H.G., and Zachau, H.G. (1984) J. Mol. Biol. 176, 189-204.
12. Pech, M. and Zachau, H.G. (1984) Nucl. Acids Res. 12, 9229-9236.
13. Klobeck, H.G., Combriato, G., and Zachau, H.G. (1984) Nucl. Acids Res. 12, 6995-7006.

14. Klobeck, H.G., Bornkamm, G.W., Combriato, G., Mocikat, R., Pohlenz, H.D., and Zachau, H.G., accompanying paper.
15. Pech, M., Smola, H., Pohlenz, H.D., Straubinger, B., Gerl, R., and Zachau, H.G. (1985) J. Mol. Biol. 183 in press.
16. Bentley, D.L. (1984) Nature 307, 77-80.
17. Stavnezer, J., Kekish, O., Batter, D., Grenier, J., Balazs, I., Henderson, E., and Zegers, B.J.M. (1985) Nucl. Acids Res. 13, 3495-3514.
18. Lenoir, G.M., Vuillaume, M., and Bonnardel, C. (1985) in Burkitt's Lymphoma, G.M. Lenoir, G. O'Conor and C.L.M. Olweny eds. (IARC Scientific Publications No.60) in press.
19. Lenoir, G.M., Preud'homme, J.L., Bernheim, A., and Berger, R. (1982) Nature 298, 474-476.
20. Levy, J.A., Virolainen, M., Defendi, V. (1968) Cancer 22, 517-524.
21. Nilsson, K. and Sundström, Ch. (1974) Int. J. Cancer 13, 808-823.
22. Bornkamm, G.W., Kaduk, B, Kachel, G., Schneider, U., Fresen, K.O., Schwanitz, G., and Hermanek, P. (1980) Blut 40, 167-177.
23. Tanigaki, N., Yagi, Y., Moore, G.E., and Pressman, D. (1966) J. Immunol. 97, 634-646.
24. Messing, J. (1983) in Methods in Enzymology, Vol 101, 20-78.
25. Norrander, J., Kempe, T., and Messing, J. (1983) Gene 26, 101-107.
26. Sanger, F., Coulson, A., Barrell, B., Smith, A., and Roe, B. (1980) J. Mol. Biol. 143, 161-178.
27. Garoff, H. and Ansorge, W. (1981) Analyt. Biochem. 115, 450-457.
28. Kabat, E.A., Wu, T.T., Bilofsky, H., Reid-Miller, M., and Perry, H. (1983) Sequences of Proteins of Immunological Interest, National Institutes of Health, Bethesda.
29. Ledford, D.K., Goni, F., Pizzolato, M., Franklin, E.C., Solomon, A., and Frangione, B. (1983) J. Immun. 131, 1322-1325.
30. Hieter, P.A., Max, E.E., Seidman, J.G., Maizel, J.V., and Leder, Ph. (1980) Cell 22, 197-207.
31. Rimm, D.L., Horness, D., Kucera, J., and Blattner, F.R. (1980) Gene 12, 301-309.
32. Breathnach, R. and Chambon, P. (1981) Ann. Rev. Biochem. 50, 349-383.
33. Falkner, F.G. and Zachau, H.G. (1984) Nature 310, 71-74.
34. Parslow, T.G., Blair, D.L., Murphy, W.J., and Granner, D.K. (1984) Proc. Natl. Acad. Sci. USA 81, 2650-2654.
35. Khoury, G. and Gruss, P. (1983) Cell 33, 313-314.
36. Frischauf, A.M., Lehrach, H., Poustka, A., and Murray, N. (1983) J. Mol. Biol. 170, 827-842.
37. Kindt, T.J. and Capra, J.D.: The Antibody Enigma, Plenum Press, New York and London 1984.