Rearrangement and Expression of T-Cell Antigen Receptor Genes in Human T-Lymphocyte Tumor Lines and Normal Human T-Cell Clones: Evidence for Allelic Exclusion of Tiβ Gene Expression and Preferential Use of a Jβ2 Gene Segment

JEFFREY M. LEIDEN,[1] DENO P. DIALYNAS,[1] ALLAN D. DUBY,[2] CORNELIUS MURRE,[2] JON SEIDMAN,[2] AND JACK L. STROMINGER[1]*

Department of Tumor Virology, Dana-Farber Cancer Institute,[1] and Department of Genetics,[2] Harvard Medical School Boston, Massachusetts 02115

The gene encoding the β chain of the human T-cell receptor for antigen is composed of variable (V), diversity (D), joining (J), and constant (C) gene segments which undergo specific rearrangements during T-lymphocyte ontogeny. Southern blot analyses of seven human T-cell tumor lines and normal human T-lymphocyte clones revealed that most of these T-cell lines rearrange their Tiβ genes differently. The T-cell tumor line HPB-MLT rearranges and transcribes both of its Tiβ genes. Cloning and sequencing of the Tiβ cDNAs corresponding to these rearrangements revealed that one of the rearranged Tiβ genes is defective, while the other is functional and corresponds to the Tiβ protein expressed on the surface of these cells. Thus, this cell line displays a pattern of allelic exclusion of Tiβ gene expression. A comparison of four Cβ2-containing Tiβ cDNAs from three different cell lines revealed that three of the four utilize the same Jβ2.5 gene segment joined to different Dβ and Vβ genes, suggesting that there may be preferential use of this J gene during Jβ2 rearrangements. Hybridization analyses with probes for the α and β genes of the T-cell receptor and the T-cell-specific Tγ gene revealed that HPB-MLT cells appear to express approximately equivalent amounts of RNA corresponding to each of the rearranged Tiα and Tiβ genes. However, they express a much lower level of Tγ RNA.

The antigen specificity of T lymphocytes is derived from their expression of clone-specific (idiotypic) receptors for antigen (Ti). These T-lymphocyte receptors have been serologically and biochemically identified as 90-kilodalton heterodimers composed of disulfide-linked acidic (Tiα) and basic (Tiβ) chains with an approximate size of 40 to 50 kilodaltons (2, 17, 28). The murine and human genes encoding both chains of this receptor have recently been independently cloned by several groups (10, 11, 19, 20, 33, 35, 44). These genes, which display significant homology to immunoglobulin genes, are composed of variable (V)-, diversity (D)-, joining (J)-, and constant (C)-region gene segments which undergo specific rearrangements during thymic development (10–12, 15, 35, 37, 38). Studies of both murine and human cDNA and genomic clones of the Tiβ gene have revealed the existence of two tightly linked C-region gene segments (Cβ1 and Cβ2) which are 95 to 97% homologous at the nucleotide level (15, 22, 26). These two C-region gene segments are apparently used interchangeably by cytotoxic and helper T-lymphocyte subsets. It is not yet clear whether suppressor T lymphocytes use these or different receptor genes (21, 24, 31). In addition to the Tiα and Tiβ genes, a third T-cell-specific immunoglobulinlike gene, Tγ, which displays a limited amount of diversity as compared with the Tiα and Tiβ genes, has recently been described (18, 32). Thus far, the role of this third gene in T-cell function remains unknown.

Although a great deal is already known about Ti gene structure, relatively little, as yet, is known about the mechanisms of Ti gene rearrangement and the control of Ti gene expression. We analyzed the rearrangement and expression

of Tiβ genes in five human acute lymphocytic leukemia (ALL) cell lines as well as in two normal human T-lymphocyte clones. Southern blot analyses showed that, except for two pairs of the ALL cell lines which appear to be identical, these cell lines appear to rearrange their Tiβ genes differently. A more detailed analysis of Tiβ gene expression in the human T-cell ALL cell line HPB-MLT indicated that this cell rearranges and transcribes both copies of its Tiβ genes. Cloning and sequencing of cDNAs corresponding to the two rearranged Tiβ genes revealed that one rearrangement is defective and is characterized by an out-of-frame V-D-J joining event which causes the generation of stop codons in downstream C-region sequences. A second cDNA represents the functional Tiβ gene from this cell line. The deduced amino acid sequence of this functional Tiβ cDNA corresponds to the previously described N-terminal protein sequence of the Tiβ polypeptide isolated from the surface of HPB-ALL cells. Thus, although this cell line rearranges and transcribes both copies of its Tiβ genes, it displays a pattern of allelic exclusion of Tiβ gene expression. A comparison of the sequences of four Tiβ cDNA clones from three different cell lines showed that, although each of the clones utilizes a different Vβ gene, three of the four utilize the same Jβ2.5 gene segment. This finding suggests that this Jβ2 gene segment is used preferentially during V-D-Jβ2 rearrangement. To determine whether Ti gene expression is regulated at the transcriptional level, a cDNA library from the HPB-MLT cell line was hybridized to a variety of T-cell-specific probes. These studies indicated that HPB-MLT appears to coordinately express all of its rearranged Tiα and Tiβ genes (whether functionally or defectively rearranged) while expressing a much lower level of Tγ RNA.
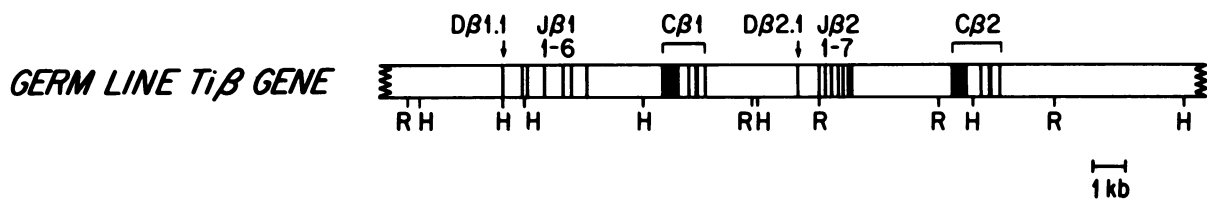
---

* Corresponding author.

FIG. 1. Partial restriction endonuclease map of the human germ line Tiβ genes (40). The relevant EcoRI (R) and HindIII (H) restriction enzyme sites are shown below the map.

MATERIALS AND METHODS

Cell lines. HPB-MLT, HPB-ALL, CEM, MOLT-3, and MOLT-4 T-cell ALL cell lines have been previously described in detail (30) and were supplied by Jun Minowada. L17 is a T3$^+$ T4$^+$ T8$^-$ human cytotoxic T-lymphocyte clone which specifically kills a subset of human cells bearing the class II major histocompatibility complex (MHC) antigen DQ1 (DC1) (Fraser et al., submitted for publication). 2G2 is a T3$^+$ T4$^-$ T8$^+$ cytotoxic human T-lymphocyte clone which specifically kills human cells bearing the class I MHC antigen HLA-B27 (8). These T-lymphocyte clones were grown and supplied by John Fraser and Michael Brenner in our laboratory.

Construction and screening of cDNA libraries. The cDNA library from HPB-MLT was constructed in bacteriophage λgt11 as previously described (22). This library is composed of 2 × 10^6 different clones with an average insert size of 1.0 kilobase (kb). The 2G2 cDNA library was prepared by a modification of the method of Gubler and Hoffman (16). Briefly, approximately 1 μg of poly(A)$^+$ RNA was prepared from 10^7 cells by the guanidium isothiocyanate-cesium chloride procedure (41). First-strand cDNA synthesis was carried out with avian myeloblastosis virus reverse transcriptase. Second-strand synthesis was carried out with RNase H and Escherichia coli DNA polymerase I (16). The ends of the double-stranded cDNA were made blunt with T4 DNA polymerase, and the cDNA was treated with EcoRI methylase. EcoRI linkers (New England BioLabs, Inc., Beverly, Mass.) were ligated to the ends of the cDNAs with T4 DNA ligase, and the resulting mixture was digested with an excess of EcoRI. The cDNA was then separated from free linkers and partially size fractionated by passage over a Sepharose CL-4B column. λgt10 DNA was digested with EcoRI and treated with calf intestinal phosphatase to prevent self-ligation. cDNA (12.5 ng) was mixed with 0.5 μg of EcoRI-digested and calf intestinal phosphatase-treated λgt10 DNA and ligated with T4 DNA ligase overnight at 12°C. The ligation mixture was packaged in vitro by standard techniques (27), and the resulting phage were amplified on lawns of E. coli C600 hfl. The 2G2 library prepared in this way is composed of 10^5 different clones with an average insert size of 1.0 kb.

Phage libraries were screened as follows. Phage (3 × 10^4) were plated on a lawn of E. coli C600 hfl on each of five 150-mm plates. The phage were grown at 37°C for approximately 6 h until they were subconfluent. Plaques were transferred to nitrocellulose filters. Phage DNA was denatured by a 60-s treatment with 0.5 M NaOH–1.5 M NaCl. Filters were neutralized for 5 min with 0.5 M Tris (pH 8.0)–1.5 M NaCl and washed with 2× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH$_2$PO$_4$, and 1 mM EDTA) for 5 min. After drying at room temperature for 30 min, the filters were baked in vacuo at 80°C for 2 h. Prehybridization was for 4 h at 68°C in 5× SSPE–5× Denhardt solution–0.1% sodium

dodecyl sulfate–100 μg of denatured salmon sperm DNA per ml–2 μg of poly(A) per ml. A 0.2-μg sample of ^{32}P-labeled nick-translated probe (2 × 10^8 to 4 × 10^8 cpm/μg) was added to the prehybridization mixture, and hybridization was continued for 16 to 20 h at 68°C. Filters were washed twice in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0)–0.1% sodium dodecyl sulfate for 15 min at room temperature, and twice in 0.3× SSC–0.1% sodium dodecyl sulfate for 1 h at 68°C. Autoradiography was carried out for 4 to 48 h at −70°C with Du Pont intensifying screens.

Positive plaques were picked and purified to homogeneity by sequential hybridization with the appropriate radiolabeled probe. Inserts were cut out of phage DNA with EcoRI and subcloned into EcoRI-digested calf intestinal phosphatase-treated pUC18 vector (42).

Isolation of human genomic Tiβ clone. A human genomic library was constructed, and clones containing the entire unrearranged Jβ1-Cβ1 and Jβ2-Cβ2 gene clusters were isolated as previously described (13b).

DNA sequencing. DNA sequencing was done by the dideoxy chain termination method of Sanger et al. (34) with [^{35}S]dATP. Appropriate restriction enzyme fragments were subcloned into M13mp18 and M13mp19 directly from low-melting-point agarose as described by Crouse et al. (13). All clones were sequenced on both strands, and all restriction enzyme sites were sequenced across.

Southern blots. High-molecular-weight DNA (10 μg) prepared by the method of Bell et al. (6) was digested for 5 h at 37°C with a fivefold excess of restriction endonuclease and separated by electrophoresis on a 0.7% agarose gel. DNA was transferred to a nitrocellulose filter by the method of Southern (39). Hybridizations were carried out as described above for the screening of cDNA libraries, except that the probe concentration was approximately 5 × 10^6 cpm/ml.

RESULTS

Tiβ gene rearrangements in human T-cell tumor lines and normal human T-lymphocyte clones. A partial restriction endonuclease map of the germ line Tiβ gene is shown in Fig. 1. By probing EcoRI- and HindIII-digested human DNAs with a Tiβ C-region probe, the number and type of Tiβ gene rearrangements in a given cell line can be determined (13b, 36, 40; J. Leiden and J. Strominger, unpublished data). Specifically, when a Southern blot containing EcoRI-digested unrearranged B-lymphocyte DNA is hybridized to a Cβ probe, two bands are seen: a 4.2-kb band containing the Cβ2 gene segment which is unchanged in size by V-D-J rearrangements, and a 10.2-kb germ line band containing the entire Jβ1-Cβ1 gene cluster. The size of this 10.2-kb germline band is altered by V-D-Jβ1 rearrangements, and it is deleted by V-D-Jβ2 rearrangements. Similarly, when a Southern blot containing HindIII-digested unrearranged B-cell DNA is hybridized to a Cβ probe, three bands are seen:
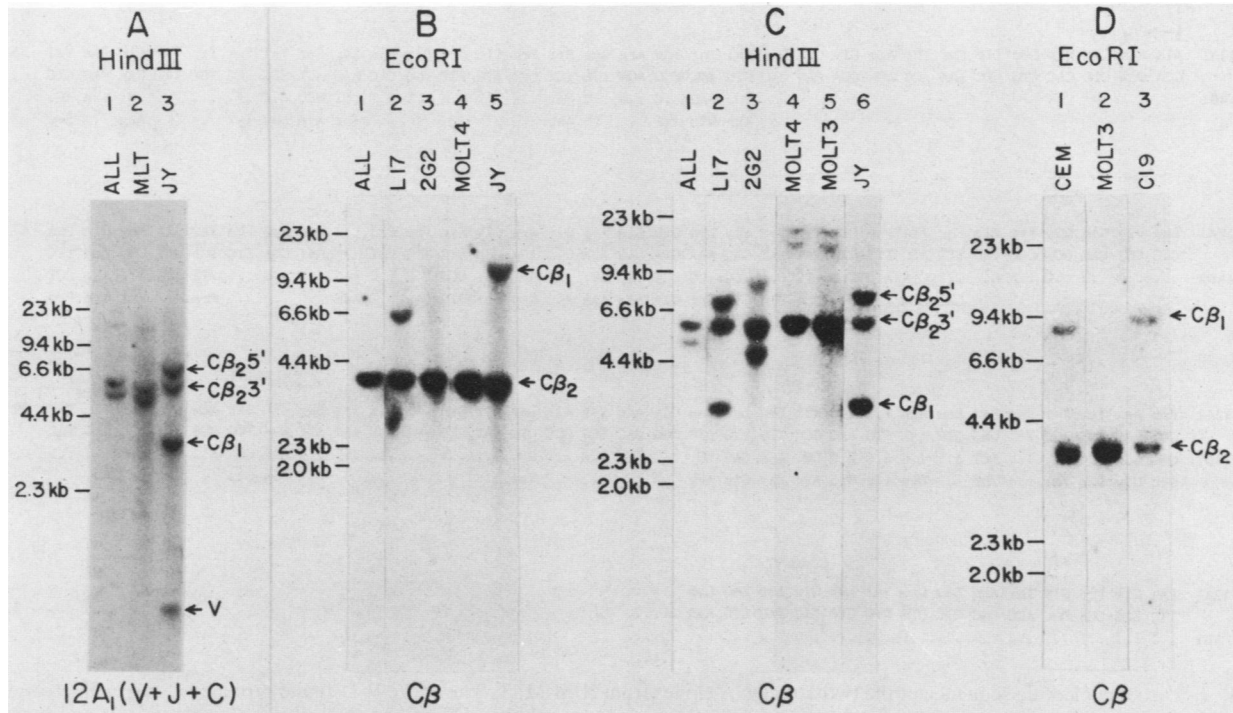
FIG. 2. Southern blot analysis of human T-cell ALL cell lines and normal human T-lymphocyte clones with Tiβ probes. DNA from the indicated cell lines was digested with *Eco*RI or *Hin*dIII and hybridized to the 12A1 (V-D-J-Cβ2) cDNA probe (A) or a Cβ probe consisting of the 280-bp *Bgl*II fragment of the 4D1 cDNA clone (B-D) (22). Bands containing the germ line Cβ1 and Cβ2 gene segments are indicated to the right of sections A to D. Because the Cβ1 and Cβ2 gene segments are 97% homologous in their coding regions, the Cβ2 probe hybridizes to fragments containing both Cβ1 and Cβ2 sequences (22). Size markers are shown in kilobases to the left of each figure. The faint band of 19 kb seen in panel A, lanes 1 and 2, is also present in panel C, lane 1, but did not reproduce well in the photograph.

(i) an 8.3-kb band containing the Jβ2 gene segments and the 5' portion of the Cβ2 gene, (ii) a 6.7-kb band containing the 3' end of the Cβ2 gene, and (iii) a 3.5-kb band containing the Cβ1 gene segment. The size of the 8.3-kb germ line band is altered by V-D-Jβ2 rearrangements.

A Southern blot analysis of five human T-cell ALL lines and two human T-lymphocyte clones is shown in Fig. 2 and summarized in Table 1. These analyses revealed that the MOLT-3 and MOLT-4 ALL cell lines display an identical pattern of Tiβ gene rearrangement which is characterized by two Jβ2-Cβ2 gene rearrangements (the germ line *Hin*dIII 8.3-kb band is replaced by novel 14.0- and 18.5-kb bands in both cell lines). This suggests that, rather than sharing a single Tiβ gene rearrangement, these two cell lines are actually identical. This finding was not unexpected as they were originally isolated from the same patient with T-cell ALL (30). Similarly, the HPB-MLT and HPB-ALL cell lines display an identical pattern of Tiβ gene rearrangement which is clearly distinct from that of MOLT-3 and MOLT-4 (Fig. 2A; Table 1). The CEM cell line and the two normal allospecific cytotoxic human T-lymphocyte clones each show novel patterns of Tiβ gene rearrangement (Fig. 2; Table 1). It is noteworthy that all the T-cell ALL cell lines examined by Southern analysis rearrange both Jβ2-Cβ2 gene segments, usually with concomitant deletion of both Cβ1 genes. The significance of this pattern is unclear. However, a similar phenomenon has been observed in murine T-cell lymphomas (24). The finding that the L17 clone, which is a cytotoxic cell specific for the class II DQ1 MHC antigen, rearranges a Jβ1-Cβ1 gene segment while the 2G2 clone, which is a cytotoxic cell specific for the class I B27 MHC antigen, rearranges two Jβ2-Cβ2 gene segments (and deletes

both gene clusters) indicates that the cytotoxic phenotype is not uniquely associated with Cβ1 or Cβ2 gene segment use. This is in agreement with the previous results of Royer et al. (31), Hedrick et al. (21), and Kronenberg et al. (24).

The HPB-MLT cell line displays a pattern of allelic exclusion of Tiβ gene expression. B lymphocytes are known to display a pattern of allelic exclusion of immunoglobulin gene expression. That is, a given B lymphocyte expresses a single immunoglobulin protein on its surface (reviewed in reference 14). A number of mechanisms are utilized by B cells to produce this pattern of allelic exclusion. Some cells rearrange a single immunoglobulin heavy- and light-chain gene. Others rearrange multiple copies of a given immunoglobulin gene, but only one of the rearrangements is productive, while the others are defective, i.e., they are characterized by out-of-frame joining events or somatic mutations which generate mRNAs which cannot be translated into functional immunoglobulin protein. Finally, some B cells express more than one functional immunoglobulin light chain in their cytoplasms but only assemble and express a single functional immunoglobulin protein on their surface (3, 4, 7, 25).

The patterns of Tiβ gene rearrangement shown in Fig. 2 reveal that, like their B-cell counterparts, some T-cell clones (e.g., L17) rearrange a single Tiβ gene, thereby producing a pattern of allelic exclusion of Tiβ gene expression. However, as noted above, most T-cell ALL lines and at least one human cytotoxic clone (2G2) rearrange both of their Tiβ genes (Table 1). To determine whether HPB-MLT cells, which rearrange two Tiβ genes (Table 1), display a pattern of allelic exclusion of Tiβ gene expression, several Tiβ cDNA clones were isolated from an HPB-MLT cDNA library and subjected to DNA sequence analysis. We have previously
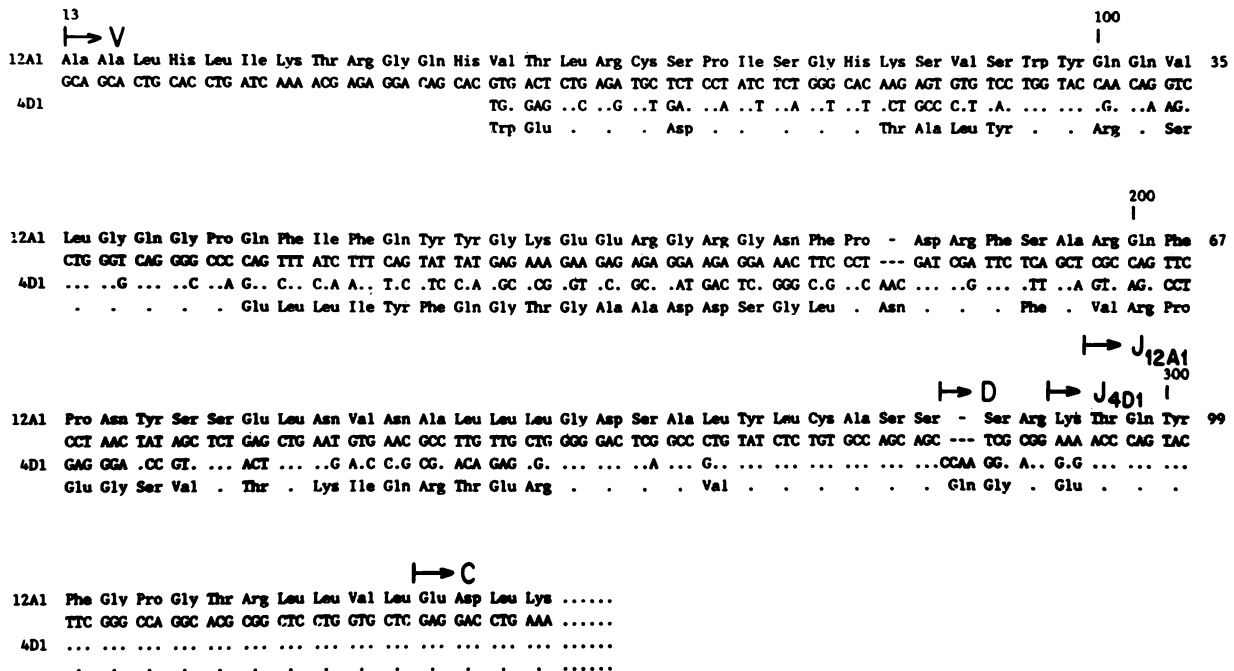
FIG. 3. Partial nucleotide sequence of the two Tiβ cDNA clones from HPB-MLT. The entire V-D-J- and proximal C-region nucleotide and predicted amino acid sequences of 12A1, the functional Tiβ cDNA clone, are shown. Differences between the defective 4D1 and functional 12A1 clones are shown in the 4D1 sequence. Dots in the 4D1 sequence represent nucleotides and amino acids which are identical between the two clones. Dashes represent nucleotides which are present in one clone and absent in the second. As described in the text, the 4D1 clone is 36 nucleotides shorter at its 5′ end than the 12A1 clone. The two clones share an identical Cβ2 nucleotide sequence at their 3′ ends which has been previously described (22) and which is not shown in detail here. Nucleotide numbers (shown above) and amino acid numbers (shown to the right of the figure) were determined by alignment of these partial-length clones with the previously described full-length human Tiβ cDNA clone YT35 (44). Approximate boundaries of the V-, D-, J-, and C-region gene segments are indicated above the 12A1 sequence.

TABLE 1. Tiβ-chain gene segment rearrangements in human T-Cell ALL and human T-lymphocyte clones

Cell line	Phenotype	Rearrangement[a]		Comments
		Jβ1-Cβ1	Jβ2-Cβ2	
HPB-MLT	T3+ T4+ T8+ T-ALL	2d	2r	
HPB-ALL	T3+ T4+ T8+ T-ALL	2d	2r	Pattern identical to HPB-MLT
MOLT-3	T3+ T4+ T8+ T-ALL	2d	2r	
MOLT-4	T3+ T4+ T8+ T-ALL	2d	2r	Pattern identical to MOLT-3
CEM	T3+ T4+ T8− T-ALL	1r, 1d	2r[b]	
L17[c]	T3+ T4+ T8−; DC1 cytotoxic clone	1r, 1d	2gl	
2G2[d]	T3+ T4− T8+; B27 cytotoxic clone	2d	2r	

[a] d, Deleted; r, rearranged; gl, germ line.

[b] Data not shown in Fig. 2C. When HindIII-digested CEM DNA was probed with Cβ, the pattern was identical to that shown for JY or L17 DNAs. However, further studies utilizing Jβ1 and Jβ2 probes have revealed that this cell line displays a complex pattern of rearrangement of both Jβ2 alleles (13c).

[c] This cell line has been previously described by Fraser et al. (submitted).

[d] This cell line has been previously described by Brenner et al. (8).

reported the nucleotide sequence of one of these Tiβ cDNA clones, 4D1 (22). This clone is defective, i.e., it is characterized by an out-of-frame V-D-J joining event which causes the generation of stop codons in the downstream C-region sequences. We now report the nucleotide and deduced amino acid sequence of the V-D-J regions of a second Tiβ cDNA, 12A1, which was isolated from this same cDNA library (Fig. 3). This partial-length clone, which begins at nucleotide 13 (44), is composed of a single open reading frame and is formed by the joining of different Vβ and Dβ gene segments to the same Jβ2-Cβ2 gene segments utilized by the defective clone 4D1. 12A1 and 4D1 presumably represent different joining events of the Tiβ gene segments present on the two copies of chromosome 7 in this cell line (9, 30a).

To confirm the hypothesis that 12A1 represents the functional Tiβ gene from HPB-MLT, the deduced amino acid sequence of this clone was compared with the previously reported (22) N-terminal sequence of the Tiβ protein expressed on the surface of HPB-ALL. These two sequences are identical except for the N-terminal three amino acids (Fig. 4). This divergence of N-terminal sequence may be due to an aberrant sequence introduced at the 5′ end of 12A1, at the hairpin loop, during the initiation of the second strand of cDNA synthesis, or during subsequent S1 nuclease processing. A comparison of the 12A1 HPB-MLT Tiβ cDNA with the previously described HPB-β2 Tiβ cDNA clone isolated from the cell line HPB-ALL (45) showed that these sequences are identical except for two nucleotides. When compared with 12A1, the previously published HPB-ALL clone has an extra nucleotide at position 159 which probably represents a sequencing error since it introduces a frameshift

12A1 cDNA Sequence: --- --- --- ---   Ala Ala Leu His Leu Ile Lys Thr Arg Gly Gln His Val Thr

Tiβ Protein Sequence: XXX Val Thr Gln Ser Pro Thr His Leu Ile Lys Thr Arg Gly Gln His Val Thr

FIG. 4. Comparison of the 12A1 Tiβ cDNA sequence with the N-terminal amino acid sequence of the Tiβ chain of HPB-ALL. The 12A1 sequence is the predicted amino acid sequence from the 12A1 cDNA clone shown in Fig. 3. The HPB-ALL sequence is the previously described amino acid sequence from the Tiβ protein purified by immunoaffinity from the surface of the T-cell tumor line HPB-ALL (22). Underlined amino acids differ between the two sequences. X's represent the N-terminal residue which could not be determined during protein sequencing. Dashes represent the 5' nucleotide sequence which was absent from the partial-length 12A1 cDNA clone.

mutation which would result in a nonfunctional clone. In addition, nucleotide 279, which is a G in 12A1, is a T in the HPB-ALL clone. This substitution is not silent, causing replacement of the Gln at position 93 of 12A1 with a His in the HPB-β2 cDNA. This may represent a sequencing error or a mutation of one of the Tiβ genes during prolonged culture in vitro.

HPB-MLT and HPB-ALL are the same cell line. The finding that HPB-ALL and HPB-MLT express nearly identical Tiβ genes was not unexpected given the previous observation that an anti-idiotypic antibody recognizing the T-cell receptor of HPB-ALL also recognized the T-cell receptor of HPB-MLT (23). Because these two cell lines were originally isolated from different patients with T-cell ALL (30), it was important to determine whether this result represented a trivial confusion of these cell lines during in vitro passaging or a real identity of Tiβ genes among different human ALL cell lines. To address this question further, we subjected DNA from HPB-MLT and HPB-ALL to Southern blot analysis with the 12A1 Tiβ cDNA probe. As described above, this analysis showed that HPB-ALL and HPB-MLT rearrange both copies of their Tiβ genes identically (Fig. 2A) suggesting that, rather than sharing a single Tiβ gene rearrangement, these two cell lines are actually identical (i.e., have been confused during in vitro culture). Further evidence of the identity of these two cell lines has been provided by more recent studies showing that HPB-ALL and HPB-MLT also rearrange the same Tiα V-region gene segment (J. Leiden and J. Strominger, unpublished data).

Expression of Tiα, Tiβ, and Tγ genes in HPB-MLT. To determine the levels of Ti and Tγ gene expression in HPB-MLT cells, the size-selected (greater than 500 base pairs [bp]) λgt11 cDNA library from this cell line was hybridized to the following probes: (i) a full-length human Tiα probe isolated from a cDNA library constructed from the functional human T-cell clone L17 (J. Leiden, J. Fraser, and J. Strominger, Immunogenetics, in press); (ii) the human Tiβ cDNA probe, 4D1 (22); (iii) a full-length human Tγ clone isolated from the HPB-MLT library by hybridization with a murine Tγ probe (13a); (iv) a 170-bp EcoRI-BamHI V-region probe from the 5' end of the 4D1 clone (Fig. 3); and (v) an 80-bp EcoRI-KpnI V-region probe from the 5' end of the 12A1 clone (Fig. 3). The results of these hybridizations are shown in Table 2 and reveal marked variations in the levels of Tiα, Tiβ, and Tγ gene expression. The Tγ gene is clearly expressed at levels that are approximately 5 to 10% of those of its Tiα and Tiβ counterparts. In addition, the level of total Tiβ gene expression is approximately double that of Tiα. This Tiβ gene expression is almost equally divided between the defective (4D1) and functional (12A1) Tiβ genes. That is, the level of expression of each of the Tiβ genes is approximately equivalent to the level of total Tiα gene expression in this cell line. The slightly lower level of functional β chain relative to defective β chain seen in this experiment is most likely due to the fact that the 12A1 V-region probe used in

these studies is located approximately 125 bp 5' of the 4D1 V-region probe and therefore would not detect partial-length cDNA clones lacking these 5' sequences. In addition, because the 12A1 V-region probe was significantly shorter than the 4D1 V-region probe, the signal intensity of the positive clones in the 12A1 hybridization was lower than that in the 4D1 hybridization. Thus, the 12A1 hybridization may have failed to detect some of the more faintly hybridizing clones.

Preferential use of a Jβ2 gene segment in Tiβ cDNAs. The rearrangement of multiple gene segments is one of the major mechanisms utilized by both B and T lymphocytes to generate diversity of their antigen receptors. Thus, as described above, the variable region of the β chain of the T-cell receptor for antigen is encoded by separate V, D, and J gene segments which rearrange during thymic development to form a functional Tiβ gene. The potential number of Tiβ variable regions and, therefore, the potential diversity of T-cell receptors is determined both by the numbers of different V, D, and J gene segments and by the relative frequencies of rearrangement of a given Vβ gene segment with individual Dβ and Jβ genes. A comparison of four previously sequenced Cβ2-containing Tiβ cDNA clones (Fig. 5) revealed that three of the four use the same or a very highly homologous Jβ gene segment, although each utilizes a different Vβ or Dβ gene segment (25a). A fourth Cβ2 cDNA clone, JURKAT β2 (45), clearly utilizes a different Jβ gene segment which differs from the sequence of the first three by 15 to 17 nucleotides (four to five amino acids). To determine whether the shared Jβ sequences used in the 2G2, 4D1, and 12A1 cDNAs are derived from a single germ line Jβ gene segment and to map this Jβ gene in the germ line, the 290-bp

TABLE 2. Levels of expression of T-cell-specific genes in the human T-cell tumor line HPB-MLT[a]

T-cell receptor gene	No. of positive clones/7 × 10⁴ plaques
Tiα	61
Tiβ (total)	106
Defective Tiβ (4D1)	56
Functional Tiβ (12A1)	47
Tγ	7

[a] The λgt11 cDNA library from HPB-MLT was plated at a density of 35,000 plaques per 150-mm plate and grown until subconfluent. Plaques were transferred to nitrocellulose filters and hybridized as described in Materials and Methods to the following ³²P-labeled, nick-translated probes: (i) α, a full-length human Tiα cDNA probe isolated from a library constructed from the functional human T-cell clone L17 (Leiden et al., in press); (ii) β (total), 4D1, a partial-length Tiβ cDNA clone (22); (iii) defective Tiβ, a 170-bp EcoRI/BamHI fragment from the 5' end of the V region of 4D1 (22); (iv) functional Tiβ, an 80-bp EcoRI-KpnI fragment from the 5' end of the V region of 12A1 (Figure 3); (v) Tγ, a full-length human Tγ cDNA probe isolated from a λgt11 cDNA library (13a). Each probe was hybridized to two filters containing a total of 7 × 10⁴ plaques.

(A)
```
Human Genomic    GGTTTTTGTGCGGGGCTCGGGGCCGTGAC CAA GAG ACC CAG TAC TTC GGG CCA GGC ACG CGG CTC CTG GTG CTC GGT
  Jβ2.5

2G2                                          GTA .G. .A. ... ... ... ... ... ... ... ... ... ... ... ... ...

4D1                                          .GG AGG ... ... ... ... ... ... ... ... ... ... ... ... ...

12A1                                         TCG .GG A.A ... ... ... ... ... ... ... ... ... ... ... ...

Jurβ2                                        AG. AC. ..T ..G ... ..T ..T ..C ... ... ..C G.. ..G ACA ... ...

Murine Jβ2.5                                 A.. ... ..C ... ... ... ..T ... ... ... ..T ... ... ..C ... T.A
```

(B)
```
Human Genomic        -  Q  E  T  Q  Y  F  G  P  G  T  R  L  L  V  L
  Jβ2

2G2                  V  R  .  .  .  .  .  .  .  .  .  .  .  .  .  .

4D1                  G  R  .  .  .  .  .  .  .  .  .  .  .  .  .  .

12A1                 S  R  K  .  .  .  .  .  .  .  .  .  .  .  .  .

Jurβ2                S  T  D  .  .  .  .  .  .  .  G  .  T  .  .

Murine               N  .  D  .  .  .  .  .  .  .  .  .  .  .  .  .
  Jβ2.5
```

FIG. 5. Comparison of the J-region nucleotide and deduced amino acid sequences from four human Tiβ cDNA clones and the homologous human and murine germ line Jβ 2.5 gene segments. The 12A1, 2G2, and human germ line Jβ 2.5 sequences are from this paper. The 4D1 sequence is from Jones et al. (22). The Jurβ 2 sequence is from Yoshikai et al. (45). The murine Jβ 2.5 sequence is from Malissen et al. (26). Dots represent nucleotides and amino acids which are identical to those of the human genomic Jβ 2.5 sequence. The nanomer-heptamer sequences involved in Jβ recombination are overlined (11, 26). The underlined sequence is a potential splice donor sequence. (A) Comparison of nucleotide sequences. (B) Comparison of deduced amino acid sequences.

XhoI-EcoRI fragment of the 4D1 Tiβ cDNA clone containing V-, D-, and J-region sequences was hybridized to a Southern blot of human genomic clones previously shown to contain the entire unrearranged Jβ1-Cβ1 and Jβ2-Cβ2 gene clusters (13b). This probe hybridized to a single 300-bp SmaI fragment located approximately 4 kb upstream of the Cβ2 gene (data not shown). This fragment, which has been mapped within the Jβ2 gene cluster (A. Duby and J. Seidman, unpublished data), was subsequently subjected to DNA sequence analysis (Fig. 5). A comparison of this sequence with previously published murine germ line Jβ sequences revealed that the preferentially used human Jβ2 gene segment was highly homologous to the murine Jβ2.5 germ line gene (Fig. 5). While this work was in progress, the sequence of the entire human germ line Jβ2 cluster was reported (40). A comparison of these sequences revealed that the preferentially used human Jβ2 gene reported here is identical to the human germ line Jβ2.5 gene segment. Also, while this work was in progress, the homologous murine Jβ2.5 gene segment was shown to be used in a similarly preferential fashion in murine Tiβ cDNAs isolated from a mouse thymocyte cDNA library (Jβ2.5 was used in 6 of 11 Jβ2-containing murine Tiβ cDNAs) (5).

A comparison of the J-region sequences of the 12A1, 4D1, and 2G2 cDNAs (Fig. 5) showed that although they are identical at their 3' ends, they differ, both from each other and from their germ line counterpart, by one to seven nucleotides at their 5' ends. Because these differences are contiguous and tightly clustered at the 5' end of J, we favor the hypothesis that they reflect imprecise V-D-J joining and N-region diversity events. Interestingly, there are no sequence differences in the 3' ends of the three Jβ2.5-containing cDNAs or the two genomic Jβ2.5 genes which have been sequenced, supporting the hypothesis that somatic hypermutation of genomic Jβ genes does not play a major role in the generation of Tiβ gene diversity (11).

DISCUSSION

The present findings regarding Tiβ gene rearrangement and expression are relevant to the issue of the diversity of the repertoire of human T-cell antigen receptors in both functional T-cell clones and T-cell ALLs. First, previous studies have suggested that acute T-cell leukemias display a limited repertoire of T-cell receptors. Specifically, the sequence of a Tiβ cDNA clone reportedly isolated from the MOLT-3 cell line was shown to be identical to the amino acid sequence of the Tiβ chain isolated from the T-cell tumor cell line REX, as well as to the nucleotide sequences of Tiβ cDNA clones isolated from the T-cell tumor lines JURKAT and JM (1, 36, 44, 45). In addition, Minden et al. (29) have reported that Southern blot analyses of human T-cell ALL blood samples revealed a limited number of patterns of Tiβ gene rearrangement. The finding that the MOLT-3 and MOLT-4 cell lines as well as the HPB-ALL and HPB-MLT lines are actually identical and may have been confused during prolonged in vitro culture highlights the importance of ruling out trivial cross-contamination of cell lines during in vitro culture as the cause of similar Tiβ sequences or rearrangements among different T-cell lines. This caveat is emphasized by the results of Sims et al. (36) who have shown that the YT35 cDNA clone reportedly isolated from MOLT-3 was, in fact, probably isolated from JURKAT, presumably as a result of a similar confusion during in vitro culture. Given these results, the possibility that REX and JURKAT are an identical culture must also be considered.

The present findings also demonstrate that T lymphocytes, like their B-cell counterparts, can utilize different mechanisms to produce a pattern of allelic exclusion of T-cell receptor gene expression. Some T cells rearrange a single T-cell receptor β gene (e.g., the L17 cell line), while others, such as HPB-MLT, rearrange one Tiβ gene nonproductively and a second in a productive manner. Like defectively

rearranged immunoglobulin genes, a defectively rearranged Tiβ gene can be transcribed and processed into mature mRNA, but is not expressed as protein on the cell surface. Thus far, no T-cell tumor or clone has been identified which expresses more than one functional T-cell receptor on its surface.

Previous studies of murine Tiα and Tiβ gene expression have suggested that these genes are coordinately regulated (10). The present results concerning Tiα and Tiβ and Tγ gene expression in the HPB-MLT cell line support a model in which each fully rearranged human Tiα and Tiβ gene is expressed as RNA at an equivalent level, regardless of whether a given rearrangement is productive or defective. In contrast to Tiα and Tiβ gene expression, which appear to be coordinately regulated, expression of the Tγ gene seems to be independently regulated, at least in the HPB-MLT tumor cell line. This finding is in agreement with that of Tonegawa and colleagues (18) who have reported that the level of expression of the Tγ gene is significantly lower than that of the Tiα and Tiβ genes in murine cytotoxic T-lymphocyte clones.

The finding that the same Jβ2.5 gene segment is used preferentially in both murine and human Jβ2-containing Tiβ cDNAs suggests that although these results are based upon relatively small numbers of cDNA sequences, they in fact reflect a real phenomenon which operates in a wide range of mammalian species. The mechanisms responsible for this preferential Jβ gene utilization remain obscure. They could involve structural features of this particular Jβ gene segment or, alternatively, could reflect postrearrangement selection of T cells utilizing the Jβ2.5 gene segment. Previous studies of immunoglobulin κ light-chain genes have shown that Vκ-Jκ rearrangement is also not a random process. Specifically, the 5'-most Jκ gene segments (Jκ1 and Jκ2) are used preferentially in the formation of rearranged immunoglobulin κ genes. Together, these gene segments account for a total of 70 to 90% of murine splenic B-lymphocyte Vκ-Jκ gene rearrangements (43). Similarly, at least during early B-cell development, the 3'-most V regions are preferentially used in immunoglobulin VH gene rearrangements (46). To explain these results, it has been postulated that the 5'-most Jκ genes are preferentially recognized by an enzyme which travels down the DNA in a 3' to 5' direction until it encounters a gene segment containing the appropriate 5' rearrangement signals. In this regard, it is interesting that the preferentially utilized Jβ2.5 gene segment lies quite 3' in the Jβ2 gene cluster and would therefore be unlikely to be preferentially utilized by such a rearrangement mechanism. In addition, it has been postulated that the preferential use of Jκ immunoglobulin gene segments correlates with the presence of a TG dinucleotide in the first several bases of the coding region of a Jκ gene segment (43). However, the genomic Jβ2.5 gene segment clearly lacks such a dinucleotide (Fig. 5). When taken together, these results suggest either that these structural features are not essential for either Jκ or Jβ preferential gene usage or that the mechanisms used to generate preferential J utilization in immunoglobulin genes are different from those used in Tiβ gene rearrangements.

As described above, the potential diversity of Tiβ genes is dependent both upon the numbers of Tiβ V, D, and J genes and upon the randomness of their association. The number of murine germ line Vβ genes has been estimated to be between 20 and 30 (5). There are 13 human germ line Jβ genes (six Jβ1 and seven Jβ2 gene segments) and at least two human germ line Dβ genes (40). Thus, there are potentially at least 520 (20 × 13 × 2) possible Tiβ gene combinations.

However, the present finding of preferential Jβ gene utilization indicates that more information is needed concerning preferential Vβ and Jβ gene usage both during thymic development and in mature peripheral blood T cells before meaningful estimates of Tiβ gene diversity can be made.

ACKNOWLEDGMENTS

LITERATURE CITED

1. Acuto, O., M. Fabbi, J. Smart, C. B. Poole, J. Protentis, H. D. Royer, S. F. Schlossman, and E. Reinherz. 1984. Purification and NH₂-terminal amino acid sequencing of the β subunit of a human T-cell antigen receptor. Proc. Natl. Acad. Sci. USA 81: 3851–3855.

2. Allison, J. P., B. W. McIntyre, and D. Bloch. 1982. Tumor-specific antigen on a murine T-lymphoma defined with monoclonal antibody. J. Immunol. 129:2293–2300.

3. Alt, F. W., V. Enea, A. L. Bothwell, and D. Baltimore. 1980. Activity of multiple light chain genes in murine myeloma cells producing a single, functional light chain. Cell 21:1–14.

4. Altenburger, W., M. Steinmetz, and H. G. Zauchau. 1980. Functional and non-functional joining in immunoglobulin light chain genes of a mouse myeloma. Nature (London) 287: 603–607.

5. Barth, R. K., B. S. Kim, N. C. Lan, T. Hunkapiller, N. Sobieck, A. Winoto, H. Gershenfeld, C. Okada, D. Hansburg, I. L. Weissman, and L. Hood. 1985. The murine T-cell receptor uses a limited repertoire of expressed Vβ gene segments. Nature (London) 316:517–523.

6. Bell, G., J. H. Koram, and W. J. Rutter. 1981. Polymorphic DNA region adjacent to the 5' end of the human insulin gene. Proc. Natl. Acad. Sci. USA 78:5759–5763.

7. Bernard, O., N. M. Gough, and J. Adams. 1981. Plasmacytomas with more than one immunoglobulin kappa mRNA. Proc. Natl. Acad. Sci. USA 78:5812–5816.

8. Brenner, M. B., I. S. Trowbridge, J. McLean, and J. L. Strominger. 1984. Identification of shared antigenic determinants of the putative human T lymphocyte antigen receptor. J. Exp. Med. 160:541–551.

9. Caccia, N., M. Kronenberg, D. Saxe, R. Haars, G. A. Biuas, J. Goverman, H. Malissen, H. Willard, Y. Yoshikai, M. Simon, L. Hood, and T. W. Mak. 1984. The T cell receptor β chain genes are located on chromosome 6 in mice and chromosome 7 in humans. Cell 37:1091–1099.

10. Chien, Y., D. M. Becker, T. Lindsten, M. Okomura, D. I. Cohen, and M. M. Davis. 1984. A third type of murine T-cell receptor gene. Nature (London) 312:31–35.

11. Chien, Y., R. J. Gascoigne, J. Kavaler, N. Lee, and M. M. Davis. 1984. Somatic recombination in a murine T cell receptor gene. Nature (London) 309:322–326.

12. Clark, S. P., Y. Yoshikai, S. Taylor, G. Siu, L. Hood, and T. W. Mak. 1984. Identification of a diversity segment of human T-cell receptor β chain and comparison with analogous murine element. Nature (London) 311:387–389.

13. Crouse, G. F., A. Forschauf, and T. Lehrach. 1983. An integrated and simplified approach to cloning into plasmids and single-stranded phages. Methods Enzymol. 101:78–89.

13a. Dialynas, D., C. Murre, T. Quertermous, J. Boss, J. Leiden, J. G. Seidman, and J. Strominger. 1986. Cloning and sequence analysis of complementary DNA encoding an aberrantly rearranged human T-cell γ-chain. Proc. Natl. Acad. Sci. USA 83:2619–2623.

13b. Duby, A. D., K. A. Klein, C. Murre, and J. G. Seidman. 1985. A novel mechanism of somatic rearrangement predicted by a human T-cell antigen receptor β-chain complementary DNA. Science 228:1204–1206.

13c. Duby, A. D., and J. G. Seidman. 1986. Abnormal recombination

products result from aberrant DNA rearrangement of the human T-cell antigen receptor β-chain gene. Proc. Natl. Acad. Sci. USA 83:4890–4894.

14. Early, P., and L. Hood. 1981. Allelic exclusion and non-productive immunoglobulin gene rearrangements. Cell 24:1–3.

15. Gascoigne, N., Y. Chien, D. Becker, J. Kavaler, and M. Davis. 1984. Genomic organization and sequence of T-cell receptor β-chain constant- and joining-region genes. Nature (London) 310:387–391.

16. Gubler, U., and B. J. Hoffman. 1983. A simple and very efficient method for generating cDNA libraries. Gene 25:263–269.

17. Haskins, K., R. Kubo, J. White, M. Pigeon, J. Kappler, and P. Marrack. 1983. The major histocompatibility complex-restricted antigen receptor on T cells. J. Exp. Med. 157: 1149–1169.

18. Hayday, A. C., H. Saito, S. Gillies, D. M. Kranz, G. Tanigawa, H. Eisen, and S. Tonegawa. 1985. Structure, organization and somatic rearrangement of T cell gamma genes. Cell 40:259–269.

19. Hedrick, S., D. Cohen, E. Nielsen, and M. Davis. 1984. Isolation of cDNA clones encoding T cell-specific membrane associated proteins. Nature (London) 308:149–153.

20. Hedrick, S., E. Nielsen, J. Kavaler, D. Cohen, and M. Davis. 1984. Sequence relationships between putative T-cell receptor polypeptides and immunoglobulins. Nature (London) 308: 153–158.

21. Hedrick, S. M., R. N. Germain, M. J. Bevan, M. Dorf, I. Engel, P. Fink, N. Gascoigne, E. Herber-Katz, J. Kapp, Y. Kaufman, C. Sorensen, M. Taniguchi, and M. M. Davis. 1985. Rearrangement and transcription of a T-cell receptor β chain gene in different T cell subsets. Proc. Natl. Acad. Sci. USA 82:531–535.

22. Jones, N., J. Leiden, D. Dialynas, J. Fraser, M. Clabby, K. Kishimoto, J. Strominger, D. Andrews, W. Lane, and J. Woody. 1985. Partial primary structure of the alpha and beta chains of human tumor T cell receptors. Science 227:311–314.

23. Kappler, J., R. Kubo, K. Haskins, C. Hannum, P. Marrack, M. Pigeon, B. McIntyre, J. Allison, and I. Trowbridge. 1983. The major histocompatibility complex-restricted antigen receptor on T cells in mouse and man: identification of constant and variable peptides. Cell 35:295–302.

24. Kronenberg, M., J. Goverman, R. Haars, M. Malissen, E. Kraig, L. Phillips, T. Delovitch, N. Suciu-Foca, and L. Hood. 1985. Rearrangement and transcription of the β-chain genes of the T-cell antigen receptor in different types of murine lymphocytes. Nature (London) 313:647–653.

25. Kwon, S., E. Max, J. G. Seidman, P. Leder, and M. D. Scharff. 1981. Two kappa immunoglobulin genes are expressed in the myeloma J107. Cell 26:57–66.

25a.Leiden, J. M., and J. Strominger. 1986. Generation of diversity of the β-chain of the human T-lymphocyte receptor for antigen. Proc. Natl. Acad. Sci. USA 83:4456–4460.

26. Malissen, M., K. Minard, S. Mjolsness, M. Kronenberg, J. Goverman, T. Hunkapiller, M. Prystowsky, Y. Yoshikai, F. Fitch, T. W. Mak, and L. Hood. 1984. Mouse T cell antigen receptor: structure and organization of constant and joining gene segments encoding the β polypeptide. Cell 37:1101–1110.

27. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

28. Meuer, S. C., K. A. Fitzgerald, R. E. Hussey, J. C. Hodgdon, S. F. Schlossman, and E. L. Reinherz. 1983a. Clonotypic structures involved in antigen-specific human T cell function. J. Exp. Med. 157:705–719.

29. Minden, M. D., B. Toyonaga, K. Ha, Y. Yanagi, B. Chin, E. Gelford, and T. Mak. 1985. Somatic rearrangement of T-cell antigen receptor gene in human T cell malignancies. Proc. Natl. Acad. Sci. USA 82:1224–1227.

30. Minowada, J., G. Janossy, M. F. Greaves, T. Tsubota, B. I. Srivastoca, J. Morikowa, and E. Tatsumi. 1978. Expression of an antigen associated with acute lymphoblastoic leukemia in human leukemia-lymphoma cell lines. J. Natl. Cancer Inst. 6:1269–1276.

30a.Morton, C. C., A. D. Duby, R. L. Eddy, T. B. Shows, and J. G. Seidman. 1985. Genes for β-chain of the human T-cell antigen receptor map to regions of chromosomal rearrangement in T-cells. Science 228:582–585.

31. Royer, H. D., A. Bensusson, O. Acuto, and E. Reinherz. 1984. Functional isotopes are not encoded by the constant region genes of the β subunit of the T cell receptor for antigen/major histocompatibility complex. J. Exp. Med. 160:947–952.

32. Saito, H., D. M. Kranz, Y. Takagaki, A. Hayday, H. Eisen, and S. Tonegawa. 1984. Complete primary structure of a heterodimeric T-cell receptor deduced from cDNA sequences. Nature (London) 309:757–762.

33. Saito, H., D. M. Kranz, Y. Takagaki, A. C. Hayday, H. N. Eisen, and S. Tonegawa. 1984. A third rearranged and expressed gene in a clone of cytotoxic T lymphocytes. Nature (London) 312: 36–40.

34. Sanger, F., S. Nicklen, and A. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74:5463–5467.

35. Sim, G. K., J. Yogue, J. Nelson, P. Marrack, E. Palmer, A. Augustin, and J. Kappler. 1984. Primary structure of human T cell receptor α-chain. Science 312:771–775.

36. Sims, J. E., A. Tunnacliffe, W. J. Smith, and T. H. Rabbits. 1984. Complexity of human T-cell antigen receptor β chain constant and variable region genes. Nature (London) 312: 541–545.

37. Siu, G., S. P. Clark, Y. Yoshikai, M. Malissen, Y. Yanagi, E. Strauss, T. W. Mak, and L. Hood. 1984. The human T cell antigen receptor is encoded by variable diversity and joining gene segments that rearrange to generate a complete V gene. Cell 37:393–401.

38. Siu, G., M. Kronenberg, E. Strauss, R. Haars, T. W. Mak, and L. Hood. 1984. The structure, rearrangement and expression of Dβ gene segments of the murine T-cell antigen receptor. Nature (London) 311:344–350.

39. Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503–517.

40. Toyonaga, B., Y. Yoshikai, V. Vadasz, B. Chin, and T. W. Mak. 1985. Organization and sequences of the diversity, joining, and constant region genes of the human T-cell receptor β chain. Proc. Natl. Acad. Sci. USA 82:8624–8628.

41. Ullrich, A., J. Shone, T. Chirgwin, E. Pickett, E. Tischer, W. J. Ruffer, and H. M. Goodman. 1977. Rat insulin genes: construction of plasmids containing the coding sequences. Science 197:1313–1319.

42. Vieira, J., and J. Messing. 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene 19:259–268.

43. Wood, D. L., and C. Coleclough. 1984. Different joining region J elements of the murine K immunoglobulin light chain locus are used at markedly different frequencies. Proc. Natl. Acad. Sci. USA 81:4756–4760.

44. Yanagi, Y., Y. Yoshikai, K. Leggett, S. Clark, I. Alexander, and T. Mak. 1984. A human T cell-specific cDNA clone encodes a protein having extensive homology to immunoglobulin chains. Nature (London) 308:145–149.

45. Yoshikai, Y., D. Anatoniou, S. Clark, Y. Yanagi, R. Songster, P. Von den Elsen, C. Terhorst, and T. W. Mak. 1984. Sequence and expression of transcripts of the human T-cell receptor β-chain genes. Nature (London) 312:521–524.

46. Yoncopoulos, G. D., S. V. Disiderco, M. Poskind, J. F. Kearney, D. Baltimore, and F. W. Alt. 1984. Preferential utilization of the most JH-proximal VH gene segments in pre-B-cell lines. Nature (London) 311:727–733.