geostrophic transport anomaly is only 0.6×10^6 m^3/sec per 1-cm sea level difference. For the root-mean-square amplitude of 4.2 cm of the sea level difference along line E the latter assumption would result in a root-mean-square transport fluctuation of 2.5×10^6 m^3/sec, still larger by a factor of 2 than our Florida Current transport estimate, whereas for a barotropic velocity assumption this value would be about ten times as much.

One has to bear in mind here that this transport fluctuation can only show the net geostrophic flow between Tenerife and San Juan, not any recirculation of southward flow in midbasin within line E east of Puerto Rico.

Time series anomalies of Sverdrup transport between Africa and the western boundary, again low-passed with an 18-month cutoff period, are given in Fig. 2b for 30°N and 20°N. They are calculated from the monthly means of wind stresses of Bunker and Goldsmith (*22*) for a 10° by 10° grid. Despite this rather coarse resolution, the integrated wind stress curl fields agree reasonably well with the finer resolution curls from Hellerman and Rosenstein (*15*), at least at the annual period (Fig. 1d). Hence, the time series for transports across 20°N and 30°N as shown in Fig. 2b should give an idea of the magnitude of interannual variations. Root-mean-square amplitudes are 4.8×10^6 m^3/sec at 30°N and 2.1×10^6 m^3/sec at 20°N. This is the same order of magnitude as the geostrophic transport estimate across line E (Fig. 1a) that one would attain from sea level differences (Fig. 2a), assuming the baroclinic profile, but correlation between the Sverdrup transports (or their geostrophic component after elimination of the Ekman transport) and sea level differences is not significant. Even if wind forcing were the only factor causing this variability with periods of a few years, the lack of correlation would not be unexpected. Although at the annual period the response is mostly barotropic and therefore generates a western boundary current immediately, Anderson and Corry (*17*) have estimated that it would take about a decade to establish Sverdrup equilibrium in a basin of the scale of the subtropical North Atlantic because of the slow propagation speed of the baroclinic Rossby wave. This conclusion is also supported by a recent numerical model calculation with a six-layer model, 2° resolution, driven by the winds of Bunker and Goldsmith (*22*) in which Willebrand and Olbers (*23*) obtained a small interannual variation of the western boundary current transport that was not correlated with the Sverdrup transport of Fig. 2b. In an earlier study with a barotropic model and winds of Bunker and Goldsmith (*22*), Willebrand *et al.* (*24*) showed for a time segment of several years that the resulting western boundary current transport closely resembled the Sverdrup transport across 40°N.

Another mechanism to be considered is thermohaline forcing, which drives a meridional circulation cell at 24°N of about 15×10^6 m^3/sec of warm water going north above 1000 m and returning at depth (*25*). The variability of this vertical gyre on the interannual time scale is not known, except that transports and heat flux in 1957 (the International Geophysical Year) and 1981 as determined by inverse methods (*25*) were very similar; this similarity could have been a coincidence. Modeling with interannual thermohaline-forcing anomalies is needed, but the data base is much sparser and more doubtful than for the wind fields.

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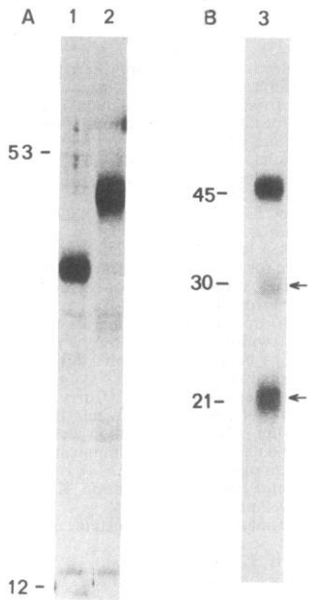
Partial Primary Structure of the Alpha and Beta Chains of Human Tumor T-Cell Receptors

Abstract. *The T-cell receptor for antigen (Ti) was purified from the human tumor cell line HPB-ALL. Amino-terminal sequence analysis of an acid-cleaved peptide of the Tiα chain showed that it is highly homologous to a putative murine α chain recently described. Amino-terminal sequence analysis of the Tiβ chain revealed that it shares 50 percent homology with the Tiβ chain amino acid sequences from two other human T-cell tumors. Nucleotide sequence analysis of a complementary DNA clone encoding the Tiβ chain from the HPB-MLT cell line showed that this chain represents a second human constant region gene segment and suggested that it arises from direct joining of the variable and joining gene segments without any intervening D region sequences.*

The T-cell receptor for antigen (Ti) has been serologically and biochemically identified as a 90-kilodalton (kD) heterodimer composed of disulfide-linked acidic (α) and basic (β) glycoprotein chains of approximately 40 to 50 kD (*1–3*). Using differential hybridization techniques, two groups of investigators have identified a T-cell specific complementary DNA (cDNA) clone with a deduced amino acid sequence that is homologous to the amino-terminal sequence of the β chain of Ti (*4–6*). This gene, which shows significant homology to immunoglobulin genes, is composed of variable (V), diversity (D), joining (J), and constant (C) region segments that undergo specific rearrangements during T-cell ontogeny (*4–5, 7–11*). Analysis of the murine genomic Tiβ gene, has revealed the existence of two constant region gene segments ($C_{\beta 1}$ and $C_{\beta 2}$) which are more



A

HPB-ALL β: X Val Thr Gln Ser Pro Thr His Leu Ile Lys Thr Arg Gly Gln His Val Thr

REX β : X Val Ile Gln Ser Pro Arg His Glu Val Thr Glu X Gly X Glu Val Thr

MOLT-3 β : Gly Val Ile Gln Ser Pro Arg His Glu Val Thr Glu Met Gly Gln Glu Val Thr

B

HPB-ALL α: (Asp)Pro Ala X Tyr Gln Leu Arg Asp Ser Lys Ser

MURINE Cα: Glu Pro Ala Val Tyr Gln Leu Lys Asp Pro Arg Ser

Fig. 1 (left). (A) Separated subunits for Ti after SDS-PAGE and electroelution. Purified Ti, containing radioactive tracer Ti, was applied to a preparative 10 percent polyacrylamide gel under reducing conditions. Gel pieces were counted and those containing α or β subunits were electroeluted. One-half percent of the eluted material was analyzed on a 10 percent gel by a silver staining method (*17*). The separated subunits are Tiβ chain (lane 1) and Tiα (lane 2). (B) Acid cleavage of ^{125}I-labeled Tiα chain. Tiα chain was treated with 70 percent formic acid for 32 hours at 37°C (*18*). The acid hydrolyzed protein was lyophilized and then resolved on a 13 percent polyacrylamide gel (lane 3). The arrows indicate the two cleavage products generated from Tiα. Standards for each gel are indicated by the numbers to the left of each gel. Fig. 2 (right). (A) Comparison of NH_2-terminal amino acid sequences of the Tiβ chain from three human T-cell tumors. The first sequence was obtained from the β chain isolated from HPB-ALL cells; the second was obtained from protein isolated from the tumor line REX (*6*); the third sequence was deduced from the nucleotide sequence of the β chain from MOLT-3 (*4*). An underlined amino acid in the HPB-ALL sequence indicates one that is identical to one at the same position in the sequence of REX and MOLT-3. An "X" indicates an unassigned amino acid. (B) Comparison of the amino acid sequence from the acid fragment of HPB-ALL Tiα chain (first sequence) with that deduced from the nucleotide sequence from the C region of a putative murine Tiα chain gene (*13*). The "Asp" in parentheses is assumed since acid preferentially cleaves proteins at Asp-Pro bonds. The unassigned "X" at residue 4 in the HPB-ALL α chain sequence was Val in two sequencing runs but appeared to Glu in a third.

than 95 percent homologous (*9–10*). More recently, using similar subtractive hybridization techniques, several groups have identified two different candidates for the murine Tiα chain gene (*12–13*). Both of these murine genes are specifically expressed in T cells and rearrange during T-cell ontogeny.

We report here partial amino acid sequence data for both the α and β chains of Ti from the T3+, T4+, T8+ human T-cell tumor line HPB-ALL. Comparison of our Tiα chain amino acid sequence with those deduced from putative α chain genes allows identification of the "bona fide" murine α chain gene. In addition, we have cloned the cDNA encoding the Tiβ chain from the T3+, T4+, T8+ human T-cell tumor line HPB-MLT. Sequence analysis of this cDNA shows that it is encoded by a second Cβ gene ($C_{\beta 2}$) which is highly homologous to the previously described $C_{\beta 1}$ gene expressed in the T-cell tumor MOLT-3 (*4*). In addition, this clone appears to result from direct joining of the V, J, and C region gene segments without the presence of D region sequences. However, because of a frameshift mutation occurring at the V-J junction, this clone appears to encode a nonfunctional Tiβ chain protein.

The T-cell receptor for antigen was purified in large quantities from HPB-ALL solubilized membranes by immunoaffinity chromatography over a column of Sepharose-linked T40/25, a monoclonal antibody (*14*) which recognizes the Ti of HPB-ALL and HPB-MLT (*15*). These cell lines express identical surface phenotypes when tested with both T cell–specific and HLA reagents. Approximately 1 μg of purified Ti was obtained from 1×10^9 cells. After reduction, the two chains of the receptor were separated by SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) and eluted from the gel as described (*16*); the relative purity of the separated α and β subunits of Ti was determined by SDS-PAGE and a silver staining method (*17*) (Fig. 1A). The separated α and β chains were then subjected to NH_2-terminal amino acid sequence analysis on a gas phase sequenator (Applied Biosystems model 470A). Comparison of the Tiβ chain from HPB-ALL and the Tiβ chains from REX and MOLT-3 (Fig. 2A), which were previously shown to be identical at their amino terminal (*4, 6*), indicates ~50 percent homology. This finding suggests that MOLT-3 and REX share a common V region gene segment, while HPB-ALL has a homologous yet distinct V region segment.

Amino-terminal sequence analysis of the purified Tiα chain from HPB-ALL revealed that it has a blocked NH_2-terminus. Therefore, the purified α chain was subjected to formic acid hydrolysis (*18*). The resulting fragments of approximately equal size, 28 and 22 kD (Fig. 1B), were subjected to NH_2-terminal sequence analysis. Because the native NH_2-terminus of the molecule remains blocked, such an analysis yields only a single set of internal sequence data. As shown in Fig. 2B, 7 of 11 amino acids of the HPB-ALL α chain are identical to those deduced from a portion of the sequence at the beginning of the C region of a putative murine Tiα chain cDNA clone obtained by Chien *et al.* (*13*). Moreover, of the four nonhomologous amino acids, three are very highly conservative substitutions (Asp for Glu, Arg for Lys, and Lys for Arg). This degree of homology is similar to that reported for the C regions of the murine and human Tiβ constant region gene segments (*4, 5*). There is no apparent homology between the Tiα amino acid sequence shown in Fig. 2B and that deduced from the nucleotide sequence of the putative murine Tiα reported by Saito *et al.* (*12*).

For a more complete study of the structure of the Tiβ chain gene, a cDNA library was constructed from the HPB-MLT cell line in λgt 10 and probed by hybridization with a human Tiβ cDNA clone from JURKAT cells (*19*). Approximately 0.04 percent of the clones hybridized to this probe. A single positive clone, 4D1, was plaque-purified by sequential hybridization to the JURKAT cDNA probe and subsequently subcloned into the vectors pBR322, M13mp18, and M13mp19 for restriction enzyme and sequence analysis by the dideoxy method (*20*). The nucleotide and deduced amino acid sequences of this partial length clone (Fig. 3) were compared to those of the previously reported Tiβ sequence from a human T-cell tu-

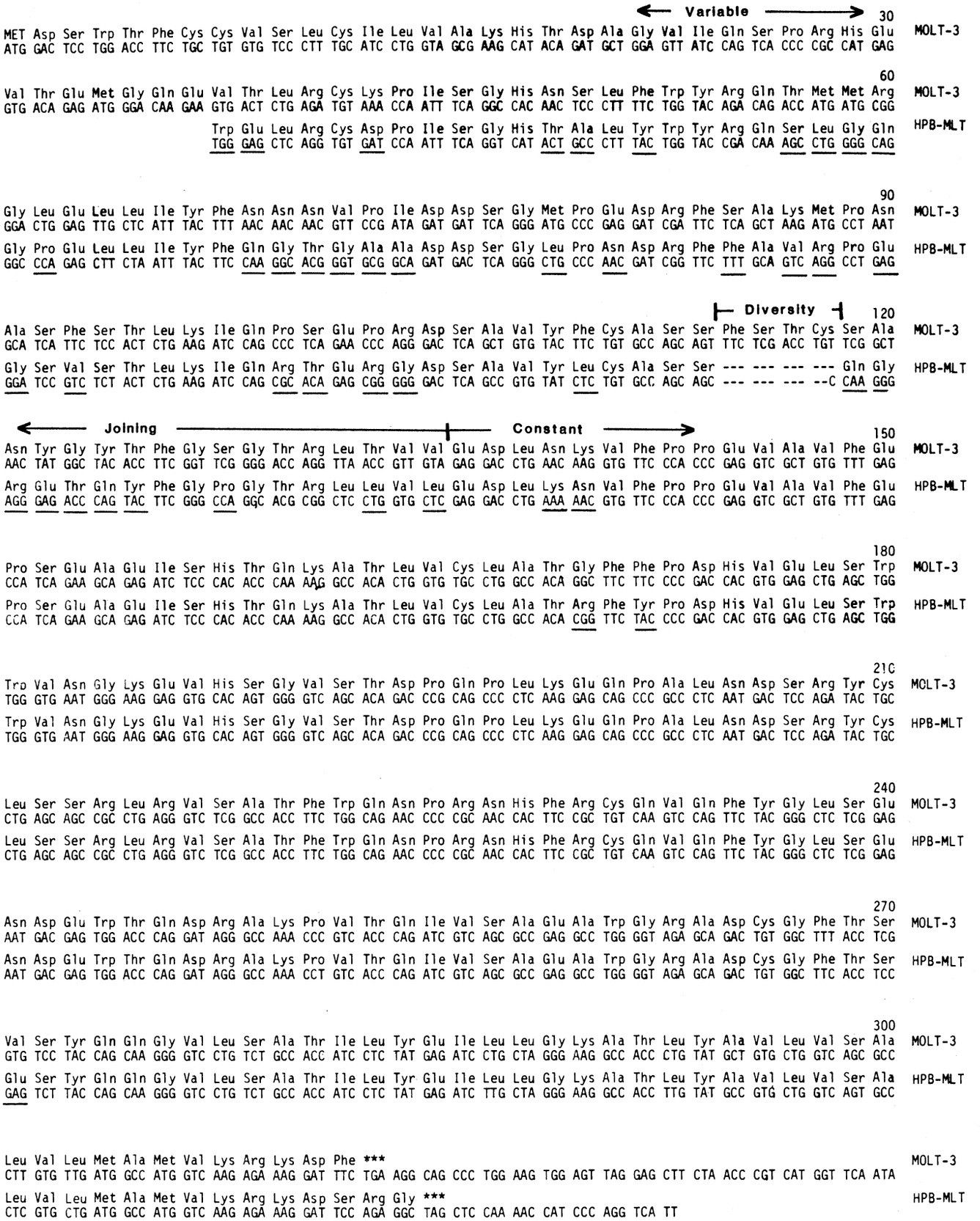


Fig. 3. Nucleotide and deduced amino acid sequences of the Tiβ chain genes from HPB-MLT and MOLT-3. Eco RI–Bam HI, Eco RI–Bgl II and Bgl II restriction enzyme fragments of 4D1 were subcloned into M13mp18 and M13mp19 and sequenced using the dideoxy method of Sanger *et al.* (*20*). The MOLT-3 sequence is from Yanagi *et al.* (*4*). The proper reading frame was inferred from comparison to that of the MOLT-3 Tiβ cDNA. An underlined amino acid in the HPB-MLT sequence indicates one that differs from one at the same position in the MOLT-3 sequence.

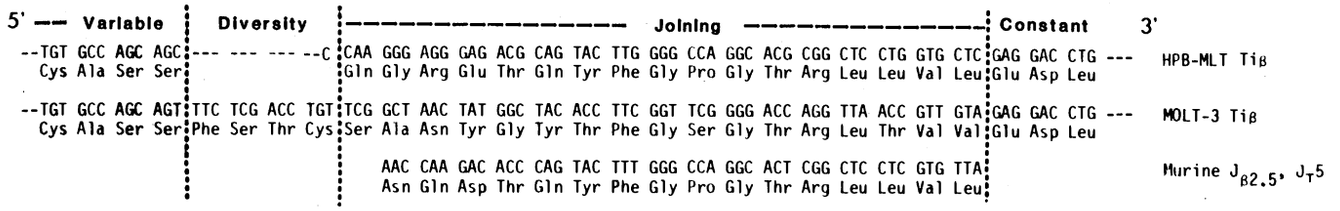


Fig. 4. V-J-C joining in the Tiβ gene of HPB-MLT. The HPB-MLT Tiβ nucleotide sequence was obtained as described in the legend to Fig. 3. The MOLT-3 Tiβ sequence is that determined by Yanagi *et al.* (*4*). The $J_{\beta 2.5}$ or $J_T 5$ sequences, which are identical, are those determined by Malissen *et al.* (*10*) and Chien *et al.* (*11*). The boundaries of V, D, and J regions were inferred by analogy to their murine counterparts (*10, 11*).

mor, MOLT-3 (*4*). This comparison showed that the 4D1 constant region sequence differs from that of MOLT-3 by approximately 3 percent (6 of 177 amino acids and 17 of 351 nucleotides). In addition the carboxyl terminus (the presumed cytoplasmic domain) of the Tiβ chain of HPB-MLT is two amino acids longer than that of MOLT-3. This amount of divergence is more than expected of an allele, and suggests the existence of two highly homologous but distinct human C_β gene segments. These results are analogous to those of Gascoigne *et al.* (*9*) who identified two C region gene segments in the mouse differing by only 4 of 173 amino acids. While all of the murine amino acid substitutions occur at the 3′ end of the molecule, in the human genes amino acid substitutions occur at both the 3′ and 5′ ends of the C regions. As suggested by Gascoigne *et al.*, this difference could be due to a recent gene conversion event in the mouse which has not taken place in the human (*9*). Overall, the V region segments of the two different β chain genes show 60 percent nucleotide homology. However, there is a highly conserved 80-base-pair (bp) region of 84 percent nucleotide homology located at the 3′ end of the V segments of these two genes.

In the V-J-C joining region of clone 4D1 (Fig. 4), the 3′ ends of the V regions of HPB-MLT and MOLT-3 Tiβ chains are highly homologous (see above). Interestingly, the J region of the human 4D1 clone is very highly homologous to the previously reported murine genomic J region, $J_{\beta 2.5}$ or $J_T 5$ (*10–11*). If we align the homologous V, J, and C regions of the Tiβ chains of 4D1, MOLT-3, and $J_{\beta 2.5}$, it is apparent that 4D1 lacks the four amino acids that correspond to the D region of the Tiβ gene of MOLT-3. This suggests that 4D1 arose by direct joining of germ line V, J, and C gene segments with concomitant deletion of all D region sequences. Formal proof of this direct V-J joining requires sequencing of the germ line genomic V, J, and C region genes used in the 4D1 gene rearrangements. However, it should be noted that the possibility of such direct V-J-C joining has been suggested on theoretical grounds by Malissen *et al.* (*10*) and Chien *et al.* (*11*). Their analyses of murine genomic Tiβ sequences showed that the nonconserved spacer sequences surrounding the V, D, J, and C gene segments are ordered in a V–23bp-12 bp–D–23bp-12bp–J arrangement. According to the rules that govern immunoglobulin gene rearrangements (*21, 22*), this arrangement should allow for direct V-J-C as well as D-D joining during the formation of the Tiβ chain gene. In addition to the probable lack of D region sequences in 4D1, there is a single nucleotide insertion between the V and J regions of 4D1 (Fig. 4) which causes a frameshift in J and C region sequences downstream. This frameshift, in turn, causes the generation of two stop codons in the C region of 4D1. Because both strands of this region of 4D1 have been sequenced numerous times, a sequencing error is unlikely to be the source of this frame shift. Instead 4D1 may represent a nonfunctional cDNA caused by faulty V-J joining. Such nontranslatable β gene clones have been found in a murine thymocyte library and have been postulated to arise during the early stages of thymocyte differentiation (*5*). In this regard it is noteworthy that HPB-MLT expresses the early thymocyte T4+, T6+, T8+ phenotype. Additional Tiβ cDNA clones from this cell line are being sequenced in an attempt to define a second Tiβ chain allele which is expressed on the surface of HPB-MLT.

In summary, we have presented amino acid sequence analysis of the α chain of the human T-cell receptor for antigen which by sequence homology confirms the identity of the putative Tiα chain gene recently cloned by Davis and colleagues. In addition, we have determined the nucleotide sequence of a second human Tiβ constant region gene segment and have demonstrated that direct V-J-C joining can occur in the formation of the Tiβ chain gene during T-cell ontogeny.

Note added in proof: While this report was in press, Saito *et al.* (*23*) reported the cloning of a murine gene which also appears to encode the Tiα chain and Hannum *et al.* (*24*) reported amino acid sequences of several peptides of the Tiα chain from HPB-ALL.

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