Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 6884–6888, October 1987
Immunology

Diversity and structure of human T-cell receptor α-chain variable region genes

(nucleotide sequences/variability/repertoire size)

MICHEL H. KLEIN*†, PATRICK CONCANNON‡, MARGARET EVERETT*, LEONARD D. H. KIM‡, TIM HUNKAPILLER‡, AND LEROY HOOD‡

‡Division of Biology, 147-75, California Institute of Technology, Pasadena, CA 91125; and *Departments of Immunology, Biochemistry, and Pathology, University of Toronto, Toronto, ON M5S 1A8, Canada

Contributed by Leroy Hood, June 24, 1987

ABSTRACT **The nucleotide sequences of 27 T-cell receptor α-chain variable region (V_α)-containing cDNA clones isolated from a cDNA library derived from human peripheral blood lymphocytes were determined. Eighteen different V_α and 26 different joining (J_α) gene segments are utilized in these clones. The V_α gene segments belong to 12 different subfamilies, each containing from one to seven members. Comparisons with the 16 different V_α and 21 different J_α sequences previously reported suggest that the germ-line repertoires for these gene segments are greater than previously estimated. Flexibility in the sites of gene segment joining and possibly N-region diversification also contribute to human α-chain diversity. Comparisons of human V_α regions indicate a high degree of variability spread uniformly across the entire V_α region without obvious hypervariable regions. However, amino acids important for the maintenance of *V* gene structure are conserved.**

The T-cell antigen receptor (TCR) functions to recognize foreign macromolecules (antigens) presented by accessory cells in the context of molecules encoded by the major histocompatibility complex (1). The TCR is displayed on the surface of T cells as a disulfide-linked α-/β-chain heterodimer (2, 3). Each chain is divided into a variable (V) region that recognizes antigen and a constant (C) region that attaches the V region to the cell surface. The V_α regions are encoded by discontinuous V_α and joining (J_α) gene segments that recombine during T-cell differentiation to create functional V_α genes (4, 5). Likewise, the V_β regions are encoded by rearranging V_β, diversity (D_β), and J_β gene segments that form V_β genes (6–11). The TCR must be capable of enormous diversification to recognize the universe of foreign antigens. This diversification occurs by four major somatic mechanisms: (*i*) combinatorial joining of any *V* to any *D* or *J* gene segment, (*ii*) flexibility in the positions within the gene segments at which joining occurs (junctional diversity), (*iii*) insertion of non-germ-line nucleotides into the junctions during joining (N-region diversity), (*iv*) combinatorial association of any α chain with any β chain. Somatic hypermutation does not seem to play a significant role in generating TCR diversity (11). Therefore, it is important to have an accurate estimate of the total number of germ-line gene segments and to understand the somatic mechanisms that operate to generate variability in order to estimate the true range of potentially expressed TCR diversity.

There is substantial information regarding the number of β gene segments in humans and mice. Each has two D_β–J_β–C_β clusters. These clusters contain single D_β and C_β elements and either six or seven J_β segments (12–14). Statistical analyses of pools of V_β gene segments suggest that the mouse has 20–30 (15, 16) and humans have ≈60 V_β gene segments (17–19).

In the mouse, we identified 40 V_α and 18 J_α gene segment sequences through nucleotide sequence analysis and hybridization techniques (6). An analysis of 21 human V_α genes from a cDNA library derived from the peripheral lymphocytes of a single individual suggested that there were only 40 V_α and <55 J_α gene segments (20). Because the gene segments in this initial analysis did not appear to be randomly distributed, statistical estimates of germ-line diversity were unreliable. Therefore, we examined 27 V_α genes from a peripheral lymphocyte cDNA library derived from a second individual. This analysis indicates that the human V_α and J_α repertoires may be substantially larger than previously estimated.§

MATERIALS AND METHODS

Isolation of α-Chain cDNA Clones. A *Pvu* II restriction fragment corresponding to the C_α region from the cDNA clone PY14 (21) was used to screen a phytohemagglutinin-stimulated human peripheral lymphocyte cDNA library (17). Phage DNAs from C_α-positive plaques were purified on DEAE columns (22). *Eco*RI-excised cDNA inserts were sized on 0.7% agarose gels and those >1.5 kilobases (kb) long were subcloned into the *Eco*RI site of M13mp18. Orientation of inserts in M13mp18 was determined by hybridization with strand-specific oligonucleotide probes.

DNA Sequence Analysis. The nucleotide sequences of the V_α and J_α regions of the isolated cDNA clones were determined by the Sanger *et al.* method (23). DNA sequence reactions were primed with a C_α-specific oligonucleotide primer and with the M13 universal sequencing primer. All nucleotide sequences reported were determined multiple times on each strand.

Southern Blot Analysis. *Eco*RI inserts for use as hybridization probes were isolated from M13 phage clones by freeze–thaw and phenol extraction from low melting temperature agarose gels. Blots of *Eco*RI- and *Bam*HI-digested human germ-line DNA were prepared on nylon membranes (Gelman Scientific) by the procedure of Reed and Mann (24) and hybridized as described (25).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: TCR, T-cell antigen receptor; MHC, major histocompatibility complex; V, C, J, and D, variable, constant, joining, and diversity regions.

†On leave sabbatical from the Toronto Western Hospital, Toronto, Canada.

§These sequences are being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J02992).

RESULTS

V_α Gene Segment Repertoire. We randomly selected 30 cDNA clones isolated from a cDNA library constructed from phytohemagglutinin-stimulated human peripheral blood lymphocytes with a C_α probe and determined their nucleotide sequences. Twenty-seven of these clones contained nucleotide sequences derived from a V_α gene segment. Their sequences are presented in Fig. 1. There are 18 unique V_α gene segments represented among these sequences, 12 of which have not been previously reported. These sequences bring to 28 the total number of different human V_α gene segments that have been directly identified by nucleotide sequence analysis. Some V_α gene segments were frequently isolated. For example, the $V_\alpha 1.2$ gene segment is used in 6 of the 27 clones listed in Fig. 1. The $V_\alpha 2.1$ and $V_\alpha 2.2$ gene segments are used two and four times, respectively. In a similar analysis, Yoshikai *et al.* (20), isolated four clones out of 21 containing the $V_\alpha 11.1$ and three clones containing the $V_\alpha 8.1$ gene segments. In contrast, we isolated only a single $V_\alpha 11.1$-containing clone and saw no examples of the $V_\alpha 8.1$ gene segment. These observations demonstrate that the distribution of V_α gene segments among the sequences reported here, or when pooled with those previously described (20), is nonrandom. Hence, it is not possible to use statistical methods to estimate the expressed V_α gene segment repertoire size. The fact that 28 different V_α gene segments have been derived from 49 independently selected cDNA clones certainly suggests that the germ-line repertoire is much larger than 40.

The nucleotide sequences of three additional clones were also determined (data not shown). Two of these appear to be germ-line J_α transcripts (26), extending through the J_α gene segment and containing the heptamer and nonamer sequences that mediate gene rearrangement as well as additional 5′ flanking DNA. The third clone contained only a truncated C_α transcript.

V_α Gene Segment Subfamilies. Subfamilies of V gene segments are defined as groups of V gene segments that share at least 75% nucleotide identity. Twelve different human V_α gene segment subfamilies have been defined thus far (20). The 18 different V_α gene segment sequences we report here include 12 novel V_α sequences and 6 that have been previously identified. These additional V_α gene segments define 7 V_α gene segment subfamilies, $V_\alpha 13$–$V_\alpha 19$ by the nomenclature of Yoshikai *et al.* (20), and provide nucleotide sequence information for three new members of the $V_\alpha 1$ subfamily and two new members of the $V_\alpha 2$ subfamily. To determine the sizes of these V_α subfamilies, we used cDNA clones each containing a V_α gene segment from one subfamily to probe human germ-line DNA on genomic blots (Fig. 2). Two different restriction digests (*Eco*RI and *Bam*HI) were probed to clearly establish how many hybridization bands corresponded to distinct V_α gene segments. These subfamilies appear to include only one or two members under hybridization conditions that yielded similar results to those reported by Yoshikai *et al.* (20) for the sizes of subfamilies $V_\alpha 1$ and $V_\alpha 2$. There are three subfamilies with two members ($V_\alpha 13$, $V_\alpha 16$, and $V_\alpha 18$) and four single-member subfamilies ($V_\alpha 14$, $V_\alpha 15$, $V_\alpha 17$, and $V_\alpha 19$). The hybridization pattern with clone AD2.10 ($V_\alpha 19.1$) was consistent with it representing a new V_α gene segment subfamily, although the amount of available nucleotide sequence is limited and derived from the most conserved portion of the V_α gene segment. As a result, this clone shares apparently artifactual nucleotide identity with members of five different V_α subfamilies. A total of 48 V_α gene segments could be estimated from the analysis of hybridization experiments reported here and previously (20).

Structure of V_α Gene Segments. The translated amino acid sequences of human V_α gene segments from subfamilies $V_\alpha 1$–$V_\alpha 19$ are aligned and displayed in Fig. 3. The protein sequence similarity between different V_α gene segment subfamilies ranges from 16% to 58%, in the same range as that observed in comparisons of similarly aligned human V_β subfamily representatives. Despite this extreme range of variability, a series of 10 amino acid positions known to be important for the maintenance of the protein structure characteristic of immunoglobulin V domains, indicated by asterisks in Fig. 3, are conserved by >75% of characterized V_α and V_β gene segments (1). Conservation of these positions as well as five others can also be seen in comparisons between human and murine V_α gene segments (5).

A great range of variability in the choice of amino acid residues without compromise of structure and function is characteristic of V regions of immunoglobulin and T-cell receptor chains. The distribution of variability, position by position, along the primary structure of the molecules is typically nonrandom when V-region amino acid sequences are aligned and compared. The standard means by which this distribution is displayed is through the use of variability plots based on the algorithm of Wu and Kabat and coworkers (27, 28). In these plots, most V-region families reveal the presence of three or four regions of relative hypervariability. These regions correspond to sites of antigen contact in immunoglobulin molecules (27, 28) and peaks at similar positions occur in human V_β regions (17–19). However, such an analysis (not shown) for V_α sequences as aligned in Fig. 3 fails to reveal obvious hypervariable regions other than at the V_α–J_α junction where somatic mutation mechanisms generate extensive diversity. While this result does not preclude that V_α regions use similar sites for antigen recognition, it does indicate that variability is more uniformly distributed in V_α regions than in the sample of other known V regions, making detection of potential antigen combining sites by this technique more difficult.

J_α Contributions to Diversity. There are 26 different J_α gene segments found in 29 J_α-containing cDNA clones whose nucleotide sequences we determined. One clone is nonproductive by virtue of having incorporated a germ-line J_α pseudogene (AP511). J_α gene segment usage appears to be random with respect to combinatorial joining to V_α gene segments. For example, each of the six different cDNA clones we examined that utilized the $V_\alpha 1.2$ gene segment used a different J_α gene segment. From this distribution of J_α α gene usage, a statistical calculation (15) indicates that there may be 100 or more germ-line J_α gene segments. When these J_α gene segments are added to those previously reported (20, 26), a total of 37 different J_α gene segments have been identified.

On average, human J_α gene segments are longer than their J_β counterparts, primarily at the 5′ end. J_α and J_β segments share a highly conserved core sequence Phe-Gly-Xaa-Gly-Thr-Xaa-Leu-Xaa-Val, where Xaa can be any amino acid. Despite this protein conservation, the J_α gene segments are highly variable at the nucleotide level, with an average nucleotide identity ranging from 40% to 60%. Hence, none of the human J_α gene segments sequenced appear to be simple alleles, nor can any be grouped into subfamilies as can the V_α gene segments.

Within the cDNA clones, variability is particularly great at the 5′ end of the J_α gene segments. A comparison of J_α sequences within isolated cDNA clones to those germ-line J_α gene segment sequences available indicates that some of this variability results from flexibility at the sites at which the gene segments are joined (Fig. 4). Also, a comparison of the sequences of two $V_\alpha 1.2$-containing clones (PY14 and AB17) to the germ-line sequence of $V_\alpha 1.3$ and the appropriate J_α gene segments reveals the presence of nucleotides at the junctions not encoded in either germ-line sequence. These nucleotides most likely reflect N-region diversification (29),

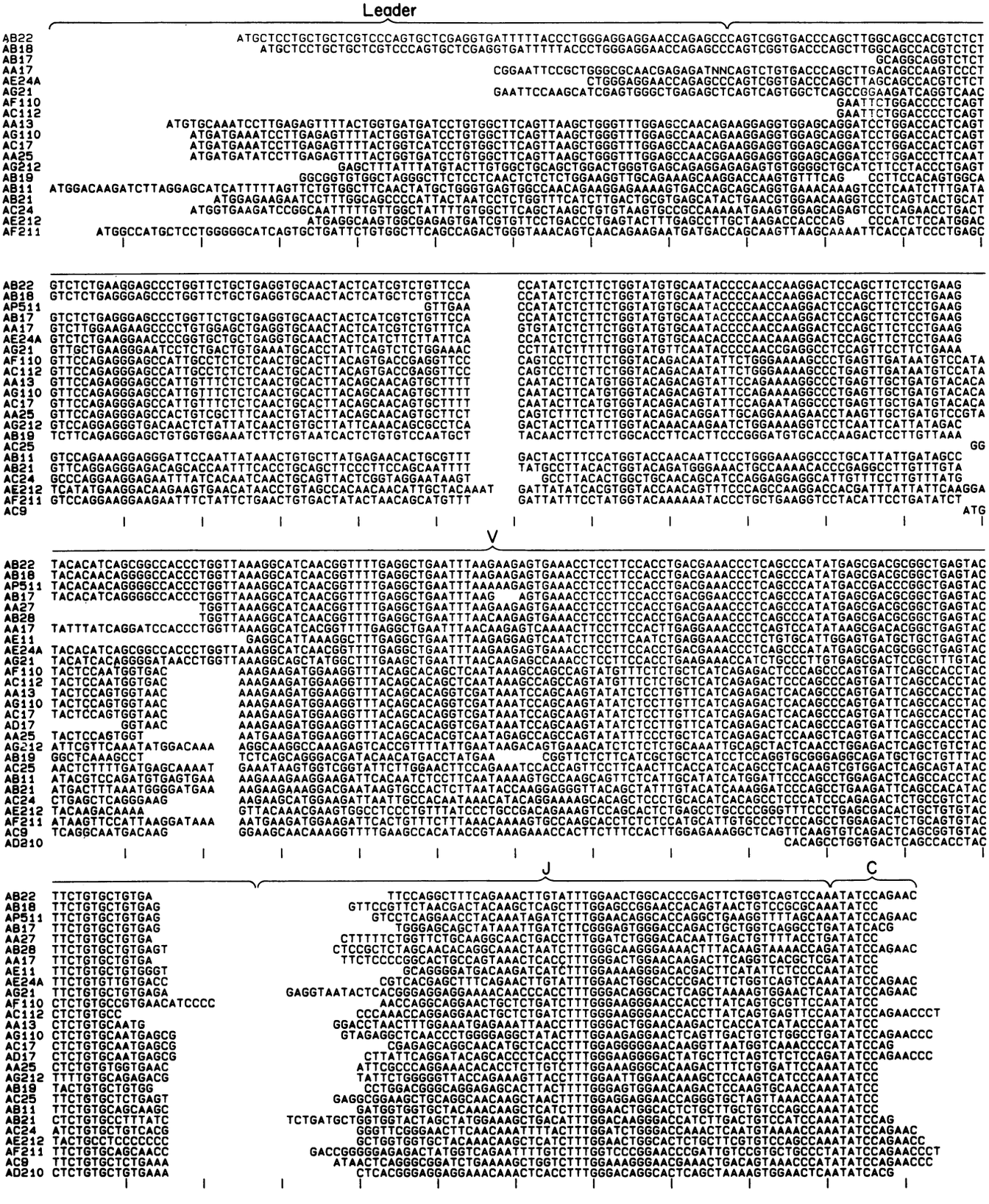

FIG. 1. Nucleotide sequences of human V_α gene segments. Nucleotide sequences were translated and aligned to maximize amino acid identity. Sequences previously reported (4, 20) were included in this analysis and are responsible for the positioning of some gaps but are not shown. The 3′ ends of V_α gene segments were assigned as the last nucleotide of continuous identity between multiple examples of the same V_α gene segment or V_α segments from the same subfamily. Clones AB11–AD210 correspond to V_α gene segment subfamilies $V_\alpha 13$–$V_\alpha 19$.

although polymorphism or the presence of a D_α gene segment cannot be excluded (Fig. 4). However, no conserved junctional sequences that might indicate at least a limited number of D_α elements are observed.

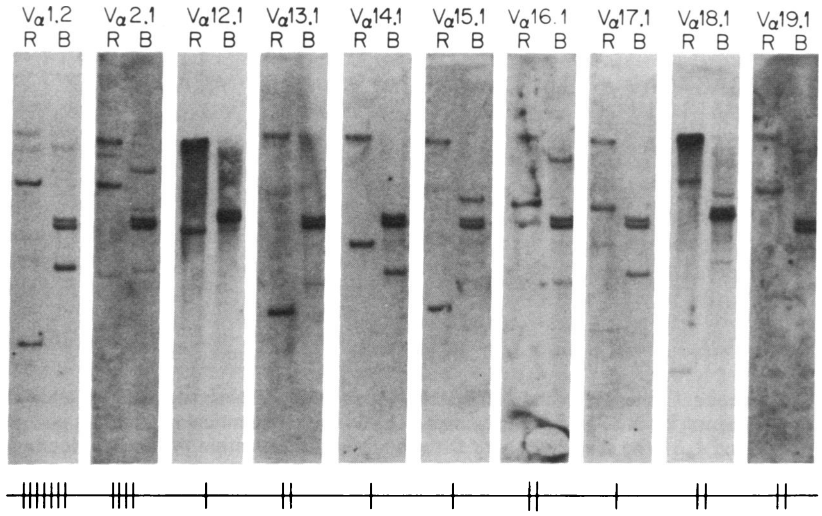

FIG. 2. Southern blot analysis of human germ-line DNA with V_α gene probes. HeLa cell DNA was digested with *Eco*RI (R) and *Bam*HI (B), electrophoresed, blotted to nylon membranes, and hybridized with cDNA clones containing the indicated V_α gene segments. Vertical lines below the gels represent the estimated number of members within the indicated V_α gene segment subfamily.

DISCUSSION

We have reported here the analysis of 30 α-chain cDNA clones isolated from a library constructed from phytohemagglutinin-stimulated human peripheral blood lymphocytes. These clones contain 18 different V_α gene segments, 12 of which have not been previously identified. These V_α gene segments define seven V_α gene segment subfamilies, $V_\alpha 13$ to $V_\alpha 19$. The V_α gene segments are not randomly utilized in these clones. Twenty-six different J_α gene segments are utilized in these cDNA clones, 16 of which have not been seen previously. We draw several conclusions from these data. First, the human germ-line V_α gene segment repertoire is substantially larger than previously reported. Nonrandom expression of the germ-line repertoire compromises our ability to estimate the total size of the repertoire by statistical methods. However, the fact that we see 28 different V_α gene segments in 49 α cDNA clones suggests the repertoire is substantially greater than 40. The same is not true of human V_β gene segments (15, 16). Therefore, the results with V_α gene segments may reflect distinct patterns of T-cell clonal expansion due to distinct antigenic histories. It is interesting that J_α gene segment expression does not appear to be as significantly constrained. This may imply that somatic variation at the V_α–J_α junction is more critical to antigen/major histocompatibility complex specificity than are particular J_α gene segments. Second, although the range of variability between V_α regions is similar to that observed between V_β regions on a percentage basis, V_α variability is distributed more uniformly along the primary sequence. Obvious hypervariable regions that are often observed for other *V* gene segment families are not detected. Third, the total number of J_α gene segments identified by direct nucleotide sequencing is 37. Based on their apparently random usage in the clones we have studied, we estimate that there may be on the order of 100 J_α sequences. Fourth, comparison of J_α sequences in the cDNA clones we have isolated to known germ-line J_α gene segment sequences provides evidence for a significant role for junctional and probably N-region diver-

```
            *         *      *             ** *                                      *            * **
Vα 1.1  QSVSQHNHHVILSEAASLELGCNYSYGGT  VNLFWYVQYPGQHLQLLLK YFSGDPLVKGIKGFEAEFIKSKFSFNLRKPSVQWSDTAEYFCAVN
Vα 1.2  QSVTQLGSHVSVSEGALVLLRCNYSSSVP  PYLFWYVQYPNQGLQLLLK YTSAATLVKGINGFEAEFKKSETSFHLTKPSAHMSDAAEYFCAV?
Vα 1.3  QSVTQLDSQVPVFEEAPVELRCNYSSSVS  VYLFWYVQYPNQGLQLLLK YLSGSTLVESINGFEAEFNKSQTSFHLRKPSVHISDTAEYFCAVS
Vα 1.4                                                             ?GIKGFEAEFKRSQSSFNLRKPSVHWSDAAEYFCAVG
Vα 1.5  QSVTQLSSHVSVSEGTPVLLRCNYSSSYS  PSLFWYVQHPNKGLQLLLK YTSAATLVKGINGFEAEFKKSETSFHLTKPSAHMSDAAEYFCVVT
Vα 1.6  QSVAQPEDQVNVAEGNPLTVKCTYSVSGN  PYLFWYVQYPNRGLQFLLK YITGDNLVKGSYGFEAEFNKSQTSFHLKKPSALVSDSALYFCAVR
Vα 2.1      ?NSGPLSVPEGAIASLNCTYSDRGS  QSFFWYRQYSGKSPELIMSIYSNGD   KEDGRFTAQLNKASQYVSLLIRDSQPSDSATYLCAVN
Vα 2.2  KEVEQDPGPLSVPEGAIVSLNCTYSNSAF  QYFMWYRQYSRKGPELLMYTYSSGN   KEDGRFTAQVDKSSKYISLFIRDSQPSDSATYLCAMS
Vα 2.3  KEVEQDPGPFNVPEGATVAFNCTYSNSAS  QSFFWYRQDCRKEPKLLMSVYSSG    NEDGRFTAHVNRASQYISLLIRDSKLSDSATYLCVVN
Vα 3.1  QQGEEDPQALSIQEGENATMNCSYKTSIN   NLQWYRQNSGRGLVHLIL IRSNERE KHSGRLRVTLDTSKKSSSLLITASRAADTASYFCAT
Vα 4.1  AKTTQ PNSMESNEEEPVHLPCNHSTISGT DYIHWYRQLPSQGPEYVIHGLTSN    VNNRMASLAIAEDRKSSTLILHRATLRDAAVYYCIRA
Vα 5.1         EALNIQEGKTATLTCNYTNYSP  AYLQWYRQDPGRGPVFLLL IRENEKE KRKERLKVTFDTTLNQSLFHITASQPADSATYLCALA
Vα 6.1  QKITQTQPGMFVQEKEAVTLDCTYDTSDQS YGVFWYKQPSSGKYFSYLS GSYDEQN ANRRSLLINFQKARKSANLVISASQLGDSAMYFCASR
Vα 7.1  QSLEQ PSEVTAVEGAIVQINCTYQTSGF  YGLSWYQQHDGGAPTFLSYNGLDGL   EETGRFSSFLSRSDSYGYLLLQELQMKDSASYFCAVF
Vα 7.2  QNIDQ PTEMTATEGAIVQINCTYQTSGF  NGLSWYQQHAGEAPTFLSYNVLDGL   EEK
Vα 8.1  ENVEQHPSTLSVQEGDSAVIKCTYSDSAS  NYFPWYKQELGKRPQLIID IRSNVGE KKDQRIAVTLNKTAKHFSLQITETQPQDSAVYFCAAS
Vα 8.2  ESVGLHLPTLSVQEGDNSIINCAYSNSAS  DYFIWYKQESGKGPQFIID IRSNMDK RQGQRVTVLLNKTVKHLSLQIAATQPGDSAVYFCAET
Vα 9.1  QRVTQPEKLLSVFKGAPVELKCNYSYSGS  PELFWYVQYSRQRLQLLLR HISR    ESIKGFTADLNKGETSFHLKKPFAQEEDSAMYYCALS
Vα 10.1 QLLEQSPQFLSIQEGENLTVYCN SSSVF  SSLQWYRQEPGEGPVLLVTVVTGGE  VKKLKRLTFQFGDARKDSSLHITAAQPGDTGHYLCAGV
Vα 11.1 DQVFQ PSTVASSEGAVVEIFCNHSVSNA  YNFFWHLHFPGCAPRLLVK GSKP    SQQGRYNMTYE  RFSSSLLILQVREADAAVYYCAV?
Vα 12.1 QKVTQAQTEISVVEKEDVTLDCVYETRDTT YYLFWYKQPPSGELVFLIRRNSFDEQN EISGRYSWNFQKSTSSFNFTITASQVVDSAVYFCAL?
Vα 13.1 QQVKQSPQSLIVQKGGIPIINCAYENTAF  DYFPWYQQFPGKGPALLIA IRPDVSE KKEGRFTISFNKSAKQFSLHIMDSQPGDSATYFCAAS
Vα 14.1 LNVEQGPQSLHVQEGDSTNFTCSFPSSNF  YALHWYRWETAKTPEALFV MTLNGDE KKKGRISATLNTKEGYSYLYIKGSQPEDSATYLCAFI
Vα 15.1 NEVEQSPQNLTAQEGEFITINCSYSVGIS   ALHWLQQHPGGGIVSLFM LSSGK   KKHGRLIATINIQEKHSSLHITASHPRDSAVYICAVT
Vα 16.1 AKTTQ PISMDSYEGGQEVNITCSHNNIATN DYITWYQQFPSQGPRFIIQGYKTK    VTNEVASLFIPADRKSSTLSLPRVSLSDTAVYYCLPP
Vα 17.1 QQVKQNSPSLSVQEGRISILNCDYTNSMF  DYFLWYKKYPAEGPTFLIS ISSIKDK NEDGRFTVSLNKSAKHLSPCIVPSQPGDSAVYFCAAT
Vα 18.1                                                  MSGNDK    GSNKGFEATYRKETTSFHLEKGSVQVSDSAVYFCALK
Vα 19.1                                                                                  ?QPGDSATYLCAVK
                 |         |         |         |         |         |         |         |         |
```

FIG. 3. Amino acid sequences of human V_α gene segments. Representative nucleotide sequences for each of the different human V_α gene segment subfamilies were translated and aligned to maximize identity. The V_α*1.1*, *-1.3*, *-3.1*, *-4.1*, *-5.1*, *-6.1*, *-7.1*, *-7.2*, *-8.1*, *-9.1*, and *-10.1* gene segments were derived from published sequences (4, 20). Asterisks indicate residues conserved at the 75% level in a comparison of human V_α, V_β, and murine V_α gene segments. Amino acids are identified by the single-letter code.

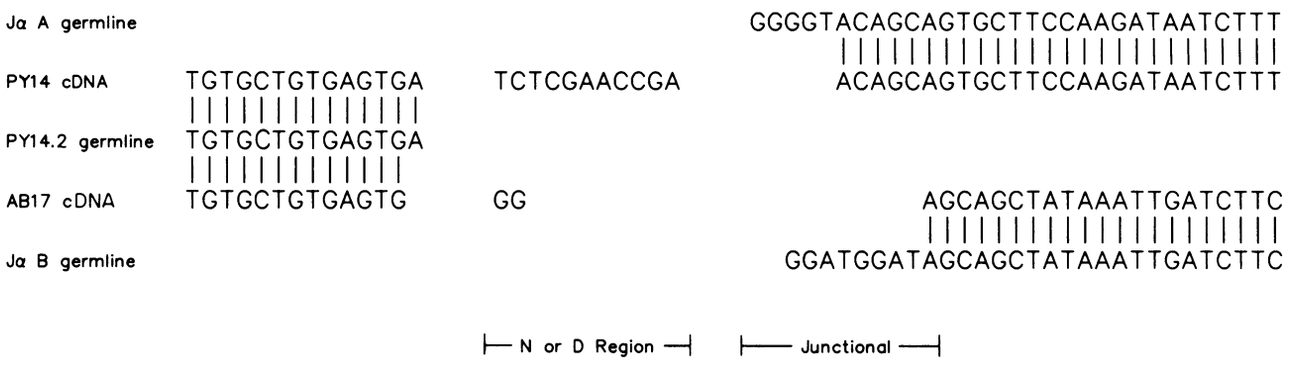

FIG. 4. Diversity at the V_α–J_α junction. Clones PY14 and AB17 each contain the $V_\alpha 1.2$ gene segment (21) and either the $J_\alpha A$ or $J_\alpha B$ sequence (26), respectively. The germ-line sequence for the $V_\alpha 1.3$ gene segment, which is >90% identical to $V_\alpha 1.2$, is indicated as PY14.2 germ line (26) as are germ-line sequences for $J_\alpha A$ and $J_\alpha B$ gene segments. N or D region, nucleotides within the cDNA clones that cannot be accounted for by reference to germ-line sequences; Junctional, nucleotides from J_α gene segments not represented within the cDNA clones.

sification mechanisms in human V_α chain gene diversification.

A minimal estimate of the number of potential human TCR α-/β-chain heterodimers can be derived by simple multiplication of germ-line gene segment numbers assuming that germ-line pseudogenes are infrequent and that all possible combinations both at the level of gene segment rearrangement and the level of chain association are permitted. Current nucleotide sequences of human V_α gene segments and genomic blot analyses with V_α subfamily probes provide firm evidence for the existence of at least 48 human V_α gene segments. The infrequent occurrence of pseudogenes in the fully characterized sequences may argue that most of these are functional V_α gene segments. Given the limited overlap (6/18) in V_α gene segment usage in our analysis and that of Yoshikai *et al.* (20), it is likely that substantially more V_α gene segments may exist. Nucleotide sequence analyses indicate the existence of at least 37 different J_α gene segments, and we have estimated, based on distribution, that there could be more than 100 such segments. Therefore, we can calculate that there are minimally 50×100 or 5000 possible α chains. A similar calculation for the β chain utilizing a germ-line repertoire of 60 V_β gene segments, two D_β gene segments used in all three reading frames, and 13 functional J_β gene segments in two clusters yields $\approx$3500 possible β chains. Assuming random association of α and β chains, there are potentially 1.8×10^7 possible heterodimers. This calculated value is similar to that derived for murine T-cell receptors and immunoglobulins (30). When somatic variation is considered, it becomes clear that B cells and T cells have the potential to code for similar numbers of antigen–receptor molecules.

Note Added in Proof. Kimura *et al.* (31) have recently published the nucleotide sequences of additional human V_α-containing cDNA clones.

The authors wish to thank Dr. B. Arden for assistance in library screening, Dr. Suzanna Horvath for synthesis of oligonucleotide sequencing primers, Dr. T. Mak for providing the PY14 clone, and Cathy Elkins for assistance in the preparation of this manuscript. This work was supported in part by a grant (MT-6780) from the Medical Research Council of Canada and grants from the National Institutes of Health. M.H.K. is the recipient of a Biotechnology Retraining Award from the Medical Research Council of Canada.

1. Kronenberg, M., Siu, G., Hood, L. & Shastri, N. (1986) *Annu. Rev. Immunol.* **4**, 529–591.
2. Haskins, K., Dubo, R., White, J., Pigeon, M., Kappler, J. & Marrack, P. (1983) *J. Exp. Med.* **157**, 1149–1169.
3. Meuer, S. C., Fitzgerald, K. A., Hussey, R. E., Hodgdon, J. C., Schlossman, S. F. & Reinherz, E. L. (1983) *J. Exp. Med.* **157**, 705–719.
4. Kim, G. K., Yague, J., Nelson, J., Marrack, P., Palmer, E., Augustin, A. & Kappler, J. (1984) *Nature (London)* **312**, 771–775.
5. Arden, B., Klotz, J. L., Siu, G. & Hood, L. E. (1985) *Nature (London)* **316**, 783–787.
6. Yanagi, Y., Yoshikai, Y., Legett, K., Clark, S. P., Aleksander, I. & Mak, T. W. (1984) *Nature (London)* **308**, 145–149.
7. Hedrick, S. M., Cohen, D. I., Nielsen, E. & Davis, M. M. (1984) *Nature (London)* **308**, 149–153.
8. Siu, G., Kronenberg, M., Strauss, E., Haars, R., Mak, T. W. & Hood, L. (1984) *Nature (London)* **311**, 344–350.
9. Clark, S. P., Yoshikai, Y., Taylor, S., Siu, G., Hood, L. & Mak, T. W. (1984) *Nature (London)* **311**, 387–389.
10. Siu, G., Clark, S., Yoshikai, Y., Malissen, M., Yanagi, Y., Strauss, E., Mak, T. & Hood, L. (1984) *Cell* **37**, 393–401.
11. Ikuta, K., Ogura, T., Shimizu, A. & Honjo, T. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 7701–7705.
12. Gascoigne, N. R. J., Chien, Y.-H., Becker, D. M., Kavaler, J. & Davis, M. M. (1984) *Nature (London)* **310**, 387–391.
13. Malissen, M., Minard, M., Mjolsaess, S., Kronenberg, M., Goverman, J., Hunkapiller, T., Prystowsky, M., Yoshikai, Y., Fitch, F., Mak, T. & Hood, L. (1984) *Cell* **37**, 1101–1110.
14. Toyonaga, B., Yoshikai, Y., Vadasz, V., Chin, B. & Mak, T. W. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 8624–8638.
15. Barth, R., Kim, B., Lan, N., Hunkapiller, T., Sobieck, N., Winoto, A., Gershenfeld, H., Okada, C., Hansburg, D., Weissman, I. & Hood, L. (1985) *Nature (London)* **316**, 517–523.
16. Behlke, M. A., Spinella, D. G., Chou, H., Sha, W., Hartl, D. L. & Loh, D. Y. (1985) *Science* **229**, 566–570.
17. Concannon, P., Pickering, L. A., Kung, P. & Hood, L. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 6598–6602.
18. Tillinghast, J. P., Behlke, M. A. & Loh, D. Y. (1986) *Science* **233**, 879–883.
19. Kimura, N., Toyonaga, B., Yoshikai, Y., Triebel, F., Debre, P., Minden, M. D. & Mak, T. W. (1986) *J. Exp. Med* **164**, 739–750.
20. Yoshikai, Y., Kimura, N., Toyonaga, B. & Mak, T. (1986) *J. Exp. Med.* **164**, 90–103.
21. Yanagi, Y., Chan, A., Chin, B., Minden, M. & Mak, T. W. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 3430–3434.
22. Helms, C., Graham, M. Y., Dutchik, J. E. & Olson, M. W. (1985) *DNA* **4**, 39–49.
23. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
24. Reed, K. C. & Mann, D. A. (1985) *Nucleic Acids Res.* **13**, 7207–7221.
25. Concannon, P., Gatti, R. A. & Hood, L. (1987) *J. Exp. Med.* **165**, 1130–1140.
26. Yoshikai, Y., Clark, S. P., Taylor, S., Sohn, U., Wilson, B. I., Minden, M. D. & Mak, T. W. (1985) *Nature (London)* **316**, 837–840.
27. Kabat, E. A., Wu, T. T., Bilofsky, H., Reid-Miller, M. & Perry, H. (1983) in *Sequences of Immunological Interest* (U.S. Department of Health and Human Sciences, Washington, DC).
28. Wu, T. T. & Kabat, E. A. (1970) *J. Exp. Med.* **132**, 922–930.
29. Alt, F. & Baltimore, D. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4118–4122.
30. Goverman, J., Hunkapiller, T. & Hood, L. (1986) *Cell* **45**, 475–484.
31. Kimura, N., Toyonaga, B., Yoshikai, Y., Du, R.-P. & Mak, T. W. (1987) *Eur. J. Immunol.* **17**, 375–383.