Structure of the human gene encoding the invariant γ -chain of class II histocompatibility antigens

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ABSTRACT

The primary structures of a cDNA and the genomic DNA of a gene selectively expressed in chronic lymphocytic leukemia were determined. A computer search of the nucleotide sequence data bank identified this gene as the invariant γ -chain associated with class II histocompatibility antigens. The invariant γ -chain genomic sequence spans about 11 kilobases, with eight exons and seven introns. Three of the introns contain members of the *Alu* repeat family. A putative cap site and promoter sequence were identified at the 5' end of the gene. One or two copies of the gene is present in each haploid genome, and no evidence for amplification or polymorphism was obtained.

INTRODUCTION

The human class II histocompatibility antigens consist of polymorphic α and β chains and an associated invariant γ -chain (1). They are found on the cell surface of B-lymphocytes, a subset of activated T-lymphocytes and macrophages. The three glycoproteins have similar molecular weights (α ; 34,000; β ; 29,000; γ ; 33,000); however, the α and β chains show high electrophoretic polymorphisms and the γ chain is invariant (2). The genes for α and β chains are in the major histocompatibility complex (MHC) cluster on chromosome 6 (3) but the γ chain gene is on chromosome 5 (4). Monoclonal antibodies directed against the γ chain have been isolated (5) and cDNA clones encoding the γ -chain have been isolated and characterized (6-8).

In a previous study to identify the cDNAs that are preferentially expressed in human leukemias, we selected a clone (p7-2D-1) that was more highly expressed in chronic lymphocytic leukemia (CLL) than in several other tissues (9). Subsequent experiments have shown that this gene is expressed in human blood cells and their precursors but not in placenta, liver, kidney, pancreas, gut, or muscle (10). In normal cells the highest expression is found in resting peripheral blood B-lymphocytes.

In this communication we present the nucleotide sequence of the structural gene with its 5' and 3' flanking regions. Comparing of the cDNA sequence with the sequences in the sequence data bank identified the gene as that encoding the

invariant γ chain associated with class II histocompatibility antigens. This information is essential to understanding both the tissue-specific regulation and cell cycle-regulation of γ chain gene expression.

MATERIALS AND METHODS

Preparation of Poly(A) RNA, Northern Blot Hybridization, and Construction of cDNA library

The invariant γ -chain clone (p7-2D-1) previously isolated (9) contained a cDNA insert of 583 bp, which was shorter than the cDNA insert of the corresponding size (1440 nucleotides) of 7-2D mRNA. Therefore, cDNA libraries from CLL and acute lymphocytic leukemia ALL cells that expressed the 7-2D message were constructed again. Total RNA was extracted from the cells and poly(A⁺)RNA was isolated from total RNA by two passages over oligo(dT)-cellulose as previously described (11). The poly(A⁺)RNA was used to prepare double-stranded cDNA, which was inserted into the PstI site of the bacterial plasmid pBR322 after dG:dC homopolymeric tailing as described by Land *et al.* (12) and Lopez *et al.* (13). The recombinant plasmids were used to transform *Escherichia coli* strain RR1.

Screening and Sequencing

Bacterial colonies containing recombinant plasmids were screened with a 5'-end, ³²P-labeled PstI fragment (440 bp) of 7-2D-1 cDNA essentially according to the colony hybridization assay as described by Grunstein and Hogness (14). Approximately 4000 colonies of the CLL cDNA library and 2600 colonies of the ALL cDNA library were screened. The previously constructed (9) CLL cDNA library of 2300 colonies was also screened using a 3'-end probe (160 bp) of 7-2D-1 cDNA to obtain the 3'-end sequence. To decrease nonspecific hybridization, unlabeled pBR322 and oligo(dG) were added to the hybridization buffer at concentrations of 2 μ g/ml and 0.5 μ g/ml, respectively. Plasmids from colonies hybridizing with the 7-2D-1 probe were isolated from minilysates as described (15,16) but with some modification. The modified procedure was as follows: 3 M potassium acetate, pH 5.0, was used as a high-salt solution; after collecting the supernatant, ethanol precipitation was done at room temperature. The precipitate was then dissolved in 0.1 M sodium acetate in 50 mM Tris-HCl, (pH 8.0) and precipitated with ethanol again. The pellet was washed twice with 70% ethanol, dissolved in water, and treated with RNase A (40 μ g/ml) for 30 min at 37°C. The purified plasmids were analyzed after PstI digestion by electrophoresis on a 4% polyacrylamide gel. The relatively long inserts of several clones from the ALL cDNA library were separated by preparative gel electrophoresis and subcloned into bacteriophage vector M13. DNA was subjected to sequence analysis by the Sanger dideoxy- chain-termination

method (17). Sequence data were compiled and searched with the Intelligenetics IFIND command.

Phage Screening and Sequencing

The cDNA clone p7-2D-1 was labeled with ^{32}P by nick translation (18) to a specific activity of $1.5 - 2 \times 10^8$ cpm/ μg and used to screen a human genomic DNA library (19). The library, 6×10^5 plaques in bacteriophage λ Ch4A, was transferred to nitrocellulose filters and screened as described (20). A positive clone $\lambda\text{h7-2D-1}$ selected, analyzed for restriction enzyme cleavage sites, and sequenced. After being sequenced and mapped it was apparent that this phage did not contain the 5' exon of the 7-2D gene. A second screening of the library with p7-2D-1446, which contains 250 bp of the 5' end of 7-2D cDNA, resulted in the selection of $\lambda\text{h7-2D-2}$, a genomic clone containing 1.5-kb 5' flanking and 3.5-kb 3' flanking sequences.

Determination of Gene Copy Number and Analysis of 7-2D Genome

In order to determine the copy number of the invariant γ -chain gene, high-molecular-weight DNA was isolated from various normal and leukemic tissues as described (21). Samples were digested with BamH1 and analyzed by Southern blot hybridization with ^{32}P -labeled p7-2D-1 that contained the 3'- end sequence of cDNA. Copy number controls consisted of various amounts of $\lambda\text{h7-2D-1}$ DNA digested with BamH1 and coelectrophoresed adjacent to the genomic DNA samples. Amounts were loaded to correspond to 1,3,5, and 10 copies of 7-2D per haploid genome in 20 μg of DNA (22). To examine the 5'- end structure of the 7-2D gene in normal and CLL cells, high-molecular-weight DNA was separated from normal peripheral blood leukocytes and 5 cases of CLL. Southern blot hybridization of genomic DNA was carried out using 20 μg of DNA digested with HindIII. The filter contents were hybridized with ^{32}P -labeled p7-2D-1446 that contained the 5' end of the 7-2D cDNA.

RESULTS

Characterization of γ -chain mRNA and cDNA Sequence

Northern blot hybridization of total RNA from normal peripheral blood leukocytes, normal bone marrow cells and CLL lymphocytes with ^{32}P -labeled p7-2D-1 revealed a single band of about 1440 nucleotides (Figure 1). The 7-2D message was more abundant in CLL cells; this result was confirmed by the results of hybridization of many samples of poly(A⁺)RNA as well as of total RNA from leukemic cells (Narni et al., data not published). We were unsuccessful in isolating a longer sequence of 7-2D cDNA from the CLL library; however, two out of six positive clones in the ALL cDNA library had longer inserts than that of p7-2D-1. They were designated p7-2D-1446 and p7-2D-324. Sequence analyses of these clones revealed that the cDNAs had

Figure 1. Characterization of invariant γ -chain mRNA. Twenty micrograms of total RNA from normal peripheral blood leukocytes, normal bone marrow cells and CLL peripheral blood lymphocytes and 2 ng of *Hinf*I digested pBR322 was subjected to electrophoresis on 1.2% agarose gel after its denaturation in 1 M glyoxal and 50% dimethyl sulfoxide. Nucleic acids were transferred to nitrocellulose paper and hybridized with 32 P-labeled p7-2D-1 probe (specific activity: 1×10^8 cpm/ μ g) and autoradiographed for 16 hr at -70°C .

identical 5'- end sequences. After the three clones were sequenced, the primary structure of 1275 nucleotides of the cDNA was determined. The 1440 nucleotide mRNA is composed of a 1282 nucleotide transcript and ~160 nucleotide poly A tail. Using the computer program of Intelligenetics IFIND, a high homology with human leukocyte antigen-DR-associated invariant γ chain was found (6,7).

Primer Extension and Examination of 5'- End of Transcript

To determine the 5' end of the 7-2D mRNA, primer extension experiments were carried out using poly(A⁺)RNA from CLL cells as the template. We find that the first AUG in the longer mRNA is found at the tenth nucleotide, generating a

Figure 2. Structure of the γ -chain genomic clones. The relative sizes and positions of two genomic clones (λ 7-2D-1 and -2) described in the text are illustrated by the overlapping lines. The closed boxes indicate exon regions. White arrows indicate the Alu repeat sequences and their direction. Alu-2, and Alu-3 have the same direction as the consensus sequence (30). The black arrows indicate the direction and extent of sequences determined by the dideoxy-chain sequencing method. The precise sequence of 3.7 kb of the first intron, indicated by the gap in arrows, is unknown. Restriction sites in the map are abbreviated as follows: B, BamHI; E, EcoRI; H, HindIII; K, KpnI.

hypothetical protein with an open reading frame of 232 amino acids. There are five AUG codons in the 103 nucleotides at the 5'-end region of this mRNA. The first, second, and fifth AUG codons are in the same open reading frame, and the third and fourth AUG codons are in the same open reading frame. Kozak (24) summarized the sequences surrounding the functional AUG and found a consensus sequence of CC(A/G)CCAUG(G). Only the second and fourth AUG codons are in agreement with the consensus sequence and they generate open reading frames of 216 and 30 amino acids, respectively. Therefore, the most likely reading frame in γ -chain mRNA begins at position 58 and terminates at nucleotide 706, generating a protein of 216 amino acids. We searched for homology between the two hypothetical polypeptides generated from the third and fourth AUG codons and the known sequences in the Bionet data bank, however, no similar sequence was found that was similar to either polypeptide sequence.

Restriction Map and Sequence of the γ chain Gene

Several genomic clones were selected by screening a human genomic DNA library with the 7-2D cDNA clone. As shown in Figure 2, the mapping and sequencing strategy of genomic clones λ h7-2D-1 and λ h7-2D-2 revealed that the 7-2D gene spans approximately 11 kb and contains eight exons and seven introns. Three of the introns contain members of the Alu repeat family. Most of the large first intron has been sequenced; however, the lack of sufficient overlapping sequences precludes identification on the map of Figure 2. Figure 3 shows the

nucleotide sequence of the exons and their flanking sequences. All introns begin with the sequence GT at the 5' end and terminate with the sequence AG, and there is a reasonable concordance with the consensus sequence for exon/intron boundaries of other eukaryotic genes (25). The first putative cap site, as indicated by the primer extension experiment, is located at position 1, which is the first G residue of the sequence T-C-A-G-G-T-C-C. The second possible cap site is the first A residue of the sequence T-C-C-C-A-G-A-T-G, a position corresponding to the 5'-terminal residue in cDNA clones p7-2D-1446 and p7-2D-324. Because mapping by primer extension is subject to an error of 1 or 2 nucleotides, the positions indicated in the map may differ that much from the actual location. Inspection of the 5' flanking region of the cap site revealed a putative promotor sequence of T-T-T-A-A at -24 to -20 flanked by GC- rich regions at both the 5' and 3' sides, as well as a CATCT sequence at -85 to -81. These sequences were not typical of T-A-T-A and CAT boxes; however, their locations correspond to the previously characterized TATA box and C-A-A-T box in eukaryotic genes (26). At position 1289 near the 3' end of the gene there is an A-A-T-A-A-A sequence thought to be important in polyadenylation (27). A short distance (15 bp) downstream of the polyadenylation recognition site, the CA dinucleotide begins a GT cluster which has been proposed to be involved in correct polyadenylation (28). Figure 4 shows that the two outer Alu repeats in the introns are in opposite polarities. Alu-1, Alu-2, and Alu-3 have homologies with the consensus Alu repeat (29) of 87, 86, 82%, respectively. Direct repeat sequences flank the Alu-1, Alu-2, and Alu-3 repeats (Figure 5). Alu-3 has an AT-rich sequence of about 90 bp in the 5' flanking region and 35 bp in the 3' flanking region. Direct repeats are located adjacent to those AT-rich sequences. Alu-1 and Alu-2 have direct repeat sequences adjacent to the 5' end of the consensus sequence and at the 3' end of the A-rich sequence.

Determination of Gene Copy Number and Characterization of γ chain Gene in Various Tissues

Southern blot hybridization using p7-2D-1 probe revealed one band of approximately 6.7 kb (Figure 6) which corresponds to the size of the BamH1 fragment containing the 3' end of the gene (Figure 2). This blot shows that the 7-2D gene was present in only one or two copies per haploid genome in each of the six tissues examined. Comparison of the signal intensity of the fragment in the genomic samples with the intensity of the similar size band in the control lanes permitted an estimation of the number of γ -chain genes in normal and leukemic tissues. A genomic blot of one normal and five CLL DNAs digested with HindIII and probed with p7-2D-1446 showed a similar hybridization pattern with each DNA sample (data not

GGATCCTGGATCAGACTTGACTGTTCCATACATTCACTTCCACATTTCCAGCCCTTTGGAACCTGA -719
 AGATATCCTCTCTCTGCTGTCACTCTATGGGACTTACGGCTCTTTGTTAAAACTATTAAAGCAAG
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 CCCCACATCTGCCCCGAAAAGGCAGCTTCAACAAAGTGGGGTATTTCCAGCCTTTGTAGCTTTCACTT
 CCACATCTACCAAGTGGGGGAGTGGCCCTTCTGTGGAAGAATCAGATTCTCTCCAGCACCGACTTTAAG - 19

1 Exon 1

AGCGGAGCCGGGGGTCAGGGTCCAGATGCACAGGAGGAGAAGCAGGAGCTGTGGGAAGATCAGAAGC 51
 * MetHisArgArgArgSerArgSerCysArgGluAspGlnLysP

100

CAGTCATGGATGACAGCGCGAOCCTTATCTCCAACAATGAGCAACTGCCCATGCTGGGCGCGCGCCCTGG 121
 roValMetAspAspGlnArgAspLeuIleSerAsnAsnGluGlnLeuProMetLeuGlyArgArgProGlu

GGCCCCGAGAGGgtatgtgagcaccaggaaagggcacaccgatcctggactgcagaagccgtgcgcattgt 191
 yAlaProGluSe

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Exon 2

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Exon 3

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Exon 5
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ValP

Exon 6
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Exon 7

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Exon 8

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 TACCTGCAGGCTGAGCAGCTCTCTTCCCTTTCCCCAGCATCACTOCCCAAGGAAGAGCCAATGTTTTCOA
 CCATAATCTCTTCTGCCGACCCCTAGTTCCCTCTGCTCAGCCAAGCTTGTATCAGCTTTTCAGGGCCAT
 GGTTCAGATTAGAAATAAAGGTAGTAATTAGAACACTCTGGTTCTTGGCTCTTCTGTTGAGAGATCCAGT
 TCATTTATCCTAAAGAGGTAACTAAAGACGCTAAATCCAGTCAGTGAGGTGGGGAGAACGCAGCCCAAGT
 AACCCACAGAGATGCTGCGGGCCAGGCAGGCAAGGGGGGGGCTGTAGTCTTTGGACACAGACATGAC
 TT 11511

Figure 3. DNA sequence of the γ -chain gene. The DNA sequences of all exons, intron/exon boundaries, and 5'- and 3'-flanking regions are shown. Numbering is from the putative mRNA cap site and introns are not numbered. The larger letters represent exons of the gene. The underlining indicates putative CAT box, T-A-T-A box and A-A-T-A-A sequence. The asterisk represents the second putative cap site. Nucleotide position +1 is assigned to the G, which is the 5'- terminal nucleotide in primer extension experiments (Figure 4).

shown). These data suggest that there is no amplification or rearrangement of the invariant γ -chain gene in various tissues including CLL cells, which express the γ chain message in high abundance.

	1	20	40
Consensus	GGCGGGGCGGGTGGCTCACGCGCTGTAATCCAGCACTTT		
Alu-1	***A***A***-***T*****TT*****		
Alu-2	***T***T***A***-***T*****T*****		
Alu-3	***T*****A*****T*****		
	60	80	
Consensus	GGGAGGCGGAGGCGGGCGGATCACCTGAGGTCAGGAGTTC		
Alu-1	T*****T*****T*****T*****		
Alu-2	*****T***A***A*****C*****		
Alu-3	*****T***T***T*****_*****		
	100	120	
Consensus	GAGACCAGCCTGGCCAACATGGTGAAACCCCGTCTCTACT		
Alu-1	A*****T*****T*****		
Alu-2	*****A*****-_-*****		
Alu-3	*****C-----*****TC		
	140		
Consensus	AAAAATACAAAA--ATTAGCTGGGCGTGGTGGCGGCGGCC		
Alu-1	*****_*****_*****A*AT**		
Alu-2	*****_*****C*AT*****AGT*A**		
Alu-3	*****TA*****CA*****G*T**		
	160	180	
Consensus	TGTAATCCAGCTACTCGG-GAGGCTGAGGCAGGAGAATC		
Alu-1	*****C*****T*****		
Alu-2	*****AT_*****T*****		
Alu-3	***G***_*****A*****A*****G		
	200	220	
Consensus	GCTTGAACCGGGAGGGCGAGGTTGCAGTGAGCCGAGATT		
Alu-1	*****CT*****T*****C		
Alu-2	***T*****A*TG*****C		
Alu-3	**A*A***A*****TG*****C		
	240	260	
Consensus	G---CGCCACTGCACTCCAGCCTGGGTAACAGA-GCGAGA		
Alu-1	*---*A***A*****CG*****C***		
Alu-2	ATCA*****G*****T***		
Alu-3	*---*****CG*****G*A***		
	280	296	
Consensus	CTCCGTCTCAAAAAAAAAAAAAA		
Alu-1	***TA*****C***C***TC		
Alu-2	***A*T*****T***T*		
Alu-3	*****C***C**		

Figure 4. Alignment of Alu repeats in γ -chain gene. The sequences of three Alu family members containing 5' and 3' flanking regions are compared with consensus sequences. Asterisks denote homology; deletions (-) and insertions have been introduced so as to maintain maximum homology in the figure.

DISCUSSION

We have determined the cDNA as well as genomic nucleotide sequence of the differentially expressed gene in CLL cells. A homology search of the nucleic acid data bank revealed this cDNA as an invariant γ -chain of class II histocompatibility antigen (7,30). The cDNA sequence differs from those reported previously in that our sequence does not contain a 5' end palindromic structure, which was suggested to

Figure 5. Alignment of direct repeats flanking Alu-1, Alu-2 and Alu-3. The beginning of the consensus Alu repeat sequence is noted by +1 and the terminal nucleotide in the consensus sequence is designated by +286.

be an artifact of snapback (7). Further, we found nucleotide differences at positions 795 (G to C), 853 (G to T), and 1204 (C to G) unlike Strubin *et al.* (30), and deletion of a C residue at position 825 (8). All nucleotide differences are in the noncoding region of the mRNA. The transmembrane character of the polypeptide chain deduced from the cDNA sequence has been discussed in previous reports (7,30).

A 17 kb genomic DNA clone that contains the entire γ -chain gene was isolated and sequenced except for 3.7 kb of the first intron. The genomic structure revealed that this gene spans approximately 11 kb and contains eight exons and seven introns, with three of the introns containing members of the Alu repeat family. The first putative cap site determined by primer extension was a G residue at position 1, however, the second putative cap site at position 8 was an A residue flanked by pyrimidines, which agreed closely with the consensus cap site (31). This result suggests that the γ -chain gene may have two transcripts *in vivo*. There are three GC-rich sequences in the 5' flanking region close to the cap site, which are G-G-G-C-G-G at positions -73 to -68, G-C-C-C-C-G-C at -147 to -141 and G-C-C-C-C-C-C-C-G-C-C-C at -167 to -155. Similar sequences serve as control elements in the thymidine kinase gene (32).

Alu repeat sequences are found in three of the seven introns of the γ -chain gene (Fig. 2). These sequences share high homology with the consensus Alu repeat sequence (Fig. 4). One of the Alu repeats is in opposite orientation to the other two, a circumstance that could lead to frequent deletion of segments between the Alu

Figure 6. Analysis of number of genomic sequences in *Bam*HI digests complementary to clone 7-2D-1. The lanes a and b were loaded with 10 and 20 μ g, respectively, of high-molecular weight DNA digested with *Bam*HI from acute myelocytic leukemia (AML), chronic myelocytic leukemia (CML), chronic myelocytic leukemia in blastic crisis (CML-BC), placenta acute monocytic leukemia (Plac), acute monocytic leukemia (AMoL), and CLL cells from the left, respectively. The central 4 lanes were loaded with cloned phage λ 85 (λ h7-2D-1) digested with *Bam*HI in amounts corresponding to one, three, five, or ten copies of insert per haploid genome in 20 μ g of total human DNA. DNA fragments were transferred to nitrocellulose and hybridized with 32 P-labeled p7-2D-1. The low-molecular-weight bands are at a molecular weight consistent with plasmid contamination.

repeats. No evidence for this possibility has been obtained. In examination of more than 40 human DNA samples we have seen no restriction fragment length polymorphism in the γ -chain gene (data not shown). The possibility that Alu repeats may identify unprocessed nuclear RNA has been suggested by Sharp (33). The three Alu repeats have the consensus structure of a head to tail dimer of two approximately 130 base pair monomers. Alu-3 repeat has a 10 bp deletion in the left monomer which is usually the most conserved.

Processing a large nuclear transcript of ~11,000 nucleotides to yield a 1282-

Table 1
Putative Site of Branch-Point Formation in γ -Chain Gene*

Intron	<u>Sequence Near 3' End of Intron</u>		
A	-35	-18	Exon 2
	ATCCTC <u>CTCTGACCT</u> ATCCTCCCCACCTCCACAG CAA ---		
B	-35	-18	Exon 3
	CGGGGCCAC <u>ACTTAC</u> CTCGTTCTGTCCCCACAG CTC ---		
C	-35	-18	Exon 4
	CCAGCTC <u>TGGTAAC</u> CTTCTGTTGCTCCTCCACAG CCC ---		
D	-35	-18	Exon 5
	TCTTGA <u>ACC</u> ATCCTTCCTGCCTGCTACCTGTAG AAT ---		
E	-35	-18	Exon 6
	CCATCGA <u>ACCTGAC</u> CTCCACCTCTATCCGTATTAG GTC ---		
F	-35	-18	Exon 7
	TTCAAT <u>TGACAA</u> ACGGACTTGCTGCTCCTTTGCAG GAG ---		
G	-35	-18	Exon 8
	TGAC <u>CCCAAC</u> CTCAACCTGCTGCTTCTCTCTCCAG GTC ---		

*Consensus branch-point sequence (32): PyXPyTPuAPy.

nucleotide, mature, γ -chain mRNA requires several cleavage and splicing events. Recent *in vitro* splicing studies (34) have shown formation of a loop or lariat structure between the 5' end of the intron and an adenine residue located 20-40 nucleotides upstream of the 3' splice site. Ruskin *et al.* (34) have shown that functional branch-point sequences in different introns conform to a consensus sequence but display considerable variability. The seven introns of the invariant γ -chain gene display variations on the consensus sequence approximately 20-30 bp upstream from the 3' splice site (Table 1). In each case at least six of the seven nucleotides in the consensus sequence are found in the branch-point site. The site in intron A is the only perfect match for the branch site and may provide a testable substrate for measuring the efficiency of splicing.

The role of the invariant γ -chain protein in immune recognition and defense is not well understood. Although the γ -chain protein has not been studied in detail, its deduced amino acid sequence is that expected of a transmembrane protein, leading to speculation that the γ -chain is involved in assembly and transport of class II HLA-DR heterodimers $A_\alpha A_\beta$ and $E_\alpha E_\beta$ chains to the cell surface (35, 36). Invariant γ -chain mRNA is made in human blood cells and their precursors but has not been detected in a variety of other human tissues (10), such as among various

leukemic leukocyte populations the highest levels of γ -chain mRNA were found in patients with CLL. These data support a relationship between high levels of γ -chain mRNA and the nonproliferating condition of the cell (10,37,38).

The coordinate induction of class II MHC α , β , and γ mRNAs has been demonstrated in murine macrophage cells (39). Coordinate expression of the human class II genes is an intriguing case since the γ -chain is on chromosome 5 and the other MHC genes are on chromosome 6, suggesting that common regulatory signals for these genes exists. We find one or two copies of the γ -chain gene (Figure 6); however, Southern blot analyses and protein sequencing indicates that there are two or more copies of most class II genes (40,41). As a step in understanding the transcriptional control of MHC class II invariant γ -chain gene expression, we have described the structural features of the γ -chain gene. A detailed analysis of the putative regulatory regions for the γ -chain gene can be accomplished by in vitro transcription and gene-transfer experiments.

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