SEQUENCES AND REPERTOIRE OF HUMAN T CELL RECEPTOR α CHAIN VARIABLE REGION GENES IN MATURE T LYMPHOCYTES

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The T cell antigen receptor (TcR),[1] which recognizes antigen and the MHC gene product, seems to be a cell surface protein heterodimer consisting of an acidic (α) and a basic (β) chain (1–3). The molecular cloning of the TcR β chain (4, 5), and subsequently the α chain (6–8), established that these genes are distinct from Ig genes. Based on sequence analysis of cDNAs and germline sequences, it appears that functional TcR genes are formed by somatic recombinations of variable (V), diversity (D), joining (J), and constant (C) gene segments (4–8, 9). Chromosomal mapping of these genes indicate that they are found at locations different from those of Ig genes, indicating that these genes are different from those used in the rearrangement of Ig genes (10–13). The germline organizations of these TcR and the Ig genes share a basic structure, but definite differences are revealed upon closer examination (14–19). Thus, TcR genes have their own set of germline genes as their basis for functional diversity.

Estimates of the repertoire of TcR V_α gene segments in mouse have been reported (20, 21). The studies suggest that there may be fewer germline V_α gene segments than the number of Ig H and κ chain variable chain segments, but more than the estimated number of TcR V_β gene segments in mice (22, 23). Similar sequence analyses to estimate the repertoire of the human TcR α or β chain V gene segments are not yet available. Since preliminary studies (18) indicate that somatic mutation does not play an important role in the generation of diversity of these genes, the generation of diversity most likely rests on the extent of recombinational joinings, and thus the number of V and J gene segments is of particular significance.

In this study, we have sequenced and analyzed 24 different α chain cDNA clones derived from human peripheral blood T lymphocytes and T cell lines. The familial organization of the V_α segments and the variability within the human V_α genes have been determined.

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[1] *Abbreviation used in this paper:* TcR, T cell antigen receptor.

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Materials and Methods

Constructure of cDNA Libraries. Double-stranded (ds) cDNA was synthesized from poly(A)$^+$ RNA derived from PHA-stimulated peripheral human T cells. After treatment with Eco RI methylase and size selection, the ds cDNA was cloned into the Eco RI site of λgt10 using Eco RI linkers as described before (13).

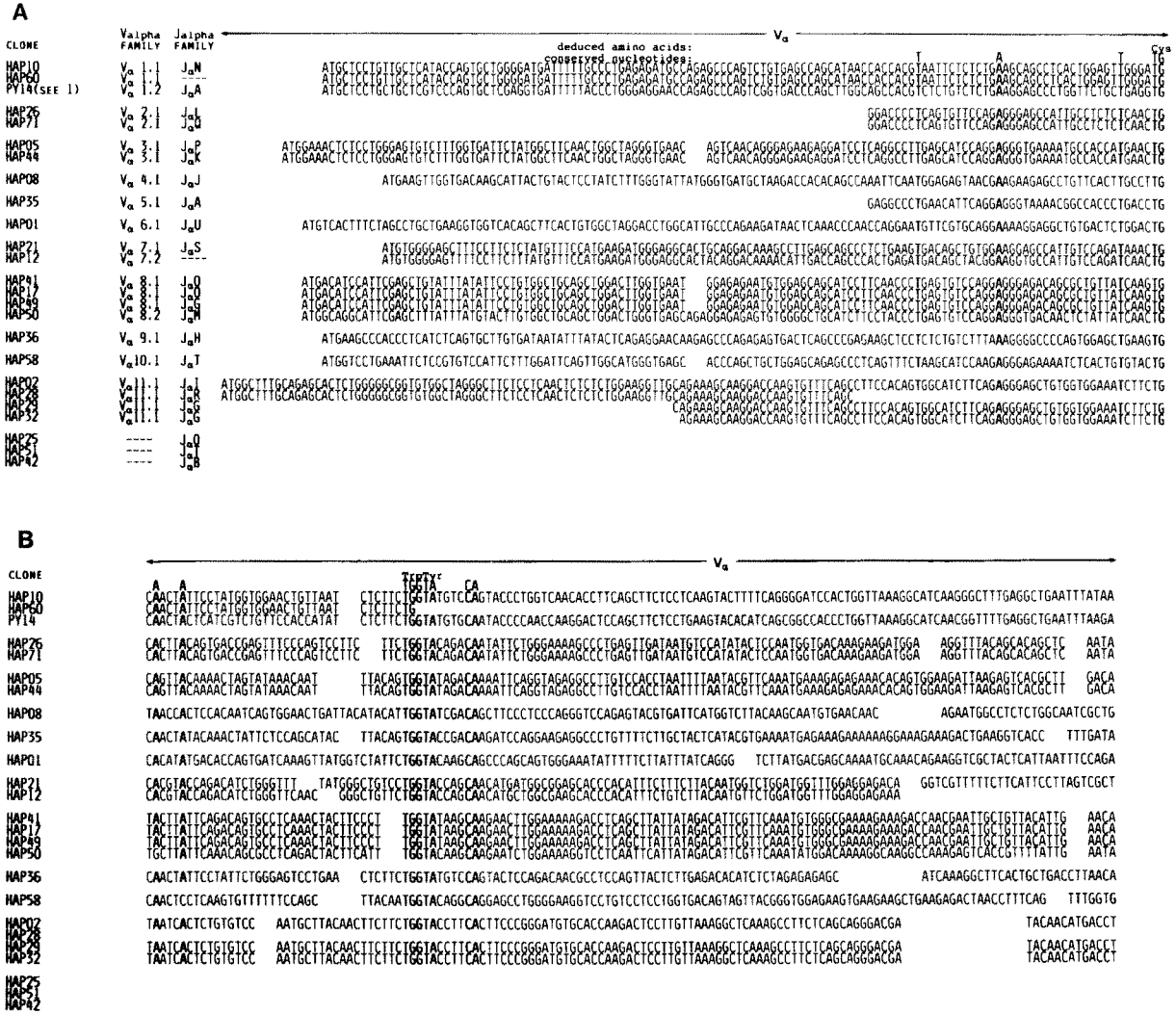
Isolation of Human α Chain cDNA Clones. The peripheral human T cell library was plated on *E. coli* C600/HFL. Screening of duplicate filters was carried out according to the standard procedure (24). Hybridizations were done for 18 h at 65°C in 5 × SSC, 5 × Denhardt's, 100 μg/ml denatured Salmon sperm DNA, and 0.5 μg ^{32}P-labelled nick-translated PY14 α cDNA probe previously described (13). Filters were washed in 2 × SSC, 0.1% SDS at room temperature several times, followed by washing in 0.2 × SSC at 65°C.

DNA Sequencing. The cDNA inserts were subcloned into M13 mp9 sites of the bacteriophage vector, and the sequences were determined using the specific-primer–directed dideoxynucleotide sequencing technique in conjunction with the dideoxy method (25).

Southern Blot Analysis. DNA was extracted from bone marrow cells and digested with Eco RI and Bam HI. DNA (10 μg) was electrophoresed through 0.8% agarose and transferred to nitrocellulose filters as described by Southern (26). Hybridization was for 24 h at 65°C in 5 × SSC, 5 × Denhardt's, 100 μg/ml denatured salmon sperm DNA, 10% dextran sulfate, and 0.5 μg ^{32}P-labelled nick-translated cDNA probe. Filters were washed at 65°C with 3 × SSC containing 0.1% SDS.

Results

Sequence of Human α Chain cDNA Clones. To examine the repertoire of the human TcR α chain genes, we have cloned α chain–homologous cDNAs from a library of human PHA-stimulated peripheral blood T lymphocytes. The library was screened using a constant region probe from the human TcR α chain, PY14 (9), and 24 cDNAs clones were randomly chosen. The inserts were subcloned into M13 mp9, and the nucleotide sequences of the cDNAs were determined (Fig. 1). The deduced protein sequence of these clones is presented in Fig. 2. The nucleotide sequence of cDNA PY14 (9) has been included for comparison. Examination of this cDNA sequences showed great variation in the *N*-terminal half, which correspond to the variable region of the TcR α chain gene. These variable genes can be divided into at least two gene segments corresponding to the V and J gene segments. The exact junctions between these sequences were determined by comparison of the cDNA sequences to those previously reported for human germline V_α and J_α genes (16). As can be seen in Fig. 1, some of the sequences of the V gene segments are identical to other V gene segments. For example, V_α gene segments of clone HAP10 and clone HAP60 contain identical V gene segments. Similarly, identical V sequences can be found between clones HAP26 and HAP71; HAP05 and HAP44; HAP41, HAP17, and HAP49; and HAP02, HAP28, HAP29, and HAP32. A high degree of sequence homology can also be found between some cDNA clones, suggesting that they belong to the same V gene family. For example, clones HAP(10,60) and PY14; HAP21 and HAP12, HAP(41,17,49,50) and HAP50 are related to each other at above 75% homology at the nucleotide level. Lower degrees of homology exist between members of the different families, with regions of conserved sequences that code for structurally important amino acids. These conserved nucleotides and deduced amino acids for which they code are also indicated in Fig. 1. On the basis of


A
B
CLONE
Valpha FAMILY
Jalpha FAMILY
deduced amino acids:
conserved nucleotides:
Vα
Cys
HAP10
HAP60
PY14(SEE 1)
HAP26
HAP71
HAP05
HAP44
HAP08
HAP35
HAP01
HAP21
HAP12
HAP41
HAP17
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HAP42

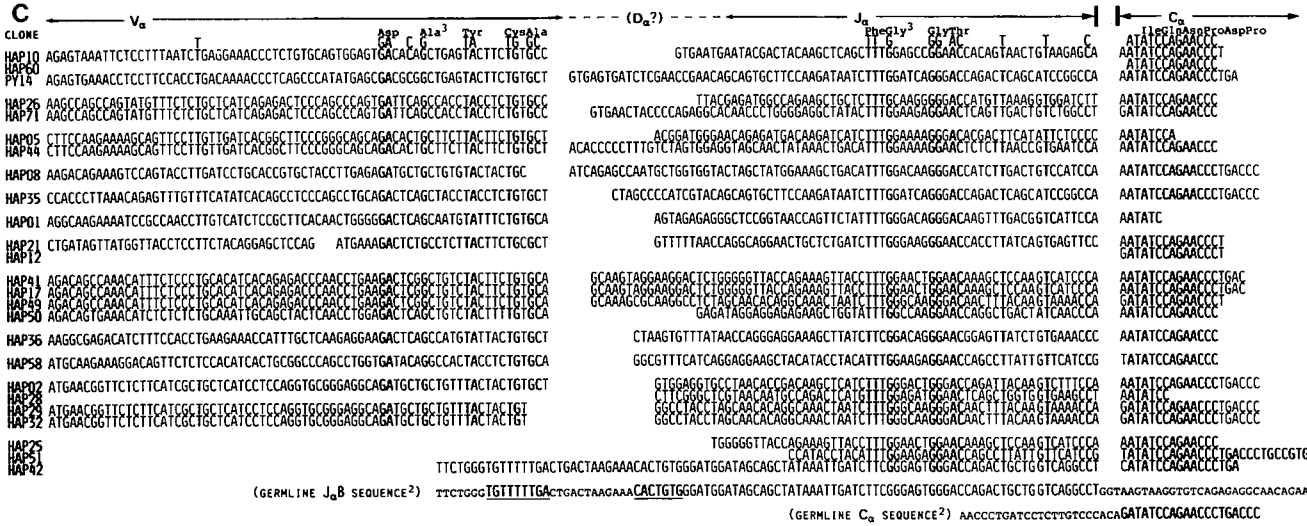

FIGURE 1. Sequences of 25 human T cell receptor α chain messages obtained from mature human T lymphocytes. 24 cDNAs from a human T cell lymphocyte library and one from human T cell line Jurkat (9) were obtained, and their sequences were determined. The sequences were aligned to obtain maximum similarity and grouped on the basis of homology to each other. Conserved nucleotides and deduced amino acids are in bold letters on top of the sequences. (*A*) Sequence obtained from PY14 (9). (*B*) Sequences of germline $J_\alpha B$ gene segment and of C_α obtained from Yoshikai et al. (16). (*C*) Deduced amino acid from all but one of the listed DNA sequences. V_α, J_α, D_α, and C_α are variable, diversity, joining segment, and constant region, respectively, of human T cell receptor α chain.

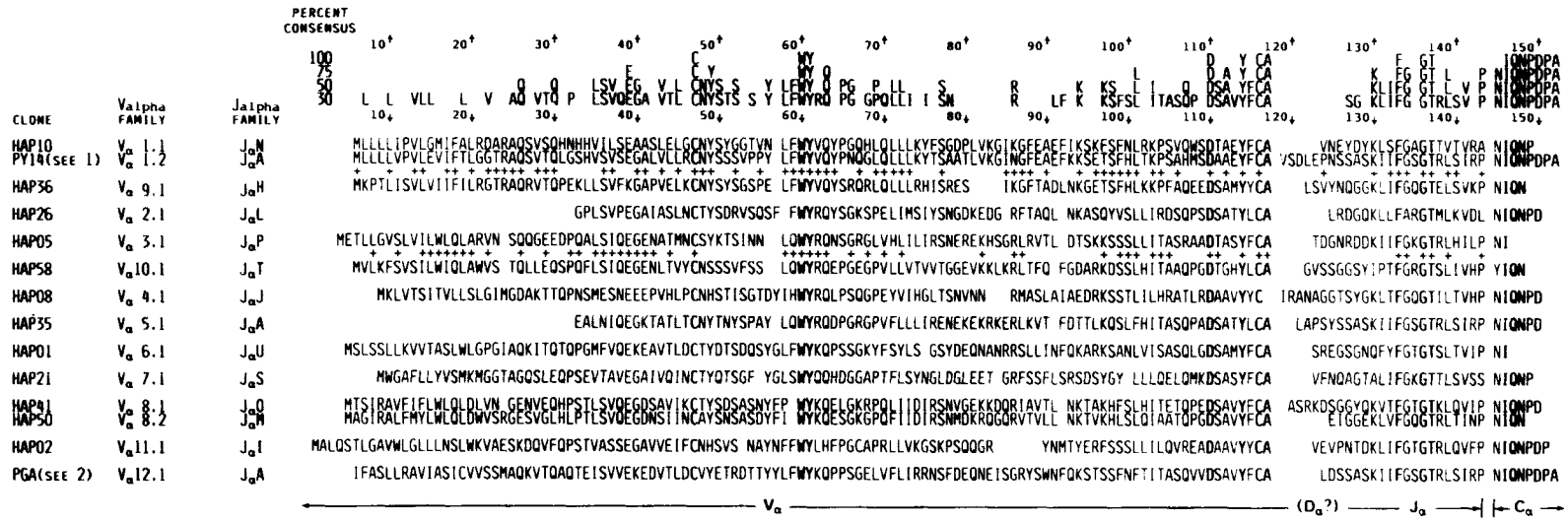

FIGURE 2. Deduced protein sequences of human T cell receptor α chain variable regions from cDNAs in Fig. 1 and sequence from HPB-MLT T cell line (8) were assembled and grouped on the basis of V_α family size (see Fig. 4). Spaces in the sequences were added to maximize homology. Frequencies of occurrence of each amino acid are designated on top of figure with first, second, third, and fourth row occurring 100, 75, 50, and 30%, respectively. Identical amino acid are indicated by + for two pairs of deduced protein sequences (PY14/HAP36 and HAP05/HAP58).

these sequence analysis, 14 of the 22 V_α gene segments isolated are unique. Thus 14 is the lower limit for the number of different V_α segments used in mature T cells.

The deduced protein sequences of the V_α gene segments have been aligned for maximum homology to each other. The deduced sequence from the cDNA clone PGA is included for comparison (8). Both inter- and intrafamilial similarities between V_α genes are even more pronounced at the protein level. Two examples of this are indicated (+) in Fig. 2.

Examination of the J gene segment sequences indicated that, although there are some segments with similar or identical sequences, a large number of distinct sequences can be found. The deduced amino acid sequences of these V and J segments is summarized in Figs. 2 and 3. These consensus sequences illustrate roughly the hypervariable and framework regions of V and J segments. Comparison of the J_α nucleotide sequences determined in this study (Fig. 1) and elsewhere (16, 28) are illustrated in Fig. 3*a*, while protein sequence comparison can be seen in Fig. 3*c*. Germline J_α gene segments from Yoshikai et al. (16) are included in Fig. 3*b* for comparison. An examination of V_α and J_α used in different clones (Fig. 1), and their respective familial origins (Fig. 3) suggest that there are no constraints on the association between V_α and J_α segments. An interesting observation is that the J_α gene segment located closest to the C_α in the germline appears to be used four times. The assignment of J_α families is arbitrary and extends the collection sequenced from genomic germline DNA by Yoshikai et al. (16). The combined number of different cDNAs and germline J_α sequences indicated that there are more than 21 independent J_α segments that can be used in the human T cell receptor.

At this time the exact source of sequence diversity at the V_α-J_α boundary is not known. The 3–20 nucleotide junctional sequences may have arisen from insertion of nucleotides, or merely by the use of as yet unknown germline V_α, D_α, or J_α sequences. The 3′ variability of the germline J_α sequences introduces further variability at the $J_\alpha C_\alpha$ junction, presumably by splicing of the germline J_α sequence into the C_α gene.

Southern Analysis of V Gene Segments in Human Germline DNA. To determine the extent of variability of V_α gene segments within germline DNA, Southern blot analyses of Bam HI– or Eco RI–digested human germline DNA was performed using the cDNAs from Fig. 1 as probes. Representative results are presented in Fig. 4. In most cases, multiple bands hybridizing to the cDNAs probes can be observed at reasonably high stringency. The fragments corresponding to the constant region are denoted. The number of V gene segments appear to range from one to seven. These results support the hypothesis that the V_α gene families have more V_α gene members than V_β gene families in mouse (22, 23). On the basis of the Southern gel results, the number and size of V_α gene families can be estimated (Table I) to contain ~40 members (12 families).

Homologies Within the Variable Regions of the Human TcR α Chain Genes. Alignment of DNA and protein sequences of the 22 cDNA V_α regions reveals regions of high and low homology reminiscent of the Ig hypervariable regions proposed by Wu and Kabat (29). A variability plot of the protein sequences in their optimized alignments (from Fig. 2) is given in Fig. 5. In this


a
Jalpha FAMILY
CLONE
deduced amino acids: PheGly GlyThr Leu5
HAP49 JαG GACTCGGCTGTCTACTTCTGTGCA GCAAAGCGCAAGGCCTCTAGCAACACAGGCAAACTAATC TTTGGGCAAGGGACAACTTACAAGTAAAACCA GATATCCAGAACCCT
HAP29 JαG GATGCTGCTGTTTACTACTGT GGCCTACCTAGCAACACAGGCAAACTAATC TTTGGGCAAGGGACAACTTACAAGTAAAACCA GATATCCAGAACCCTGACCC
HAP36 JαH GACTCAGCCATGTATTACTGTGCT CTAAGTGTTTATAACCAGGGAGGAAAGCTTATC TTCGGACAGGGAACGGAGTATCTGTGAAACCC AATATCCAGAACCC
HAP02 JαI GATGCTGCTGTTTACTACTGTGCT GTGGAGGTGCCTAACACCGACAAGCTCATC TTTGGACTGGGACCAGATACAAGTCTTTCCA AATATCCAGAACCCTGACCC
HAP08 JαJ GATGCTGCTGTGTACTACTGC ATCAGAGCCAATGCTGGTGGTACTAGCTATGGAAAGCTGACA TTTGGACAAGGGACCATCTGACTGTCCATCCA AATATCCAGAACCCTGACCC
HAP44 JαK GACACTGCTTCTTACTTCTGTGCTACACCCCCTTTGTCTAGTGGAGGTAGCAACTATAAACTGACA TTTGGAAAAGGAACTCTCTAACCGTGAATCCA AATATCCAGAACCC
HAP42 JαB ATGGATAGCAGCTATAAATTGATC TTCGGAGTGGGACCAGACGCTGGTCAGGCCT CATATCCAGAACCCTGA
SUPT1A2 JαB GATGAAAGACTCTGCCTCTTACTTCTGCGCTGTGCCCCCCTGATGGATAGCAGCTATAAATTGATC TTCGGAGTGGGACCAGACGCTGGTCAG
HAP26 JαL GATTCAGCCACCTACCTCTGTGCC TTACGAGATGGCCAGAAGCTGCTC TTTGCAAGGGGGACCATGTTAAAGGTGGATCTT AATATCCAGAACCCTGACCC
HAP50 JαM GACTCAGCTGTCTACTTTTGTGCA GAGATAGGAGGAGAGAAGCTGGTA TTTGGCCAAGGAACCAGGCTGACTATCAACCCA AATATCCAGAACCC
HAP10 JαN GACACAGCTGAGTACTTCTGTGCC GTGAATGAATACGACTACAAGCTCAGC TTTGGAGCCGGAACCACAGTAACTGTAAGAGCA AATATCCAGAACCCT
HAP41 JαO GACTCGGCTGTCTACTTCTGTGCA GCAAGTAGGAAGGACTCTGGGGGTTACCAGAAAGTTACC TTTGGAACTGGAACAAAGCTCCAAGTCATCCCA AATATCCAGAACCCTGAC
HAP25 JαO GAATTCTGGGGGTTACCAGAAAGTTACC TTTGGAACTGGAACAAAGCTCCAAGTCATCCCA AATATCCAGAACCC
PY143 JαA GACGCGGCTGAGTACTTCTGTGCTGTGAGTGATCTCGAACCGAACAGCAGTGCTTCCAAGATAATC TTTGGATCAGGGACCAGACTCAGCATCCGGCCA AATATCCAGAACCCTGACCC
PGA4 JαA GACTCAGCAGTATACTTCTGTGCT CTGGACAGCAGTGCTTCCAAGATAATC TTTGGATCAGGGACCAGACTCAGCATCCGGCCA AATATCCAGAACCCTGACCC
HAP35 JαA GACTCAGCTACCTACCTCTGTGCT CTAGCCCCATCGTACAGCAGTGCTTCCAAGATAATC TTTGGATCAGGGACCAGACTCAGCATCCGGCCA AATATCCAGAACCCTGACCC
SUPT1B2 JαA GCAGACACGGCCGTGTATTACTGTGCGAGAGTCCGTCGGAGGTACAGCAGTGCTTCCAAGATAATC TTTGGATCAGGGACCAGACTCAGCATCCGG
HAP05 JαP GACACTGCTTCTTACTTCTGTGCT ACGGATGGGAACAGAGATGACAAGATCATC TTTGGAAAAGGGACACGACTTCATATTCTCCCC AATATCCA
HAP71 JαQ GATTCAGCCACCTACCTCTGTGCC GTGAACTACCCCAGAGGCACAACCCTGGGGAGGCTATAC TTTGGAAGAGGAACTCAGTTGACTGTCTGGCCT GATATCCAGAACCC
HAP28 JαK CTTCGGGCTCGTAACAATGCCAGACTCATG TTTGGAGATGGAACTCAGCTGGTGGTGAAGCCT AATATCC
HAP21 JαS GACTCTGCCTCTTACTTCTGCGCT GTTTTTAACCAGGCAGGAACTGCTCTGATC TTTGGGAAGGGAACCACCTTATCAGTGAGTTCC AATATCCAGAACCCTG
HAP58 JαI GATACAGGCCACTACCTCTGTGCA GGCGTTTCATCAGGAGGAAGCTACATACCTACA TTTGGAAGAGGAACCAGCCTTATTGTCATCCG TATATCCAGAACCC
HAP51 JαI GAATTCCCATACCTACA TTTGGAAGAGGAACCAGCCTTATTGTCATCCG TATATCCAGAACCCTGACCC
HAP01 JαU GACTCAGCAATGTATTTCTGTGCA AGTAGAGAGGGCTCCGGTAACCAGTTCTAT TTTGGGACAGGGACAAGTTTGACGGTCATTCCA AATATC
Vα
(Dα?)
Jα
Cα

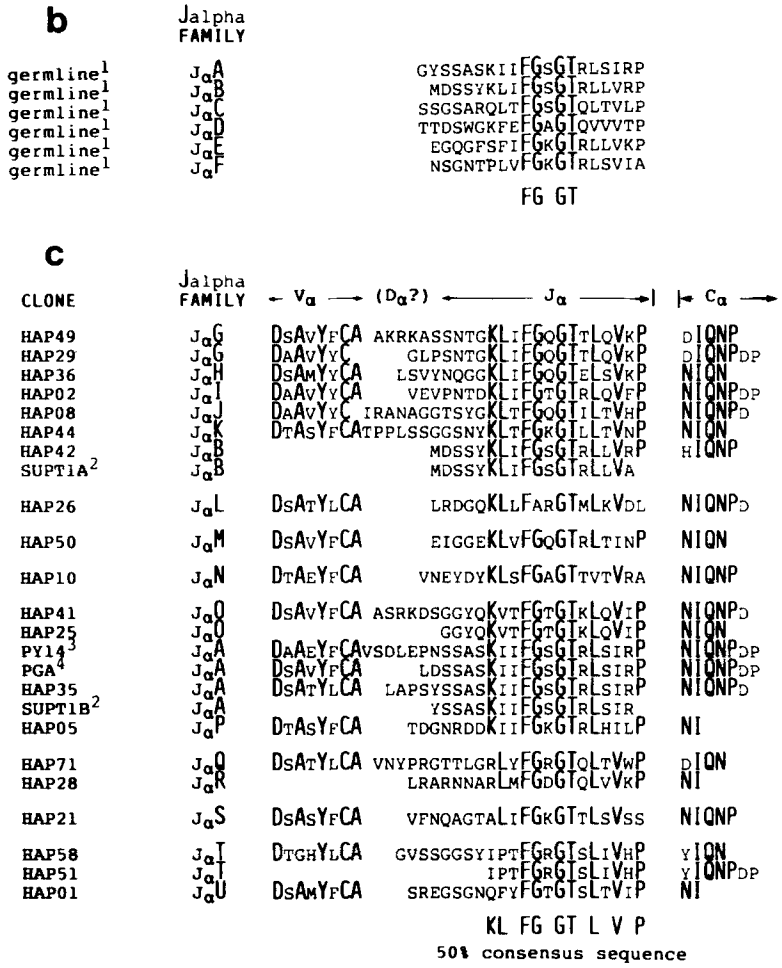

FIGURE 3. Nucleotide and deduced protein sequences from J_α gene segments of the human T cell receptor (*a*) nucleotide sequences of cDNAs, (*b*) germline J segment protein sequences, (*c*) deduced protein sequences of messages [1]Yoshikai et al. (16); [2]C. T. Denny et al. (28) SUPT1B V segment sequences are from an IgH V family; [3]Yanagi et al. (9); [4]Sim et al. (8); [5]deduced amino acid from all but one sequence, HAP10.

plot, three regions of high variability can be seen which correspond to amino acid positions 20–35, 55–75, and the region of V-D-J joining, amino acid 100–110. This pattern of variability is similar to that found upon analysis of 12 *N*-terminally blocked human Ig V_H sequences (30), with the notable exception of the additional variability at the $J_\alpha C_\alpha$ junction, which is not found in Ig V_H sequences.

Discussion

In this paper, we have presented the sequences and analyses of the variable regions of 24 different human α chain TcR cDNAs. All 18 of the 24 cDNAs that contain V segment sequences seem to be messages resulting from productive

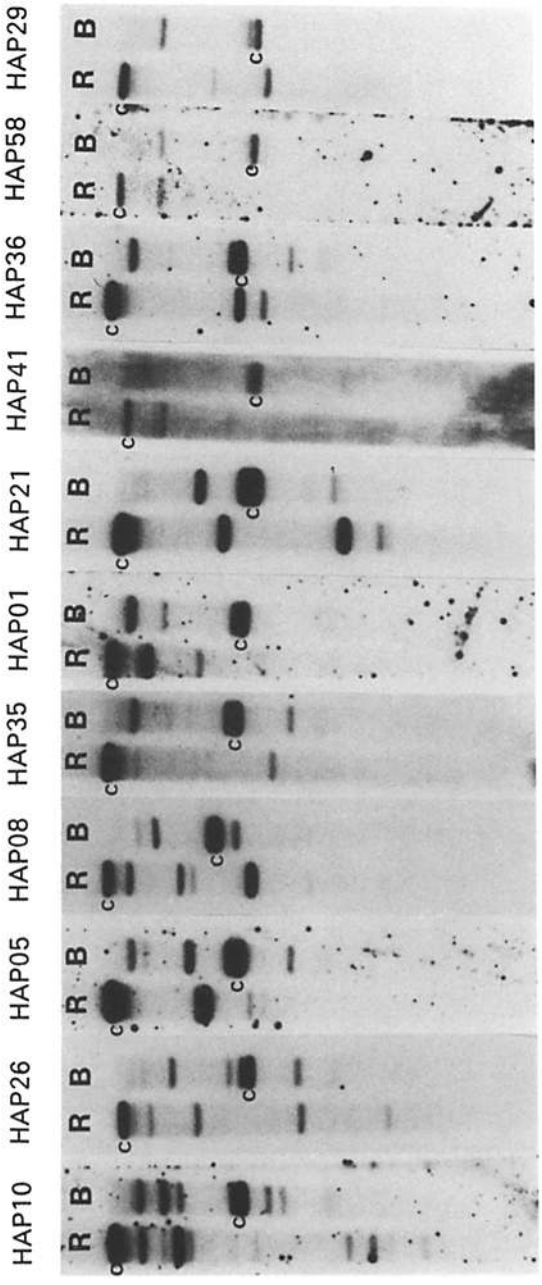

FIGURE 4. DNA was extracted from human bone marrow cells of a donor and digested with restriction enzyme Eco RI (*R*) or Bam H1 (*B*). Southern analysis was performed using cDNAs from Fig. 1 (26, 27). Individual clones used and the assigned V_α gene families are designated. (*c*) Constant region bands of ~30 kb in Eco RI–digested DNA and 5–7 kb in the Bam HI–digested DNA.

TABLE I

Human T Cell Receptor α Chain Variable Segment Gene Families

Family	Clones	Approximate family size
V_α1.1	HAP10, HAP60	7
V_α1.2	PY14.1	
V_α1.3	PY14.2	
V_α2.1	HAP26, HAP71	5
V_α3.1	HAP05	4
V_α4.1	HAP08	4
V_α5.1	HAP35	4
V_α6.1	HAP01	3
V_α7.1	HAP21	3
V_α7.2	HAP12	
V_α8.1	HAP41, HAP17, HAP49	3
V_α8.2	HAP50	
V_α9.1	HAP36	2
V_α10.1	HAP58	1
V_α11.1	HAP02, HAP28, HAP29, HAP32	1
V_α12.1	PGA	1
Estimated total		38

Human T cell receptor α chain variable segments (Figs. 1 and 2); PY14.1 (ref. 9), PY14.2 (ref. 16), and PGA (ref. 8) were grouped on the basis of crosshybridization (Fig. 4).

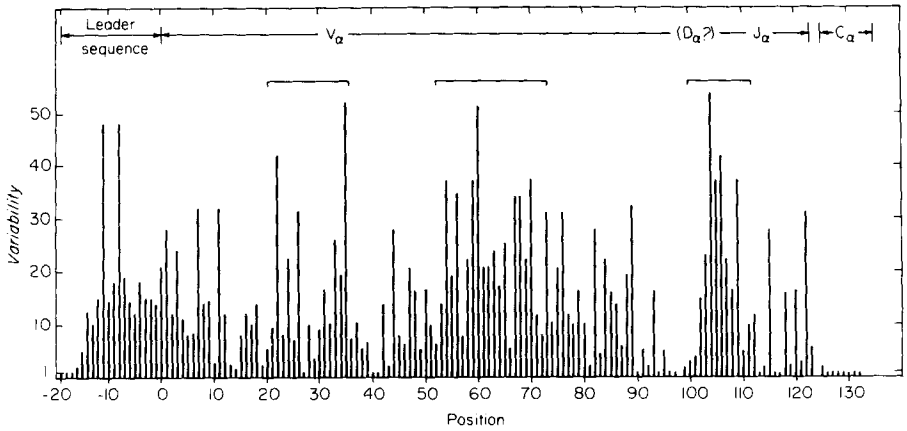

FIGURE 5. Kabat-Wu variability plot based on data presented in Fig. 2. Position: amino acid residues starting from the *N* terminus.

α chain TcR gene rearrangements, which are capable of encoding functional proteins since they show continuous open reading frames through variable, joining, and constant regions.

Examination of the V_α gene segments by DNA sequencing and Southern blot analysis of germline genomic DNA shows that there are at least 12 V_α families comprised of 40 or more V_α gene segments. It is unlikely that there are many more families used than the 12 described here, as data from our laboratory indicates that, of 10 additional α chain messages from another individual belong to these same 12 V_α families described here (Kimura, N., unpublished data). Furthermore, the number of fragments detected is similar in DNA from different individuals. Thus, although it is possible that this report may not describe all the human α chain V gene segments, it is fairly representative of the several individuals we have surveyed. The number of V regions of the human TcR α chain is considerably higher than those of the λ light chain Ig genes and the TcR β chain genes in the mouse (22, 23). However, it may be lower than that predicted by the number of heavy (31) and κ light (32) Ig V gene segments. The number of members in each family varies considerably among the Ig and TcR genes. For example, while there are 10–50 members in each V gene family of the heavy and κ light Ig chain genes (31, 32), there are very few V_β gene segments, often one per family in the mouse (22, 23). The human V_β gene families, however, are larger.[2] The murine V_α gene families are composed of one to eight members (20, 21), and our results indicate similar sizes for the human V_α families.

The human J_α gene segments differ from the other immunorecognition genes in number, lack of clustering, and in length. Our previous analysis of the germline genomic J_α organization suggested that there may be numerous J_α segments present spread over a very large distance (16). The data from the present study is consistent with this observation. In fact, the number of the J_α gene segments presented here are unique. Although the exact number of J_α in the human TcR α chain locus cannot be determined at this time, it must be considerably more than the 21 unique sequences isolated to date. A statistical estimation assuming a random assortment predicted ~55 J_α gene segments (D. Tritchler, personal communication). The J_α segments are several codons longer than those of the TcR β chain or the Ig chains. These extra codons may be accounted for by either *N*-terminal sequence diversity upon V_α-J_α joining, the incorporation of putative D_α segments, or by longer germline V_α gene segments. It is not known whether the extra codons could affect the three-dimensional structure and folding of the α and β T cell receptor heterodimer. Nonetheless, the large number and extra length of the J_α gene segments are consistent with a high level of diversity within this region of the human T cell receptor α chain gene, and may be responsible for the high levels of boundary diversity in the TcR α chain.

There are many fine differences in both function and structure between Ig and T cell receptor molecules. The former are expressed exclusively on the surface of B cells and serves as a receptor that can recognize free antigen while

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[2] N. Kimura, B. Toyonaga, Y. Yoshikai, R. P. Du, and T. W. Mak. Sequence and repertoire of the human T cell receptor β chain genes. Manuscript submitted for publication.

the latter are found solely on T cell surfaces and can recognize antigen only in the context of major histocompatibility products (30). Subtle differences, such as in the lengths of the V regions among the Ig, TcR α and β genes also exist.

In spite of these distinctions, the gross overall structures of these genes are probably quite similar, based on previous DNA and deduced protein sequence analysis. From the results reported here, this prediction can be extended, since the variable region TcR α chain gene was found to consist of three hypervariable regions, which correspond roughly to the CDR1, CDR2, and CDR3 hypervariable regions of the Ig (H or L) gene. A similar parallel between hypervariable regions is found in the murine system (20, 21). Should the T cell receptor α and β heterodimer possess no more than the same three hypervariable regions as Ig, and should the basic three-dimensional structures of these T and B cell recognition proteins be similar, then the mechanism for T cell receptor recognition of antigen only in the context of the MHC products (33) becomes even more mysterious.

Summary

24 human T cell receptor α chain messages have been examined by cDNA sequence analysis and Southern blot. The data indicate that there are ~40 α chain T cell receptor variable gene segments, which can be divided into 12 families. Comparison of the J gene segments from the cDNAs to previously determined germline J_α sequences places the number of J_α gene segments over 21, and indicates their number to be ~55. Identical nucleotide sequences in independent isolates of V_α and J_α gene segments indicate that hypermutation may not be a common mechanism for the expansion of diversity in these genes, and suggest that the major source of diversity within the α chain repertoire is a result of recombinational joinings between germline V_α and J_α sequences, combined with imprecise junctional joining. Analysis of the V regions of these α chain messages reveals the presence of three domains of hypervariability roughly analogous to the CDR1, CDR2, and CDR3 regions of immunoglobulin.

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