SEQUENCES AND DIVERSITY OF HUMAN T CELL RECEPTOR \( \alpha \) CHAIN VARIABLE REGION GENES

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During the last two years, genes of the T cell antigen receptor (TcR)\(^1\) have been cloned (1–6). The \( \beta \) chain, which was identified first (1, 2), is located on human chromosome 7 and murine chromosome 6 (7, 8). The germline DNA organization of both human (9) and murine (10, 11) TcR \( \beta \) genes has been determined. As has been shown (12–15) in the study of Ig genes, it appears that functional TcR \( \beta \) chains are encoded by genes that have undergone somatic rearrangement of noncontiguous variable (V,\( \beta \)) diversity (D,\( \beta \)), joining (J,\( \beta \)), and constant (C,\( \beta \)) gene segments. The data accumulated so far is consistent with a recombinative model for the generation of TcR \( \beta \) chain diversity. Such diversity is a fundamental characteristic of the TcR \( \beta \) component, since the receptor complex is thought (16) to be involved in the recognition of foreign antigen in the context of the product. An extensive recognition repertoire is made possible through combinatorial joining, junctional flexibility, and N region diversification of the germline \( \beta \) gene segments. Apparently, the analogy to the generation of Ig diversity does not include somatic hypermutation (17).

The extent of germline TcR V,\( \beta \) gene segment multiplicity must be determined to more fully understand the operation of the TcR. The upper limit of the V,\( \beta \) gene segment repertoire in the mouse has been estimated (18, 19) at \( \sim 20 \) different segments. Certain mutant mouse strains have been found (20) to carry even fewer germline V,\( \beta \) gene segments. The human germline V,\( \beta \) multiplicity is estimated in this study by analyzing 22 DNA sequences, and using Southern blots of germline DNA with some of the cDNAs as probes. The upper limit of human V,\( \beta \) gene segments is estimated at \( \sim 100 \) different segments, considerably greater than in mice. A similar analysis of the human TcR \( \alpha \) chain repertoire is presented elsewhere (21).

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Abbreviations used in this paper: ATL, adult T cell leukemia; ds, double-stranded; TcR, T cell receptor for antigen.
Materials and Methods

Construction of cDNA Libraries. Double-stranded (ds) cDNA was synthesized from poly(A)^+ RNA derived from PHA-stimulated peripheral human T cells (21) or a T cell clone specific for diptheria toxoid. After treatment with Eco RI methylase and size selection, the ds cDNA was cloned into the Eco RI site of λgt10 using Eco RT linkers as described before (22).

Isolation of Human β 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 a standard procedure. Hybridizations were done for 18 h at 65 °C in 5X SSC, 5X Denhardt’s, 100 μg/ml denatured salmon sperm DNA, and 0.5 μg ^32P-labeled nick-translated constant region fragment of JUR-β2 (a β cDNA probe that we have described previously [23]). Filters were washed in 2X SSC, 0.1% SDS several times at room temperature, followed by washing in 0.2X SSC at 65°C.

DNA Sequencing. The cDNA inserts were subcloned into the M13mp9 bacteriophage vector, and their sequences were determined using both the universal M13 primer and the specific-primer-directed dideoxynucleotide sequencing technique (24, 25).

Southern Blot Analysis. DNA was extracted from bone marrow cells and digested with Eco RI and Hind III. 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 5X SSC, 5X Denhardt’s, 100 μg/ml denatured salmon sperm DNA, 10% dextran sulfate, and 0.5 μg ^32P-labeled nick-translated cDNA probe. Filters were washed at 65°C with 3X SSC/0.1% SDS.

Results

Sequence of β cDNA Clones. The repertoire of the human TcR β chain genes was examined by analysis of 22 V# sequences from various sources. 13 cDNAs were obtained from the screening of a PHA-stimulated human peripheral blood T cell library using a Cβ probe (HBP series). Another two β cDNAs were obtained from human T cell clones specific for Diptheria toxoid (DT110, DT259). The remaining seven V# sequences from human T cell leukemia and T cell tumor cell line sources were taken from the existing literature (see Fig. 1). Note that the four adult T cell leukemia (ATL) sequences (MT11, ATL121, ATL122, and ATL21) shown are artificially derived from the published genomic sequences by data splicing.

Examination of the 22 V# sequences reveals that they vary in their 5’ halves, which correspond to the variable regions of the TcR β chain genes. The 3’ cDNA sequences correspond to one of the two constant regions, Cβ1 or Cβ2. The junction points between the Vβ, Dβ, Jβ, and Cβ gene segments in the sequences were determined by comparison to previously reported human germline data (9, 13). The 19 clones found to contain Vβ gene segments are compared in Fig. 1. The corresponding deduced protein sequences are compared in Fig. 2. Spaces have been introduced into the sequences to maximize intersequence similarities. The spaces at the nucleotide level are consistent with those in the protein sequences, and vice versa. This alignment process was extended to a similar set of Vβ cDNA sequences provided to us by P. Concannon, L. Pickering, and L. Hood (personal communication) for comparative purposes. In this way, a unified nomenclature was established (i.e., the first member of family 8 is called Vβ8.1). Pairwise comparisons of all Vβ DNA sequences, as shown in Fig. 1, were performed to establish Vβ gene segment families. For the comparisons, the 3’
Figure 1. Nucleotide sequences of 19 human TcR β chain variable region gene segments obtained from human T lymphocytes. Clones designated HBP were isolated from a PHA-stimulated human peripheral blood T cell cDNA library. DT110 and DT295 were obtained from human T cell clones specific for diptheria toxoid. The remaining clones from human tumor and leukemia cell lines were taken from the literature: YT35 (1), and the identical sequence is reported for a cDNA clone isolated from cell line JM (53); HPB2 (23) (from HPB-ALL); 4D1 (31) (from HPB-MLT); MT11, ATL121, ATL122, ATL21 (17) (artificially spliced genomic sequences from cell lines of patients with ATL). Corresponding Dβ and Jβ sequences for these clones are shown in Fig. 2. Spaces have been added into the sequences to maximize similarities. Those with >75% homology have been grouped into families. Deduced amino acid from the 80% DNA consensus sequence. Variable gene segment 3' boundary based upon comparison with corresponding germline sequences are indicated by a slash (/). Cytosine residue resulting in a nonfunctional message due to a translational reading frameshift in clone 4D1 is indicated by a small c. DT295 has a deletion within Jβ2.1 (†). See discussion regarding HBP54 and HBP54 family designation (+).
Figure 2. Deduced protein sequence of human TcR β chain variable regions from cDNA sequences (see Fig. 1 legend for details). Consensus sequences at 30, 50, 75, and 95% levels are displayed above the main figure. Nucleotides leading to nonfunctional messages due to translational reading frameshifts (4D1, ATL121) are printed in small letters and underlined.
**Figure 3.** VDJC region nucleotide sequences of the human TcR β chains. V<sub>a</sub> and J<sub>b</sub> boundaries, based upon comparison with germline sequences are indicated by a slash (/). (a) Sequences using C<sub>2</sub> are grouped according to J<sub>b</sub> use. Sequences identical to either germline D<sub>1</sub>1.1 gene segments are shown in smaller bold capitals, * indicates alternate D<sub>1</sub>1.1 sequences exist. Deduced amino acids from the 85% nucleotide consensus sequence. (b) Sequences using C<sub>2</sub> are grouped according to J<sub>b</sub> use. Sequences identical to either germline D<sub>1</sub>1.1 (underlined) or D<sub>2</sub>2.1 gene segments are shown in smaller bold capitals. DT295 has a deletion within J<sub>2</sub>2.1 (*). Deduced amino acids from the 85% nucleotide consensus sequence, excluding genomic intron sequences of HBP15, HBP31, and HBP22. Incomplete messages resulting from partial rearrangements (9-mer/7-mer recognition sequences are underlined).
boundary of the Vβ region was taken to be two codons after the consensus codons Tyr-X-Cys-Ala. Members of a family have at least 75% homology over their overlapping sequences. Examples of similar Vβ gene segments associated with different Dβ and Jβ gene segments were found (HPB04, 4D1, and HPB50). 17 of the 19 Vβ sequences are unique, indicating there are at least 17 different germline gene segments.

Nucleotide sequences of the region between the Vβ and Cβ gene segments are shown in Fig. 3, a and b. The 21 Jβ-containing sequences have been divided into 9 using Cβ1 and 12 using Cβ2. The location and identity of each Jβ gene segment within the sequences were determined very easily by comparison with previously determined germline Jβ data (9). Only 3 of the 13 known Jβ gene segments were not found in this study (Jβ1.4, Jβ1.6, and Jβ2.6). The exact identification of the cDNA Dβ gene segments, even with the germline counterparts (see Fig. 3, a and b, bottom), was impossible due to the combined effects of junctional and N-region diversification with the limited number of bases making up the germline segment. An attempt to assign the sequences to either Dβ1.1 or Dβ2.1 has been made in Fig. 3, a and b, respectively.

Only 2 of the 21 rearranged sequences (DT259 and HBP48) show a recombinational event that has mixed gene segments between the two distinct Dβ1ββββββ
germline clusters. In both cases, the D\(_d1.1\) gene segment has rearranged to a J\(_d2\) gene segment. In addition, it is possible that the cDNA HBP48 arose from a recombination between both D\(_d1.1\) and D\(_d2.1\). In all of the clones, the J\(_d\) gene segments have recombined with the C\(_d\) gene segment located immediately downstream to it in the germline state. The cDNAs HBP15, HBP31, and HBP22 do not contain any V\(_\gamma\)-like sequences. HBP15 and HBP31 have resulted from an incomplete rearrangement that brought only D\(_d2.1\), J\(_d2.1\), and C\(_d2\) together. The nucleotide sequence 5’ to the D\(_d2.1\) residues correspond exactly to the germline sequence associated with D\(_d2.1\). The putative nonamer/heptamer recombination signal sequences are underlined in Fig. 2b. HBP22 arose by transcription of a nonrearranged gene, with subsequent splicing leaving only J\(_d2.4\) joined to C\(_d2\). Again the nucleotides 5’ to J\(_d2.4\) correspond exactly to those found in the germline 5’ to J\(_d2.4\).

Southern Analysis of V\(_\beta\) Gene Segments in Human Germline DNA. As an alternative approach in the determination of germline V\(_\beta\) multiplicity, Southern blot analyses of Bam H1 or Eco RI-digested germline DNA was performed using selected cDNAs as probes. Representative results are illustrated in Fig. 4. Bands associated with C\(_d1\) and C\(_d2\) are marked with arrows based on comparison with published data (9, 12, 13). In most cases, multiple V\(_\beta\)-associated bands can be observed. Only HBP55 (V\(_\beta3\) family) produces a Southern blot result, which indicates that it might be the only member of the family. Familial assignments of the cDNAs based on these Southern blot results are consistent with those made by sequence analysis, as described earlier. For example, compare HBP25 and HBP50 in Figs. 1 and 4. Also, the Southern blot pattern of HBP41 (V\(_\beta8.2\)) matches that of YT35 (V\(_\beta8.1\)), described previously (12, 13). Based on Fig. 4, six families, and a total of at least 30 crosshybridizing members can be identified.

Discussion

22 human nucleotide sequences encoding the variable region of the TcR \(\beta\) chain have been analyzed in this study (Figs. 1 and 2). Three appear to be the result of incomplete rearrangement, or of transcription of unrearranged genes, since only germline intron sequences can be found where V\(_\beta\) gene segments are expected (Fig. 2b). Although several sequences are truncated at the 5’ end and do not contain leader and start codon sequences, only two, 4D1 and ATL121, lead to translational reading frameshift mutations. Note that 4D1 and ATL121 were isolated from nonfunctional leukemic T cell clones. Excluding these, all of the other sequences have the potential to code for functional TcR \(\beta\) chains.

Both of the known D\(_\beta\) gene segments are used with roughly equal frequencies in the sequences examined. In two cases (DT259 and HBP48) a D\(_d1.1\) gene segment is found joined to J\(_d2\) and C\(_d2\) gene segments. These are the only examples of recombination of gene segments between the two known D\(_d\)\(\rightarrow\)C\(_d\) germline gene segment clusters.

21 of 22 sequences contain a J\(_d\) gene segment. Each J\(_d\) is joined to the C\(_d\) of the same D\(_d\)\(\rightarrow\)C\(_d\) germline cluster. 10 of the 13 known J\(_d\) gene segments occur, with no obvious preferences. In only one case (DT259) did the cDNA J\(_d\) sequence not correspond exactly with previously reported germline data. However, the
protein sequence Phe-Gly-X-Gly, characteristic of Ig and TcR J gene segments, is conserved throughout.

Figs. 2 and 3 clearly show that junctional flexibility and N-region diversity are used fully in the recombination of Vβ, Dβ, and Jβ gene segments. No diversity is apparent at the Jβ-Cβ boundary, in contrast to the, albeit limited, Jα-Cα (21) and Jγ-Cγ (27) diversity observed.

Of the 19 Vβ-like sequences reported, 17 are unique at the nucleotide level. Two pairs differ by only one amino acid (Vβ4.2/4.3, Vβ8.1/8.3), excluding leader sequences. If they are due to polymorphism, then 15 out of 19 Vβ gene segments are unique. Assuming random expression of Vβ gene segments, an estimate of the germ line Vβ gene segment repertoire can be obtained statistically (18). The data is consistent with a maximum Vβ gene segment repertoire of 104 at the 95% confidence level, and a most probable repertoire of 38. In mice, Barth et al. (18) have estimated the Vβ repertoire to have an upper bound at 21 (at 95% confidence level), and a most probable size of 13. Similarly, Behlke et al. (19) have predicted a 95% upper bound of 30 and most probable value of 18 for the mouse. This estimate is supported by Southern blots of germ line DNA probed with selected cDNAs (Fig. 4). The majority of blots show several bands that crosshybridize with the Vβ probe. This is in marked contrast to a similar study with mice, in which most of the Vβ gene segments show little to no crosshybridization (19).

The human germline TcR β chain gene segment multiplicity is considerably higher than that of the human Igα light chain or the murine TcR β chain (16, 17, 23). It is lower than that predicted for the heavy (28) and κ (29) Ig V gene segments. The magnitude of the human Vβ gene segment repertoire appears to at least rival its human and murine α chain counterparts.

Pairwise comparisons of the Vβ sequences as aligned in Fig. 1 allowed percent homologies to be calculated over their overlapping regions. In general, the Vβ sequences homologies ranged from 30 to 100%, with most falling between 30 and 60%. The deduced protein sequences shared similar relationships, and the consensus sequences (Fig. 2) are reminiscent of other Ig-like V regions. A Kabat-Wu variability analysis (30) of the protein sequences did not define easily distinguishable regions of hypervariability, which are characteristic of Ig V regions. To a large extent, variation in Vβ chain lengths are responsible for the lack of region definition, since spaces must be introduced to maintain the structural similarities.

Even so, certain sequence similarities allowed subsets or families of Vβ segments to be defined. By definition, family members share ≥75% similarity at the DNA level. Using this criterion, the 19 Vβ sequences were divided into 11 families. The nomenclature was chosen to be consistent with those of Concannon et al. HBP54 and HBP34 Vβ gene segments are assigned to families Vβ12 and Vβ13, respectively, by comparison with data of Concannon et al. However, HBP54 and HBP34 share ≥75% similarity, and can be considered members of the same family. Thus the definition of a family is not exact, and depends on the sample size examined. The Southern blot data (Fig. 4) are consistent with the family assignments.
Based on the data of this study and Concannon et al., there are at least 16 human TcR Vβ families, each often containing several members. The total Vβ repertoire, based on number and size of families, is consistent with the estimate arrived at by statistical analysis of the sequence data described above. Again, the human TcR Vβ repertoire appears to be similar to that of human and mouse TcR Vα (this is partly due to a high number of Jα segments in both man and mouse [21, 32]) and not as small as that estimated for the mouse TcR Vβ chain.

It is possible that the number of murine TcR Vβ segments is only a reflection of the strains of mice examined. This hypothesis is supported by the findings that other strains of mice, SJL, C57/L, and C57/br are known to have fewer Vβ gene segments (20). The TcR Vβ repertoires of other murine strains, as well as wild mice, may be larger. Alternatively, the difference between the estimates may also be due to the sources of TcR cDNA sequences. If only those cDNAs obtained from a single heterogeneous (i.e., thymus, spleen, etc.) library are considered, much lower Tβ redundancy is found in both human and murine cases. Differences between human (this study) and murine (19) Vβ family sizes estimated by Southern blot data could result from differences in filter washing conditions. A final possibility is that the TcR Vβ gene segments of the mouse have diverged more rapidly than either Ig or TcR α chain variable gene segments.

In summary, it appears that the human TcR Vβ germline gene segment multiplicity could be three to four times greater than that reported for the mouse. We discuss some possible explanations for this discrepancy, which are consistent with the data. The estimates of Vβ gene segment family size and number are similar to that of human and mouse TcR Vα chains. An upper limit of ~100 germline Vβ gene segments is consistent with the data presented in this study. Even though the Vβ regions encoded by the cDNA sequences examined herein contain many Ig-like characteristics, they do not display the same uniformity in length or distribution of well-defined regions of hypervariability. Ig-like tertiary or quaternary structures for the TcR are not predicted. Thus the structural features necessary for the recognition of antigen in the context of the MHC product by the TcR remains a mystery.

Summary

The nucleotide sequences of 22 human T cell antigen receptor (TcR) β chain variable region genes isolated from various T lymphocytes have been analyzed. Of the 19 variable gene segment (Vβ)-containing sequences, 17 were unique. The Vβ gene segments were grouped into 11 families. Comparisons were made with the data of Concannon et al. to unify the nomenclature. The data is consistent with a total Vβ gene segment repertoire with a most probable value of 38 members and an upper bound of 104 members at the 95% confidence level. Southern blot data of germline DNA using selected TcR Vβ cDNAs as probes support this estimate. The human repertoire is approximately three to four times greater than that reported for the mouse. Explanations for this discrepancy are proposed.
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