A third rearranged and expressed gene in a clone of cytotoxic T lymphocytes

Haruo Saito, David M. Kranz, Yohtaroh Takagaki, Adrian C. Hayday, Herman N. Eisen & Susumu Tonegawa

Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

In addition to the two previously identified genes rearranged and expressed in a cytotoxic T-lymphocyte clone, we have identified a third gene that is also rearranged and expressed in the same clone. This new gene shows clonal diversity, codes for a polypeptide chain that contains immunoglobulin-like variable and constant domains, carries potential N*-glycosylation sites and is a particularly attractive candidate for the gene that encodes the α-subunit of the heterodimeric antigen receptor of this T-cell clone.*

IN spite of its central roles in both regulatory and effector phases of the vertebrate cellular immune system, the chemical nature of the T-cell antigen receptor has been elusive[1]. A particularly intriguing question about this receptor concerns the molecular basis for its dual recognition of antigens in conjunction with self major histocompatibility complex (MHC) products[2-4]. Unlike immunoglobulins, which recognize and bind free antigens, the T-cell antigen receptors characteristically recognize cell-bound antigens in the specific molecular context of self-MHC molecules. Recently, several groups have succeeded in making antisera and monoclonal antibodies that are specific for individual T-cell clones[5-7] and which presumably recognize clone-specific determinants on the T-cell antigen receptors. The receptor thus characterized is a heterodimeric glycoprotein of apparent relative molecular mass (M_r) ~90,000, consisting of an α-subunit (M_r 42,000-44,000) and a β-subunit (M_r 42,000-44,000) held together by an inter-chain disulphide bond(s). Peptide fingerprint analyses of α- and β-chains from several T-cell lines suggest that both subunits are composed of variable (V) and constant (C) regions[8-10].

During the past year, three groups have isolated cDNA clones [YT35 (ref. 11), TM86 (ref. 12), pHDS11 (ref. 13) and pHDS4/203 (ref. 13)] that probably correspond to T-cell receptor genes. As YT35, TM86 and pHDS11 from a human T-cell tumour, a mouse T helper hybridoma and a mouse cytotoxic T lymphocyte (CTL) clone, respectively, are all extensively homologous to each other at the 3′ half, it was concluded that they correspond to the same gene. Recently, partial amino acid sequences determined by direct analysis of the β-chain of a human T-cell receptor showed that YT35, TM86 and pHDS11 represent the gene for this subunit[14]. Although there is no extensive nucleotide homology between the fourth clone, pHDS4/203, and the other three cDNA clones, all share several common properties. (1) They are expressed in T cells but not in B cells. (2) The corresponding genes are rearranged in T cells and not in B cells. (3) In the encoded proteins there are distinctive regions that, proceeding from amino- to carboxy-terminus, correspond to a signal peptide, two immunoglobulin-like domains, a transmembrane peptide rich in hydrophobic amino acids and a short cytoplasmic peptide. (4) Their deduced amino acid sequences have low but significant homology to those of immunoglobulin chains. (5) Each gene is composed of separate *V*, *J* and *C* gene segments[15-18] (in the cases of YT35 and TM86, the corresponding genes have also been shown to have *D* segments)[19,20]. (6) Their *V*, (*D*) and *J* segments have characteristic signal sequences for gene rearrangement (heptamer and nonamer separated by either 12 or 23 base pairs (bp); refs 15, 16, 18-20 and A. Hayday *et al.*, unpublished data).

The cDNA clone pHDS4/203 therefore satisfied many of the properties expected for the α-chain of the T-cell antigen receptor. However, this clone lacks sequences that correspond to sites for *N*-linked glycosylation and it has recently been shown that both the α-chain and the β-chain of another type of T cell, helper cells, are *N*-glycosylated (J. P. Allison, personal communication). Although it was possible that the α-chain of the CTL clone from which pHDS4/203 was isolated is not *N*-glycosylated, it also seemed possible that there is still another gene (or genes) whose properties are similarly consistent with those expected for the α-subunit of the T-cell receptor. Accord-

Table 1 T cell-specific clones from a subtracted CTL cDNA library

Group	Size of mRNA (kb)	No. of clones	Rearrangement	Representative clone
T (thyl)	2.0	19	No	pHDS1
B	0.8	19	No	
C(β)	1.4	18	Yes	pHDS11
F	1.7	13	No	
E	0.8	12	No	
D	1.7	9	Yes	pHDS58
K	1.3/1.1	9	No	
G	1.2	8	No	
A	1.5	4	Yes	pHDS4/203
P	2.3	2	No	
Q	n.d.	2	No	
I	2.2	1	No	
J	1.0	1	No	
L	1.5	1	No	
N	4.4	1	No	
R	0.8	1	Maybe	pHDS86
Total		120		

Two hybridization probes were used to screen the cDNA library. The first was the 2C cDNA prepared from the poly(A)$^+$ RNA of membrane-bound polysomes by subtraction with poly(A)$^+$ RNA from a B-cell lymphoma, CH-1. The second was the cDNA prepared from the total poly(A)$^+$ RNA from another B-cell lymphoma A20-2J. After screening about 20,000 independent transformants, we have identified a total of 140 putative T cell-specific cDNA clones and grouped these clones on the basis of the corresponding genes, using the following procedures. First, plasmid DNA preparations from each of 20 randomly chosen cDNA clones were used as hybridization probes in RNA blotting analysis of 2C poly(A)$^+$ RNA and DNA blotting analysis of 2C and embryo DNA. The hybridization patterns allowed assignment of these clones to 12 groups. Second, mouse DNA inserts were dissected from a representative cDNA clone of each group and used as probes for colony hybridization of all of the cDNA clones; the results allowed assignment of 105 clones among the 12 groups. The above procedures were repeated for some of the remaining unassigned clones.

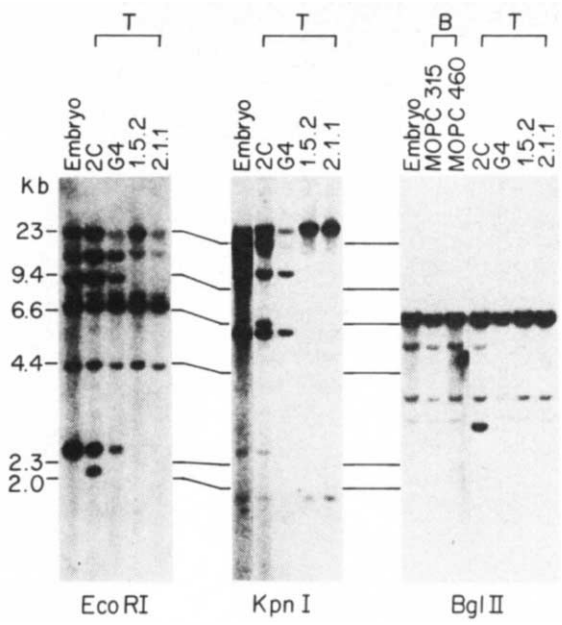

Fig. 1 Southern blot analyses[22] of DNA from BALB/c embryo, myelomas MOPC315 and MOPC460 (both BALB/c-derived) and CTL lines 2C, G4, 1.5.2 and 2.1.1 (all BALB.B-derived[21]). DNA was digested with the indicated restriction enzymes, electrophoresed through 0.8% agarose gels, blotted onto nitrocellulose and hybridized with the ^{32}P-labelled, nick-translated insert from clone pHDS58. Hybridization was carried out at 42 °C in 50% formamide and 5×SSC. The filters were washed at 65 °C in 0.2× SSC. Separate experiments showed that BALB/c and BALB.B embryo patterns are indistinguishable. Numbers on the left-hand side are M_r markers.

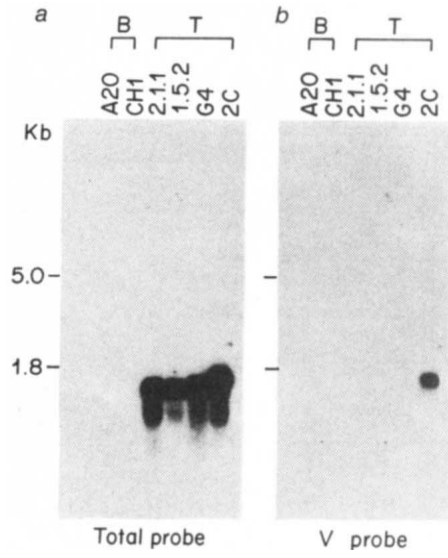

Fig. 2 RNA blot analyses[23] of poly(A)$^+$ RNA from various B- and T-cell lines. Probes: *a*, total insert of pHDS58; *b*, *Hpa*II fragment containing 270 bp at the end of the pHDS58 insert (*V*-region probe). RNA was extracted from B-cell lymphomas A20-2J and CH1 and alloreactive (H-2^b anti-H-2^d) cytotoxic T-lymphocyte clones 2.1.1, 1.5.2, G4 and 2C. Approximately 1.5 μg of poly(A)$^+$ RNA was denatured with glyoxal and electrophoresed through a 1% agarose gel in 10 mM sodium phosphate buffer (*p*H 6.5). RNA was transferred to a nitrocellulose membrane and hybridized to ^{32}P-labelled nick-translated probe DNAs. The conditions of hybridization and washing are given in Fig. 1 legend. The filter used for hybridization with the first probe was washed by boiling at 100 °C for 5 min in H_2O and re-used for hybridization with the second probe.

ingly, we have analysed further the cDNA library from CTL clone 2C and report here the isolation of a third class of cDNA clone representing a gene whose protein product is a better candidate for the α-subunit of the T-cell αβ heterodimer receptor than is pHDS4/203: the new gene is rearranged and expressed specifically in T cells, its product has the sequences expected of an integral membrane protein and it carries potential *N*-glycosylation sites.

Classification of cDNA clones

We reported previously[13] construction of a 'subtracted' cDNA library from an alloreactive CTL clone (2C) specific for the product of a gene at the *D* end of the *H-2* complex[21]. We subjected this library to differential screening using two hybridization probes (Table 1). From these experiments, it was established that 120 of 140 putative T cell-specific cDNA clones originally identified, belonging to 16 different groups, are expressed specifically in CTL clone 2C and not in B cells. Table 1 lists the number of cDNA clones in each group and the size of poly(A)$^+$ RNA they detected by Northern hybridization. Of the remaining 20 cDNA clones, 12 belonged to two gene groups whose expression in B cells was substantially lower than in T cells but still definitely detectable. The other eight cDNA clones contained relatively short inserts (less than 250 bp) and have not yet been characterized.

Among the 16 T cell-specific cDNA groups, we identified four whose genes seemed to be rearranged in CTL clones. Two (pHDS11 and pHDS4/203) of the four have been described previously[13]. The characteristics of the third group, represented by pHDS58, are the main subject of the present report. The fourth cDNA, represented by one member, pHDS86, hybridized to an extra DNA fragment when *Pvu*II digests of clone 2C DNA and embryo DNA were compared; however, nine other restriction enzymes failed to show any evidence of rearrangement (data not shown).

Gene rearrangement in T cells

Figure 1 shows the results of Southern blot analyses[22] of DNA from BALB/c embryos, four CTL clones of different specificities and two myelomas that have been digested with *Eco*RI, *Kpn*I or *Bgl*II and hybridized with the insert of pHDS58. With each of the three enzymes, clone 2C DNA showed an extra fragment that was not detected in the digest of embryo DNA. DNA from the other CTL clones gave hybridization patterns which lacked several fragments detected in embryo DNA. In contrast, the hybridization patterns of DNA from the myelomas and embryos were indistinguishable. These results strongly suggest that the gene or genes corresponding to clone pHDS58 are rearranged in cytotoxic T cells but not in myelomas.

The expression of the pHDS58 genes in CTL clones and B lymphomas was examined by Northern hybridization analyses[23] of poly(A)$^+$ RNA. When the whole insert of pHDS58 was used as the hybridization probe, no hybridization was detected in the two B-cell lymphomas, but poly(A)$^+$ RNAs of distinct sizes were detected in all four CTL clones (Fig. 2*a*). The size of the major RNA species in 2C (1.7 kilobases, kb) was slightly greater than that in the other three CTL clones (1.6 kb). In addition, minor RNA species of about 1.2 kb were detected in all four CTL clones. When a DNA fragment containing 270 bp from the 5′ end of the pHDS58 insert was used as the hybridization probe, only the 1.7-kb RNA species of 2C was detected and the other three CTL clones and the B-cell lymphomas exhibited no hybridization (Fig. 2*b*; see Fig. 3 for the region of the cDNA sequence covered by the probe). These results suggest that the 1.6–1.7-kb RNA is composed of a common 3′ sequence (*C* region) and a CTL clone-specific 5′ sequence (*V* region), as has been seen with immunoglobulin and the T-cell receptor β-chain.

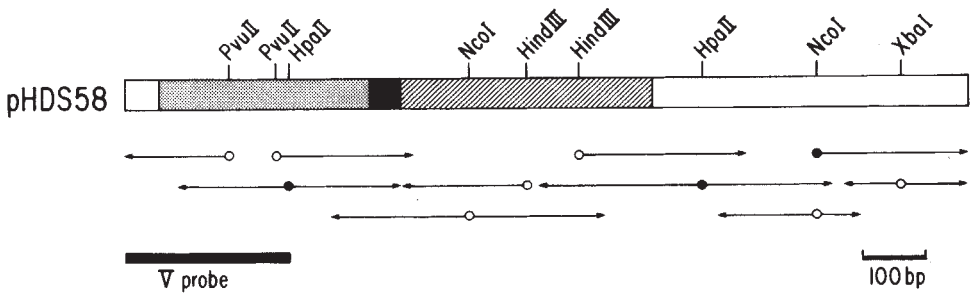

Fig. 3 Restriction map of the insert of cDNA clone pHDS58. The map was constructed by a combination of single and double restriction enzyme digestions of the plasmid DNA. The *V*, *J*, *C* and 5′- or 3′-untranslated regions are shown by shading, black, hatching and white, respectively. Also shown is the sequencing strategy used to determine the nucleotide sequence shown in Fig. 4. The arrows indicate the directions and the extent of sequence determination. The open and closed circles indicate that the ends of the DNA fragments labelled are 5′ and 3′, respectively.

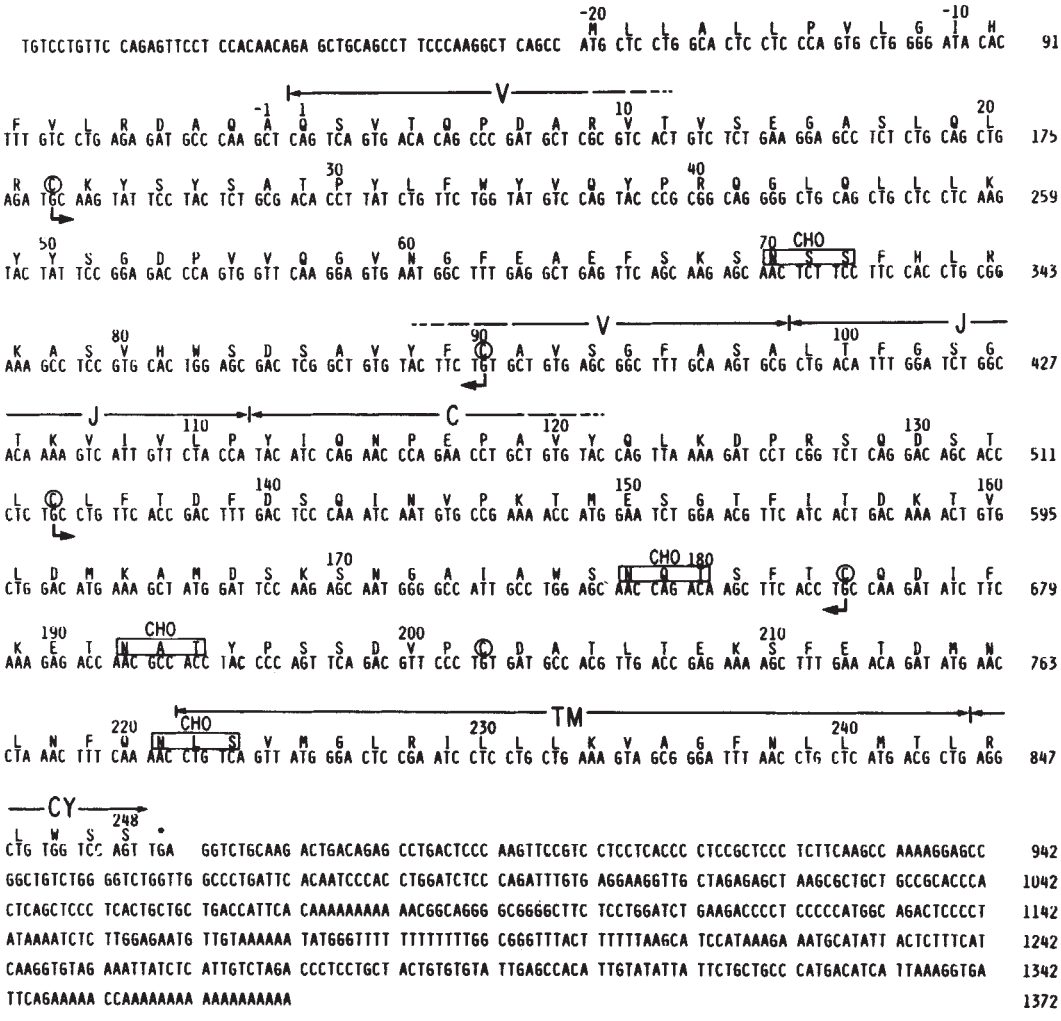

Fig. 4 The nucleotide and predicted amino acid sequences of the cDNA clone pHDS58. The nucleotide sequence was determined by the method of Maxam and Gilbert[24] according to the strategy shown in Fig. 3. Numbers given above the amino acid sequence designate amino acid residue positions. The numbers on the right show nucleotide positions. The *V*, *J*, *C*, TM (transmembrane) and CY (cytoplasmic) regions are indicated by horizontal arrows although exact boundaries are ambiguous. Cysteine residues thought to be involved in intradomain or inter-chain disulphide linkages are encircled. The potential *N*-glycosylation sites (N-X-S or N-X-T) are also indicated.

Nucleotide sequence

The restriction map and the sequencing strategy of the pHDS58 insert are shown in Fig. 3. The entire nucleotide sequence of 1,372 bp determined by the method of Maxam and Gilbert[24] is shown in Fig. 4. The longest open reading frame begins with the Met codon at nucleotide positions 56–58, extends over a stretch of 804 bp, and ends at nucleotide position 859. The amino acid sequence corresponding to this reading frame is also shown in Fig. 4. For reasons given below, the numbering of codons or amino acid residues starts with the triplet CAG (Gln) at nucleotide positions 116–118. The amino acid sequence immediately following the Met and extending to the Gln is highly hydrophobic and reminiscent of a signal peptide[25]. After the Gln, the sequence is significantly homologous to those of the T-cell

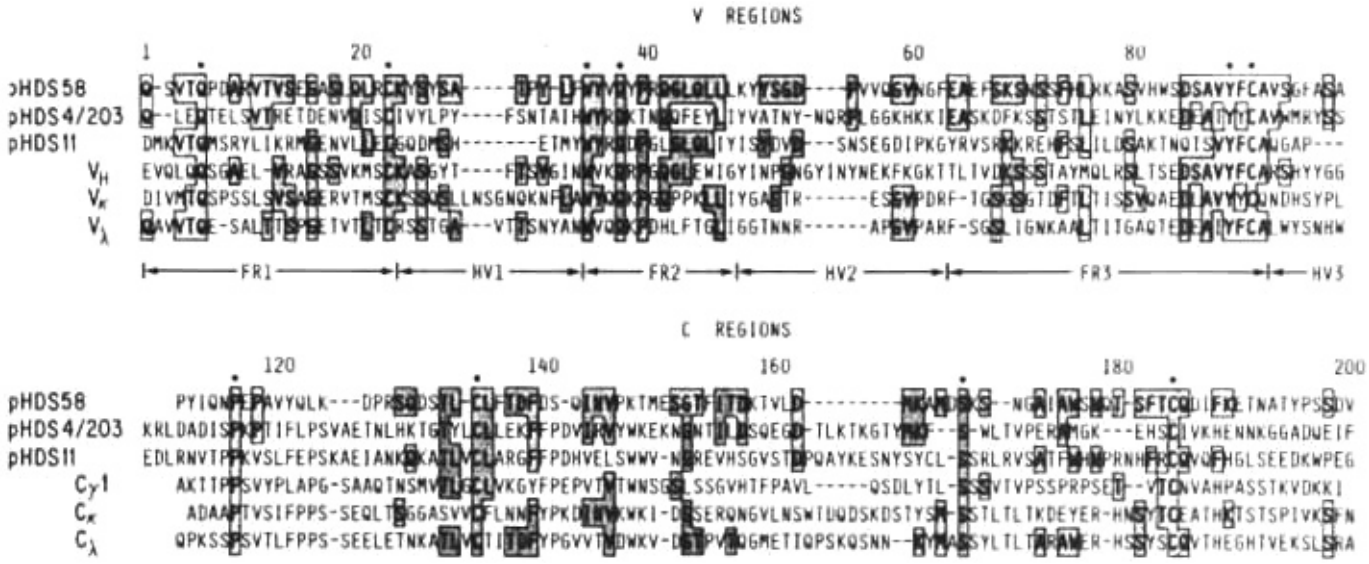

Fig. 5 Comparisons of the *V* and *C* regions of the predicted amino acid sequence of pHDS58 and the *V* and *C* regions, respectively, of the predicted or determined amino acid sequences of five other polypeptide chains: a chain encoded by pHDS4/203, the β-chain encoded by pHDS11 (ref. 13), 93G7 γ1 immunoglobulin heavy chain, MOPC603 κ light chain, and MOPC104E λ1 light chain[26]. Those residues common between the pHDS58 protein and any one of the other five chains are boxed. *, The residues common among all six chains. Approximate boundaries of framework and hypervariable regions as they appear in immunoglobulin V regions are indicated by horizontal arrows. The amino acid positions refer to those of pHDS58 protein.

receptor β-chain deduced from pHDS11 and the polypeptide chain encoded by pHDS4/203, as well as to the sequences of an immunoglobulin heavy chain (93G7), an immunoglobulin κ light chain (MOPC603) and an immunoglobulin λ1 light chain (MOPC104E)[26].

The homology is shown in Figs 5 and 6, where the pHDS58 amino acid sequence is compared with the V (or V+D), J and C region sequences of the other five polypeptide chains. Sequence homology is evident in all three regions, but is most striking in the J region, where it amounts to 38–62%. In the V regions, the pHDS58 sequence is related to the other five sequences by 22–29% homology. The six amino acid residues that are conserved in this region among pHDS11, pHDS4/203 and the three types of immunoglobulin chains (Gln, Cys, Trp, Gln, Tyr and Cys at positions 5, 22, 34, 37, 88 and 90, respectively) are also shared by pHDS58. The sequence homology is least in the C regions, where it ranges over 12–20%. Nevertheless, the relatedness is evident around the two Cys residues that form intradomain disulphide bonds in immunoglobulin chains. Throughout the V, J and C regions, the pHDS58 sequence, like the pHDS11 and pHDS4/203 sequences, is more homologous to the light chains (25% and 27% for κ and λ, respectively) than to the heavy chain (23%).

Beyond the C region, the pHDS58 polypeptide chain exhibits no obvious sequence homology to the corresponding regions of the β-chain (pHDS11) or the chain encoded by pHDS4/203. Nevertheless, the three polypeptide chains are organized in a very similar fashion in these regions. Thus, as in the pHDS11 and pHDS4/203 chains, the C region of the pHDS58 chain is followed by a peptide carrying an extra cysteine, then by a stretch of about 20 hydrophobic residues that could correspond to a transmembrane peptide and finally by a short hydrophilic C-terminal peptide that presumably extrudes into the intracytoplasmic space.

In Fig. 4 these regions of pHDS58 are indicated by horizontal arrows. The exact boundaries of a few adjacent regions are somewhat uncertain. For instance, it is not possible to determine unambiguously the N-terminus of the processed protein from the nucleotide sequence of the cDNA alone. However, the Gln at nucleotide positions 116–118 is the best candidate for the following reasons. First, the proposed assignment places the first Cys residue at position 22, while the corresponding Cys residues of most immunoglobulin chains are at position 22 or 23 (ref. 26). Second, most immunoglobulin chains carry a signal peptide 19–22 residues long[26]; the present assignment makes the proposed signal peptide 20 residues long. Finally, the pHDS58 chain is an excellent candidate for the α-subunit of the T-cell receptor, whose N-terminal residue has recently been shown to be blocked (S. Schlossman, personal communication), probably by a cyclized glutamine residue.

The proposed processed polypeptide chain is 248 amino acids long and its calculated relative molecular mass is ~28,000. The chain contains 23 negatively charged (15 Asp and 8 Glu) and 19 positively charged residues (6 Arg and 13 Lys), corresponding to an isoelectric point near neutrality in the absence of post-translation modifications. As shown in Fig. 4, it has four potential sites for *N*-glycosylation.

Conclusions

The pHDS58 gene and its product share several characteristics with the two previously identified T-cell genes, pHDS11 and pHDS4/203, and their products. (1) The gene is specifically expressed in various CTL clones but not in B lymphomas. (2) The gene is rearranged in CTL clones but not in myelomas and the rearrangement pattern varies with the CTL clone. (3) The corresponding poly(A)$^+$ RNA is composed of 5′-variable and 3′-constant regions. (4) The primary sequence suggests that the encoded protein is composed of a signal peptide, two immunoglobulin-like domains, each with one disulphide loop,

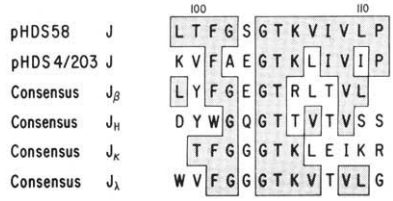

Fig. 6 Comparison of the J region amino acid sequences of pHDS58 and pHDS4/203 (ref. 13) and consensus sequences of the T-cell antigen receptor β-chain[16] and immunoglobulin J_H, J_κ and J_λ (ref. 26). Those residues common between pHDS58 and any one of the other five J segments are boxed and shaded.

a transmembrane peptide and a cytoplasmic peptide. (5) The two domains are homologous to the V and C domains of immunoglobulin chains, particularly those of λ light chains. (6) Besides having two cysteine residues in each domain, the encoded protein has a fifth cysteine residue in the region between the C domain and the transmembrane peptide, that is, at a position where the chain might be disulphide bonded to another chain[13].

The counterparts of the pHDS11 gene in a mouse T-helper hybridoma and a human T-cell tumour have been identified previously by Hedrick *et al.*[12] and Yanagi *et al.*[11] and shown to code for the β-subunit of the heterodimeric T-cell receptor[14]. As the nucleotide sequences of pHDS11 and the gene identified by Hedrick *et al.*[12] are virtually identical in the *C* region, we concluded that pHDS11 codes for the β-subunit of the CTL receptor[13].

Because pHDS4/203, the second type of rearranged gene discovered in the CTL clone 2C, had many characteristics expected for a T-cell receptor gene, it was originally thought to code for the other (α) subunit of the CTL receptor[13]. Subsequently, endoglycosidase F digestion of the αβ heterodimers of mouse helper T cells and a human T-cell tumour suggested that both subunits of these T-cell receptors are *N*-glycosylated (J. P. Allison, personal communication; C. A. Janeway, personal communication), but the pHDS4/203 gene, unlike the pHDS11 gene, has no typical *N*-glycosylation sites (namely, the tripeptide Asn-X-Ser/Thr)[13]. Although the difference in the source of T cells (that is, CTL versus helper and mouse versus human) could have explained the apparent discrepancy, our recent studies suggest that the α-subunit of CTL clone 2C is also *N*-glycosylated (unpublished data). It is therefore of particular interest that the newly discovered pHDS58 gene reported here not only possesses all of the properties expected for a T-cell receptor gene but also carries four potential *N*-glycosylation sites. Thus, while there is no direct evidence, the pHDS58 gene must be considered a better candidate than pHDS4/203 for the α-subunit.

If the pHDS58 gene encodes the α-subunit and pHDS11 the β-subunit of 2C clone αβ heterodimeric receptor, what is the function of the pHDS4/203 gene? As this gene exhibits clonal diversity, in common with the other two genes, it also is likely to be involved directly in determining T-cell specificity. If so, this raises the possibility of a second receptor. The idea of a second receptor has been previously discussed at length[27-32] but is at present less favoured than the one-receptor hypothesis[33-36]. The role of the third gene should be clarified by analysis of its product, currently underway.

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Structure, expression and divergence of T-cell receptor β-chain variable regions

Phillip Patten*, Takashi Yokota†, Jonathan Rothbard‡, Yueh-hsiu Chien‡, Ken-ichi Arai† & Mark M. Davis‡

† Department of Medical Microbiology and * Department of Biological Sciences, Stanford University School of Medicine, Stanford, California 94305, USA
† DNAX Research Institute for Molecular and Cellular Biology, Palo Alto, California 94306, USA

Analysis of three new T-cell receptor β-chain variable regions together with those in the literature indicates that they have both remarkable similarities and differences with those of immunoglobulin. Less than 10 V *regions appear to predominate in the thymus.* V_β *sequences are much more heterogeneous at the amino acid level than are immunoglobulin* V *regions and they appear to diverge between species much more quickly, apparently the result of additional hypervariable regions. Three of these putative new hypervariable regions lie outside of the classical immunoglobulin binding site, an indication that important interactions may be occurring in these regions with polymorphic MHC determinants.*

RECOGNITION of foreign entities by T cells appears to involve molecules and mechanisms strikingly similar to those involving immunoglobulins. Analysis of T-cell receptor β-chain cDNA clones and the genes encoding them has revealed *V*, *D*, *J* and *C* regions similar in size and sequence to the corresponding immunoglobulin elements[1-10]. The *V*, *D* and *J* genomic elements are rearranged during T-cell differentiation to form a single exon, as is the case for immunoglobulins, and this rearrangement