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Molecular cloning of a human immunoglobulin λ chain variable sequence

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ABSTRACT

We have cloned a human Vλ cDNA sequence from an Igλ-producing human Burkitt lymphoma cell line (BL2) by taking advantage of a cloned constant region gene as a primer for cDNA synthesis instead of an oligo(dT) primer. The amino acid sequence deduced from the nucleotide sequence of Vλ clones is highly related to that of the NEW Vλ protein of subgroup I. Southern blot hybridization of human DNAs with the $V\lambda_I$ probe showed at least 12 hybridizing Vλ fragments. These fragments are amplified in K562 cells which derive from a case of chronic myelogenous leukemia and contain an amplified c-abl oncogene and amplified Cλ sequences.

INTRODUCTION

Immunoglobulin chains are encoded by three unlinked gene families: λ light chain, κ light chain and heavy chain genes which are located on chromosomes 22, 2 and 14, respectively, in man (1-4). The formation of an active immunoglobulin (Ig) gene involves the somatic joining of two or three separated segments of chromosomal DNA (5).

In the case of the mouse κ chain locus, several hundred germ line Vκ genes (6,7) exist as units separated from a single Cκ gene and the translocation of one of the Vκ genes to one of the four active Jκ segments of the Cκ locus results in the formation of a functional κ chain gene (8,9). The organization of the human κ locus is very similar to mouse (10). On the other hand, the gene organization of the λ locus seems to differ between mouse and human. Approximately 40% of human Igs contain λ chains (11), while only 3-5% of mouse Igs do (12,13). While inbred mouse strains contain only four Cλ genes (15), at least six Cλ genes are present on each human chromosome 22 in man (16). Three of the four inbred mouse Cλ genes ($C\lambda_1$, $C\lambda_2$ and $C\lambda_3$) are functional, while the fourth gene ($C\lambda_4$) is a non-functional pseudogene (17). A single joining segment is associated with each Cλ gene in mice (14,15). The feral mice show, however, considerable variation in the number of Cλ genes (18). In inbred mouse strains the $C\lambda_2$

segment is linked with the $C\lambda_4$ segment, while the $C\lambda_3$ is associated with the $C\lambda_1$ segment (17). All inbred mouse strains which were examined have only two Vλ genes (19-21), while feral mice have at least three Vλ genes (22).

In order to analyze the germ line organization of human Vλ genes, we have attempted to clone cDNA of human Vλ sequences.

MATERIALS AND METHODS

RNA Extraction

Cytoplasmic RNA of Burkitt lymphoma cell line (BL2) was prepared by the cesium chloride method (23). The poly(A)$^+$ mRNA was isolated by oligo(dT)-cellulose column chromatography.

cDNA Cloning

The DdeI-RsaI DNA fragment (146 bp) of Cλ2 DNA (Fig. 1) was isolated. Two pmoles of DNA fragment was digested with exonuclease III (50 units) in 6.6 mM Tris-HCl (pH 7.5)/60 mM NaCl/6.6 mM $MgCl_2$/5 mM sodium phosphate (pH 7.0)/6.6 mM β-mercaptoethanol at 23°C for 6 to 10 min (about 40 to 70 bases digested). Two pmoles of Cλ primer was hybridized with 5 μg of BL2 poly(A)$^+$ RNA at less stringent conditions in 50% formamide/20 mM Tris-HCl (pH 7.5)/0.1 mM EDTA/0.1% sodium dodecyl sulfate (SDS) successively at 37°, 26°, and 15°C for 60 min each. The double-stranded cDNA was synthesized by the procedures described in ref. 24. The cDNA longer than 350 bp was selected by agarose gel electrophoresis and after ligation of EcoRI molecular linker, cloned into λgt 11 phage vector (25).

DNA Sequencing

The nucleotide sequences were determined by the chemical degradation technique of Maxam and Gilbert (26).

Southern Blotting Analysis

Cellular DNA samples were digested with EcoRI and subjected to 0.7% agarose gel. Transfer of DNA from gel to nitrocellulose sheet was performed essentially as described by Southern (27). The filters were hybridized with ^{32}P-labeled probe DNA, as described in the figure legends, and finally washed with 2X SSC at 65°C.

RESULTS AND DISCUSSION

The poly(A)$^+$ mRNA was isolated from Ig λ-producing BL2 carrying a t(8;22) chromosome translocation. As primer for cDNA synthesis, we used a DNA segment of human Cλ gene instead of oligo(dT) because a primer derived

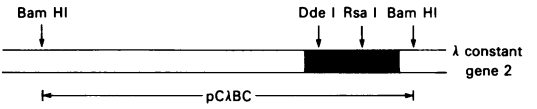

Fig. 1 Physical map of the human germ line λ light chain constant region #2 gene (16). The filled box represents the amino acid coding region. All restriction sites for DdeI and RsaI are not shown.

from a Cλ gene is specific for Igλ chain mRNA. We chose the $C\lambda_2$ gene (Fig. 1) as a DNA source of the primer although it was not known which Cλ gene is utilized in BL2 cells, since the different Cλ coding regions seem to differ by a very limited number of nucleotides (16). The DdeI-RsaI DNA fragment (146 bp) of $C\lambda_2$ DNA (28) shown in Figure 1 was isolated and digested with exonuclease III, as described in Materials and Methods. The purpose of exonuclease III digestion was twofold: i) to obtain a single-stranded region of primer to make the hybridization between mRNA and primer more effective, and ii) to increase the probability of base-matching at the 3' end of the primer. Cλ DNA primers were hybridized with BL2 poly(A)$^+$ RNA at low stringency conditions, as described in Materials and Methods. The double-stranded cDNA was synthesized using AMV reverse transcriptase and cloned into λgt11 phage vector, as described in Materials and Methods.

By screening with ^{32}P-labeled pCλBC (Fig. 1), recombinant clones containing Cλ sequence were selected. The restriction maps of three clones are shown in Figure 2.

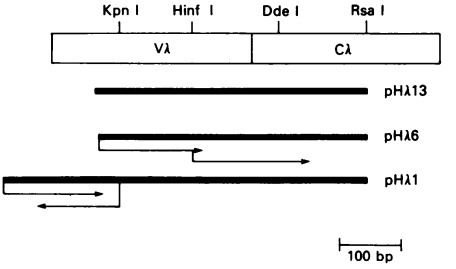

Fig. 2 Restriction map of cDNA clones representing human Ig λ light chain gene and the strategy for DNA sequencing. The regions shown by arrows were sequenced by the chemical degradation technique of Maxam and Gilbert (26). The box shown at the top indicates the amino acid coding region of Ig λ light chain gene. Each insert of three clones has EcoRI sites at both ends because EcoRI molecular linker was joined to double-stranded cDNA before cloning into λ gt11 vector (25).

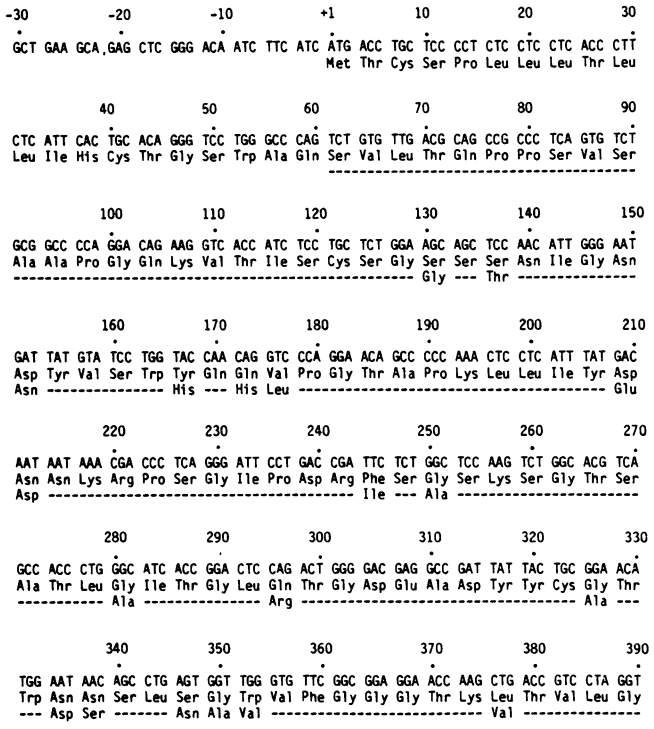

Fig. 3 Nucleotide sequence of human λ variable region. The amino acid sequences deduced are shown below the nucleotide sequence which coincides best with the amino acid sequences of NEW subgroup I light chain (29) shown at the bottom line. A dash in NEW amino acid sequence indicates identity in that amino acid position to the sequence of cloned Vλ DNA.

To confirm that these recombinant clones contain human Vλ sequences, we have determined nucleotide sequences of clones 1 and 6 (Fig. 3). Human Vλ chains have been classified into six subgroups according to their amino acid sequences. As shown in Figure 3, the amino acid sequence deduced from nucleotide sequence corresponds closely to the NEW protein (29) of Vλ subgroup I (matching of 92 amino acid residues out of 110). Thus BL2 cells seem to produce a subgroup I Vλ chain. Therefore we call this Vλ gene $V\lambda_I$. The amino acid sequence determined from the DNA sequence of pHλ1 has an additional 19 amino acid residues beginning with methionine at the N terminus of the mature Vλ sequence, which is likely to correspond to the signal peptide of precursor Igλ chain.

Since human Vλ genes may comprise a large family, we have tried to determine their multiplicity in human DNA. A DNA fragment, EcoRI (+133) to

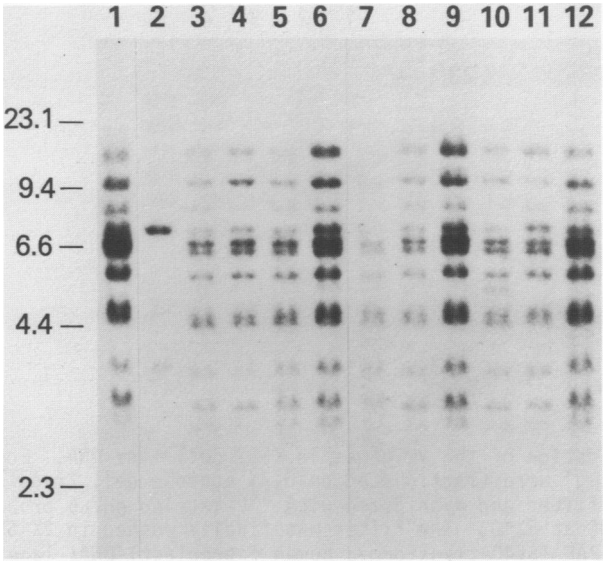

Fig. 4 Southern blot hybridization of human DNA with $V\lambda_I$ probe. Human DNAs were digested with EcoRI, fractionated on a 0.7% agarose gel and transferred to the nitrocellulose filter. The DNA on the filter was hybridized with pHVλ6 containing only Vλ sequence in the solution of 4X SSC/50% formamide at 37°C. The filter was finally washed with 2X SSC at 65°C. Lane 1, MC 116 (λ-producing Burkitt lymphoma) DNA; lane 2, BL2 (λ-producing Burkitt lymphoma) DNA; lane 3, U266 (λ-producing human myeloma) DNA; lane 4, GM1056 (λ-producing human lymphoblastoid cell) DNA; lane 5, SKO-007 (U266 HPRT$^-$) DNA; lane 6, CEM (human T-cell lymphoma) DNA; lane 7, PA682 (κ-producing Burkitt lymphoma) DNA; lane 8, JI (κ-producing Burkitt lymphoma) DNA; lane 9, Daudi (κ-producing Burkitt lymphoma) DNA; lane 10, DS178 (κ-producing Burkitt lymphoma) DNA; lane 11, PAF (SV40-transformed human fibroblast cell) DNA; lane 12, Colo 320 (human colon carcinoma) DNA (31-32).

HaeIII (+310) of pHλ6, containing only Vλ sequence (Fig. 3), was subcloned (pHVλ6) and used as a probe for the analysis of human Vλ genes in human DNA.

Southern blot filter of human DNA digested with EcoRI was hybridized with ^{32}P-labeled pHVλ6 and washed under less stringent conditions. As shown in Figures 4 and 5, at least 12 cross-hybridizing bands were detected in human DNA, suggesting that the pool of Vλ genes in man might be much larger than the Vλ gene pool in the mouse. In addition, the number and DNA sequence of human Vλ genes cross-hybridizing with $V\lambda_I$ probe seem to be well conserved among individuals. This is in striking contrast to human Cλ locus, remarkable polymorphism of which has been reported (16,30). Further, many of the λ-producing cell lines examined do not show any promi-

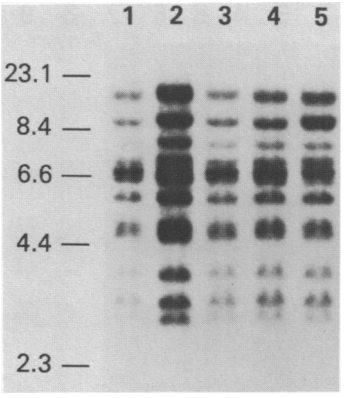

Fig. 5 Amplification of the Vλ genes in K562 cell line DNA. EcoRI-digested human DNAs (10 µg) were fractionated on 0.7% agarose gel, transferred to a nitrocellulose filter and hybridized with ^{32}P-labeled pHVλ6 probe in 50% formamide/6X SSC at 37°C. The filter was finally washed in 2X SSCS at 65°C. Lane 1, PAF (SV40-transformed human fibroblast) DNA; lane 2, K562 DNA; lanes 3, 4 and 5, human T-cell lymphoma DNA.

nent DNA rearrangement of $V\lambda_I$ gene (Fig. 4), indicating that these producer cells utilize other Vλ genes for chain production. Thus, man seems to have other functional Vλ genes which are not detectable by the $V\lambda_I$ probe.

Interestingly, we observed only two Vλ bands hybridizing with the $V\lambda_I$ probe in BL2 DNA (Fig. 4). The upper band corresponds to the productively

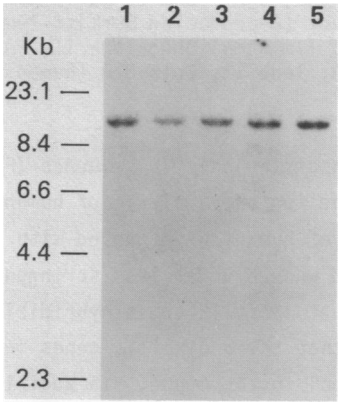

Fig. 6 The nitrocellulose filter described in Fig. 5 was rehybridized with a human myc cDNA probe specific for the two coding exons of the c-myc gene (Ryc 7.4) (28). As shown in the figure, the intensity of the c-myc band in K562 cells (lane 2) was not more prominent than in the other cell DNAs, indicating that the Vλ sequences were amplified in K562 cells (Fig. 5).

rearranged Vλ gene on chromosome 22 of BL2 cells (28) since it cross-hybridizes with a Cλ probe (data not shown). The lower band may represent a rearranged Vλ gene on the $22q^-$ chromosome of BL2 cells. In this cell line, the c-myc gene is in its germ line configuration and remains on the $8q^+$ chromosome, while a rearranged Cλ locus contained within a 12.0 kb EcoRI fragment is translocated to a chromosomal region 3' to the c-myc oncogene (28). The chromosomal breakpoint in this cell line is approximately 9 kb 3' to the c-myc oncogene on chromosome 8 and immediately 5' of a rearranged Cλ locus gene on chromosome 22 of BL2 DNA (Erikson and Croce, manuscript in preparation). Thus, most of the Vλ DNA fragments homologous to the $V\lambda_I$ DNA probes are deleted on both chromosomes 22 of BL2 cells.

Cloning and sequencing of members of other families of human Vλ genes will provide a better understanding of the structure and organization of the λ locus in man.

We have also examined the DNA of K562 human leukemic cells for the amplification of the $V\lambda_I$ sequences, since a five to ninefold amplification of both the c-abl oncogene and of the Cλ locus has been detected in these cells (31,32). The result of this amplification is a small marker chromosome, which is probably derived from a Philadelphia chromosome and carries co-amplified c-abl and Cλ sequences (32). As shown in Figure 5, we detect amplification of the $V\lambda_I$ sequences in K562 cells. The intensity of the $V\lambda_I$ bands in the K562 lane is approximately sixfold higher than the intensity of the $V\lambda_I$ bands in the other cell lines. Rehybridization of the same filter with a probe specific for the c-myc oncogene (Ryc 7.4) (28) indicates that no amplification of the c-myc oncogene occurred in K562 cells (Fig. 6). These results indicate that the amplification unit involving the joining of chromosomes 9 and 22 on the Philadelphia chromosome in K562 cells must be extremely large, since it contains all $V\lambda_I$ hybridizing DNA segments, all Cλ DNA segments (32) and the c-abl oncogene (32).

ACKNOWLEDGEMENT

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