

Future directions

The Dupal anomaly is defined on the basis of the present position of the oceanic islands and continents. If the anomaly is of ancient parentage where were these continental blocks and oceanic reservoirs in the past? Have they been anchored to the Southern Hemisphere? Could there be a mantle convection regime which would not, in billions of years, destroy the isotopic signature of the Dupal mantle, and which would not decouple the geographical positions of the Dupal continents and mantle reservoirs?

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The next step is to define the Dupal anomaly more clearly by analysis of basaltic rocks from both oceanic and continental regimes in the Southern Hemisphere. It will also be useful to search for Dupal signatures in Southern Hemisphere volcanic arcs.

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Complete primary structure of a heterodimeric T-cell receptor deduced from cDNA sequences

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Two related, but distinct, cDNA clones have been isolated and sequenced from a functional murine cytotoxic T-lymphocyte clone. The genes corresponding to these cDNA are expressed and rearranged specifically in T cells and both have similarities to immunoglobulin variable and constant region genes. It is concluded that these genes code for the two subunits of the heterodimeric antigen receptor on the surface of the T cell; its complete deduced primary structure is presented.

THE most characteristic feature of the vertebrate immune system is its capacity to respond to an enormously diverse set of antigenic determinants. At the molecular level this capacity has been traced to an equally diverse set of glycoproteins that are synthesized in B and T lymphocytes. The glycoproteins produced by B cells, called antibodies or immunoglobulins, recognize and bind free antigens and are responsible for humoral immunity, while the T-cell molecules, commonly called T-cell receptors, recognize cell-bound antigens in the specific molecular context of self major histocompatibility complex (MHC) products^{1–3} and are responsible for cellular immunity. The MHC-restriction is an acquired phenomenon^{4,5} determined not by the genotype of the T cells but by that of the host thymus in which the T cells differentiate.

During the past several years extensive studies on the biology and chemistry of immunoglobulin molecules and their genes have illuminated structural and functional properties of these physiologically critical molecules and the genetic origins of their enormous diversity⁶. However, in spite of the equally central

position they occupy in immunological phenomena, the chemical nature of the T-cell receptors has been elusive⁷. Only recently has the first glimpse of these molecules been obtained through the development of effective antisera and monoclonal antibodies that recognize and precipitate clone-specific proteins on the surface of functional T-cell clones, hybridomas or T-cell tumours^{8–10}. These studies suggested that the portion of the receptor determining specificity is a heterodimeric glycoprotein corresponding to a molecular weight (M_r) of about 90,000 (90K) consisting of a 40–45K α -subunit and a 42–44K β -subunit. Moreover, peptide fingerprint analysis suggests that both the α - and β -subunits, like immunoglobulin heavy and light chains, are composed of variable (V) and constant (C) regions^{11–13}. These studies, however, have not provided a primary structure because of the difficulty in preparing a sufficient amount of purified receptor protein.

More recently two groups of workers, using an entirely different approach, succeeded in isolating T-cell-specific cDNA clones of mouse or human origin which show significant

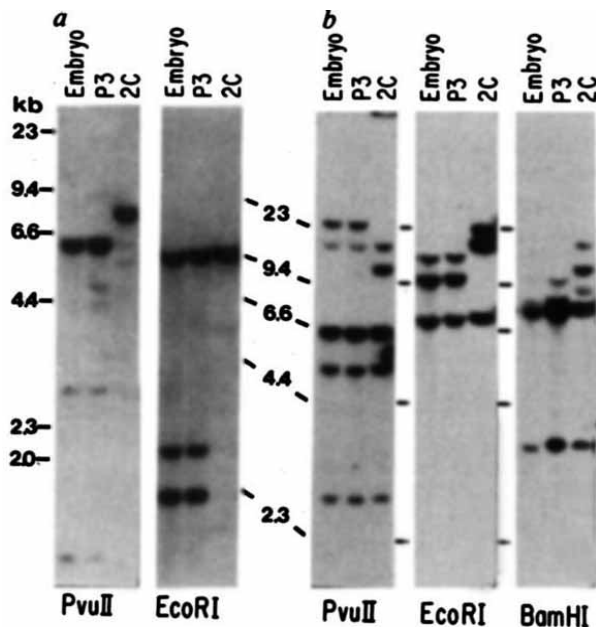


Fig. 1 Southern blot analysis of DNA from BALB/c embryos, myeloma P3 (BALB/c derived), and CTL 2C (BALB.B). DNA was digested with the indicated restriction enzymes, electrophoresed through 0.8% agarose, blotted onto nitrocellulose, and hybridized with ^{32}P -labelled, nick-translated inserts from clone pHDS11 (a) or clone pHDS4 (b). Hybridization was carried out at 42 °C in 50% formamide and $4\times\text{SSC}$. The filters were washed at 65 °C in 0.25% SSC. Separate experiments showed that BALB/c and BALB.B embryo patterns are indistinguishable.

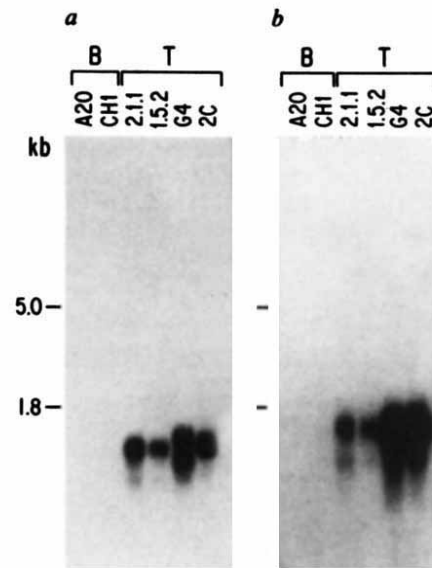


Fig. 2 RNA blot analysis of poly(A)⁺ RNA from various B- and T-cell lines. RNA was extracted from B-cell lymphomas A20-2J and CH1 and alloreactive (H-2^b anti-H-2^d), cytotoxic T-lymphocyte clones 2.1.1, 1.5.2, G4 and 2C. Approximately 1.5 µg of poly(A)⁺ RNA were denatured with glyoxal and electrophoresed through 1% agarose in 10 mM sodium phosphate buffer, pH 6.5. RNA was transferred to nitrocellulose and hybridized to ^{32}P -labelled, nick-translated inserts from clone pHDS11 (a) or clone pHDS4 (b). Positions of rRNA markers (5.0 and 1.8 kb) are indicated.

homology to immunoglobulin genes¹⁴⁻¹⁶. Consistent with their interpretation that these cDNA clones represent one of the two subunits of the receptor, one group has shown that the cDNA encodes a protein composed of an amino-terminal V and a carboxy-terminal C region¹⁶. They also showed that the corresponding genomic DNA sequences have undergone clone-specific somatic rearrangements in various T-cell lines^{15,16}. As the predicted amino acid sequences encoded by the mouse and human cDNA clones isolated by Hedrick *et al.*¹⁶ and Yanagi *et al.*¹⁴, respectively, are highly similar in the C regions, it is very likely that they represent only one of the two subunits of the T-cell receptor.

We have applied the subtraction-cloning method of Davis and his co-workers¹⁵ to a clone of murine alloreactive, cytotoxic T lymphocytes (CTL) and have identified two clearly distinct, mutually non-cross-hybridizing T-cell-specific cDNA clones. Both of these clones show significant sequence homology to immunoglobulin light and heavy chains as well as to the putative receptor genes of Hedrick *et al.*¹⁶ and Yanagi *et al.*¹⁴. In fact, one of our cDNA clones and those of Hedrick *et al.* have a virtually identical nucleotide sequence in the entire constant and 3' untranslated regions. In addition, the genes corresponding to both types of our cDNA have undergone rearrangements in this as well as other cytotoxic T-cell clones.

On the basis of these findings we believe that we have identified and sequenced the genes coding for both subunits of the heterodimeric T-cell receptor of a functional CTL clone. We report here the predicted complete primary structure of this T-cell receptor.

Isolation of T-cell-specific cDNA clones

The alloreactive CTL clone 2C, of BALB.B origin and specific for products of the D end of the BALB/c H-2 complex (d haplotype), has been described previously¹⁷. The cDNA synthesized on the poly(A)⁺ RNA from 2C was subtracted twice with

poly(A)⁺ RNA from a mouse B-cell lymphoma, A20-2J (ref. 18), according to Hedrick *et al.*¹⁵ and a library was constructed from the subtracted cDNA using the vector pBR322 and a standard G·C tailing method. A quarter of this library, containing about 20,000 independent cDNA clones, was subjected to differential screening using two hybridization probes. The first was the 2C cDNA prepared from the poly(A)⁺ RNA of membrane-bound polysomes by subtraction with poly(A)⁺ RNA from another B-cell lymphoma, CH-1 (ref. 19). The second was the cDNA prepared from the total poly(A)⁺ RNA from A20-2J. One hundred and forty 2C-specific cDNA clones were identified and their plasmid DNA was used as hybridization probes in a series of RNA blotting experiments in order to confirm the T-cell-specific expression of the corresponding genes. This permitted us to group the T-cell-specific cDNA clones into at least 10 sets according to the sizes of the corresponding mRNA present in 2C. One was judged to be for the T-cell-specific surface marker Thy-1 on the basis of its hybridization to a previously identified Thy-1 cDNA clone (a gift of Mark Davis).

Two distinct cDNA classes

As was the case in the work of Hedrick *et al.*^{15,16}, one of our assumptions was that the receptor genes in question are rearranged in CTL. Thus we used representative cDNA clones of each set as hybridization probes and compared *EcoRI*-digested genomic DNA from 2C and BALB.B embryos by Southern blot analysis (data not shown). This screening led to the identification of two distinct classes of cDNA, one represented by clone pHDS11 and the other by clones pHDS4 and pHDS203, the genes of which are rearranged in 2C. The other classes of cDNA did not give any indication of DNA rearrangement.

Figure 1a, b shows the results of more extensive Southern blot analysis carried out using clone pHDS11 and pHDS4 as hybridization probes, respectively. The results can be summarized as follows. First, the hybridization patterns obtained by the

two probes are clearly distinct, confirming that the two classes of cDNA clones represent two different sets of genes that do not cross-hybridize to a detectable level in the conditions used. Second, with either probe the hybridization patterns obtained with 2C DNA are different from the patterns obtained with BALB embryo DNA. Third, the myeloma P3 DNA patterns are the same as the embryo DNA patterns. In addition, DNA from five different CTL clones was analysed and each gave patterns different from those of embryo DNA with at least one enzyme (data not shown). Considering the multiplicity and specificity of the pattern differences, these results strongly suggest that genes corresponding to both cDNAs have undergone gross sequence rearrangement in 2C.

The T-cell-specific expression of these genes has been confirmed by analysing poly(A)⁺ RNA from 2C and three other independently derived alloreactive CTL as well as two B-cell lymphomas, A20-2J and CH1. As shown in Fig. 2a, b, each of the two cDNA probes detected RNA of distinct sizes in all four CTL but not in either of the B-cell lymphomas, although the relative content, and in some cases the size, of the RNA varied somewhat between CTL. When analysed with the clone pHDS11 probe, CTL 2C, 1.5.2 and 2.1.1 all gave a major RNA component of about 1.3 kilobases (kb) while CTL G4 contained two major components of 1.4 and 1.2 kb. On the other hand, all four CTL gave a 1.5 kb RNA component with the clone pHDS4 probe.

Nucleotide sequence analysis

The restriction maps of the three cDNA clones, pHDS11, pHDS4 and pHDS203, were constructed by standard procedures (Fig. 3), and the DNA sequences were determined by the method of Maxam and Gilbert²⁰ according to the strategy shown in Fig. 3. As both the restriction maps and DNA sequences (see below) confirm that pHDS4 and pHDS203 are derived from the same gene while pHDS11 represents a distinct one, we will describe the sequence features of the two genes separately.

The entire nucleotide sequence of the 1,054 base pair (bp) insert of clone pHDS11 is shown in Fig. 4a. The longest open reading frame is composed of 879 nucleotides whose corresponding amino acid sequence of 293 residues is also shown in Fig. 4a. For reasons given below, the codons are numbered starting with the triplet GAC at nucleotide positions 36–38. Between codons 110 (Glu) and 236 (Cys) the pHDS11 sequence is identical to the sequence of the corresponding region of Hedrick *et al.*'s thymocyte cDNA clone, 86T1 (ref. 16), except for one base pair difference in codon 159 (Arg). According to these authors this region defines the major body of the C region: codons 109 (Leu) and 110 (Glu) define the joining (J) and C region boundary while codon 236 (Cys) precedes the N-terminus of the transmembrane segment. The sequence identity is even more striking between pHDS11 and 2B4.71, the latter being a cDNA clone isolated from a T-helper cell hybridoma specific for pigeon cytochrome *c* (ref. 21). From codon 109 (Leu) the two sequences are virtually identical throughout the C regions and the entire 3' untranslated regions. The exceptions are two base pair differences, one in codon 159 (Arg) and the other at nucleotide position 992.

Between codons 97 and 109 the pHDS11 sequence is similar but distinct from the corresponding sequence of 86T1 or 2B4.71. As pointed out by Hedrick *et al.*¹⁶, this region is strikingly homologous to the J segments of immunoglobulin genes. In fact the pHDS11 J sequence corresponds exactly to the sequence of the J_H7 genomic segment recently identified and characterized by Chien *et al.*²¹.

Between codons 1 and 97 the pHDS11 sequence is quite different from the sequence of the corresponding region of either 86T1 or 2B4.71 (Fig. 5a). However, patches of conserved residues clearly exist throughout the three sequences and also between these sequences and those encoding immunoglobulin V regions of both light and heavy chains (the latter not shown). Particularly noteworthy conservations are the two Cys residues that are involved in intra-domain disulphide linkages in immunoglobulin V regions (residues 23 and 91 in Figs 4a and

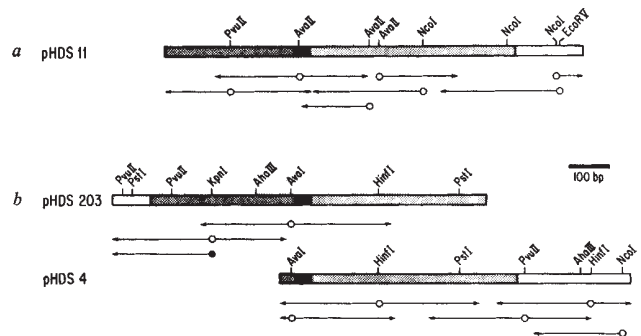


Fig. 3 Restriction maps of the inserts of cDNA clones isolated from CTL 2C. The maps were constructed by the standard single, double or triple digestions of the plasmid DNA. The V, J, C and 5'- or 3'-untranslated regions are shown by □, ■, ▨ and □, respectively. Also shown are the sequencing strategies used to produce the nucleotide sequences shown in Fig. 4. The 5'- and 3'-end labellings are indicated by ○ and ●, respectively.

5a). The Trp residue that is universally conserved in immunoglobulin V regions is also present in the corresponding position of the T-cell proteins (residue 34).

At the 5' end of the open reading frame (see Fig. 4a) there is a stretch of 11 highly hydrophobic residues that probably comprise part of a signal peptide²². The position of the N-terminal residue of the mature protein is ambiguous. However, homology between the pHDS11 protein and immunoglobulin V regions, particularly V_κ regions²³, suggests that Asp at position 1 is the N-terminal residue.

As already pointed out by Hedrick *et al.*¹⁶, another stretch of a highly hydrophobic region of about 22 residues, evidently constituting a transmembrane peptide, occurs immediately before the C-terminal 5 hydrophilic residues; the latter are thought to extend into the cytoplasm. Overall, the gene defined by pHDS11 can encode a processed protein of 282 residues with a molecular weight of 32K.

The composite nucleotide sequence of 1,286 bp defined by the two overlapping cDNA clones pHDS4 and pHDS203 is shown in Fig. 4b. The longest open reading frame begins with the Met codon at nucleotide positions 88–90, extends over a stretch of 912 bp, and ends at nucleotide position 999. The predicted amino acid sequence is also shown in Fig. 4b. This sequence is significantly homologous to those of pHDS11, 86T1, 93G7 immunoglobulin heavy chain, MOPC603 immunoglobulin κ light chain, and MOPC104E immunoglobulin λ1 light chain. This is shown in Figs 5b and 6 where the pHDS4/203 protein sequence is compared with the V (or V+D), J and C region sequences of the other five proteins. Homology is evident in all three regions. The relatedness of the pHDS4/203 protein to the other five proteins in each of the three regions ranges over 18–23% for the V or V+D region, 21–50% for the J region and 16–22% for the C region. The similarity occurs in patches and in the case of the V (or V+D) region these patches tend to correspond to the framework regions (FR) of immunoglobulin V regions. In addition, many of the residues shared by the five non-pHDS4/203 proteins are also shared by the pHDS4/203 protein. These residues (indicated in Fig. 5b by *) include the Cys residues involved in the intra-domain disulphide linkages as well as the Trp residue which is universally present at the boundary of the first hypervariable (HV-I) and FR-2 segments of all immunoglobulin V regions studied to date²³.

As in the pHDS11 protein, the peptide at the amino-terminal end of the pHDS4/203 protein is highly hydrophobic and most probably constitutes a signal peptide. Accordingly the N-terminal residue of the processed protein cannot be unambiguously determined from the cDNA sequence. However, we believe that the N-terminal residue is the Gln at position 1 in Figs 4b and 5b. This is because the N-terminal residue of the α-subunit of the T-cell receptor is blocked, probably by a pyrrolidone carboxylic acid residue (S. Schlossman, personal

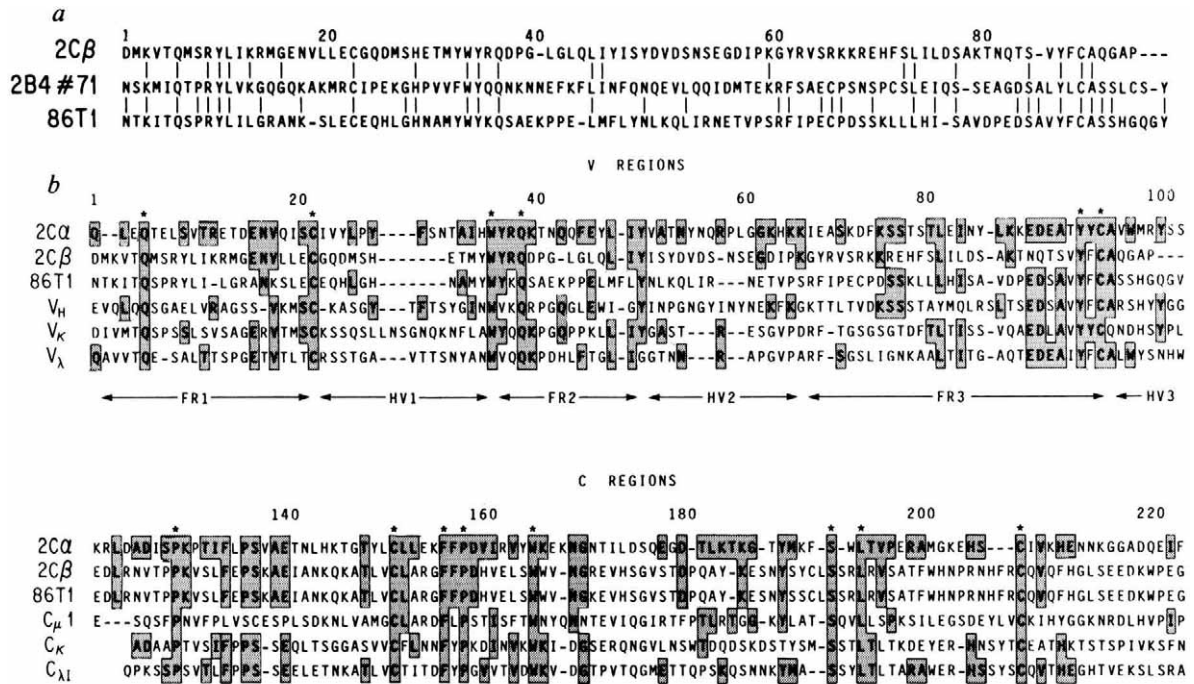


Fig. 5 *a*, Comparisons of the predicted amino acid sequences of three V_β regions, 2Cβ (pHSD11), 2B4.71 (ref. 21) and 86T1 (ref. 16). Those residues common between 2Cβ and 2B4.71, or 2B4.71 and 86T1 are indicated by vertical lines. *b*, Comparisons of the predicted amino acid sequence of pHDS4/203 (2Cα) and predicted or determined amino acid sequences of five other proteins, the β-chain encoded by pHDS11, the β-chain encoded by cDNA clone 86T1 (ref. 16), the V regions of the 93G7 γ1 heavy chain²³, the C_{H1} region of the μ heavy chain, the V and C regions of the MOPC603 κ chain²³ and the MOPC104E λ1 chain²³. Those residues common between the 2Cα polypeptide chain and any of the other five chains are shaded. The residues common among all six chains are indicated by *. Approximate boundaries of framework and hypervariable regions as they appear in immunoglobulin V regions are indicated by arrows.

immunoglobulin-like domains, a transmembrane peptide and a cytoplasmic peptide of similar sizes in the two chains. Earlier studies at the protein level indicated that the mouse receptor is composed of two different subunits of similar size held together by one or more disulphide linkage(s)⁸⁻¹⁰. In agreement with these findings, the predicted molecular weight (33K) of the pHDS4/203 protein is very similar to that (32K) of the β-chain encoded by pHDS11. Also, the pHDS4/203 protein and the pHDS11 β-chain each contain one Cys residue in corresponding positions (234 and 236, respectively) that lie outside the immunoglobulin-like domains. These residues can provide the postulated inter-chain disulphide linkage. Thus, while direct evidence is yet to be produced it is very likely that pHDS4/203 codes for the α-subunit of the CTL receptor.

Proposed structure of the T-cell receptor

The amino acid sequences deduced for the two subunits suggest the overall structure for the T-cell receptor shown in Fig. 7. In this model each receptor molecule is made up of two chains, each with two extracellular immunoglobulin-like domains, an amino-terminal variable and a carboxy-terminal constant domain. Each of these domains is stabilized by an S-S bond between cysteine residues that are separated in the linear sequence by 60-70 residues. Amino acid sequence similarity with immunoglobulin V regions (Fig. 5b) point to V (or V+D), D and J segments in the N-terminal domain of the α-subunit (V_α), as previously proposed for the corresponding domain of the β-subunit (V_β)^{16,21}.

Cysteine residues at positions 234 and 236 of the α- and β-subunits, respectively, could well form a single inter-chain S-S bond located close to the cell outer membrane. This bond would link the two subunits in the intact molecule and account for the difference in apparent molecular weight between the unreduced and reduced receptor (90K compared with 40-45K) in SDS-polyacrylamide gel electrophoresis (PAGE). More important, a stable association between two different subunits may well be required to form an effective antigen-binding region,

as is characteristic of immunoglobulins. The obligate participation of two different subunits in the formation of a single combining site means that combinatorial variability is likely to contribute to structural and functional diversity of these receptors.

The protein molecular weights of the α- and β-subunits are each about 10K less than the apparent molecular weight observed in SDS-PAGE. The difference suggests that the subunits are glycosylated. There are four potential sites for N-glycosylation of the β-subunit but none in the α-subunit. It is possible that the α-subunit is O-glycosylated on serine and threonine residues.

After the constant domain, each subunit has at its carboxyl end a hydrophobic stretch of 21-22 amino acids followed by a short stretch of 5(β) or 12(α) amino acid residues in which cationic residues abound. As these segments correspond, respectively, to the transmembrane and cytoplasmic domains that are characteristically found in transmembrane proteins, they support the proposed membrane location and receptor function of the gene products.

The presence of a cysteine residue in the cytoplasmic domain of the α-subunit might provide an SH group for dynamic

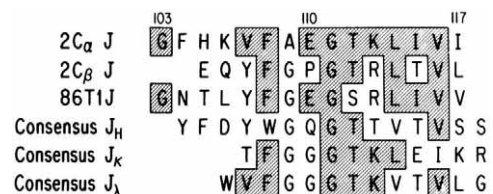


Fig. 6 Comparisons of the J region sequence of 2Cα (predicted from pHDS4/203) and those of 2Cβ (predicted from pHDS11), 86T1 (ref. 16), and immunoglobulin J_H, J_K, and J_λ (ref. 23) consensus sequences. Those residues common between 2Cα J and any one of the other five J segments are boxed in and shaded.

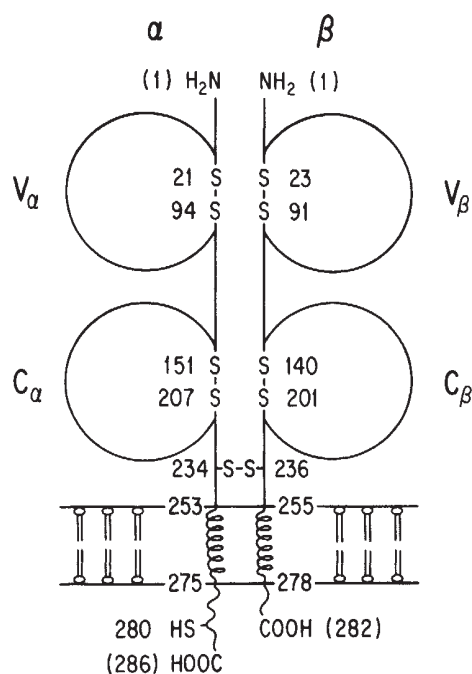


Fig. 7 Proposed overall structure of the 2C receptor. Each receptor molecule is made up of two chains, α and β , each with two extracellular immunoglobulin-like domains, an amino-terminal variable and a carboxy-terminal constant domain. Each of these domains is stabilized by an S-S bond between cysteine residues. Two chains are held by a single inter-chain S-S bond located close to the cell's outer membrane. The protein is anchored on the membrane by two (one for each chain) hydrophobic transmembrane peptides. A short carboxy-terminal peptide rich in cationic residues extends into cytoplasm in each chain. The SH group of the cysteine residue present in the cytoplasmic peptide of the α -subunit may interact dynamically with a membrane or cytoplasmic protein and thereby may be important for the cell's effector function. Both α - and β -chains are glycosylated, although the exact site(s) and extent of glycosylation are unknown.

interactions between the receptor and other molecules of the cell membrane (such as T3 (ref. 9) in human CTL) or perhaps the cytoskeleton. The need for a functional association with such accessory structures is suggested by the overall resemblance of the proposed model to Fab fragments of immunoglobulin molecules: as is well known, these fragments have ligand-binding activity but lack effector functions.

These data throw light on the question of whether CTL and T-helper cell receptors are encoded by the same or separate sets of genes. We will consider the V and C regions separately because the two regions are encoded by distinct gene segments (ref. 21 and our unpublished observations). In immunoglobulin molecules the primary function of the V_H and V_L regions is, of course, to determine antigen specificity. Each of the multiple C_H regions defines an immunoglobulin class and carries a distinct effector function. The primary function of the C_L region is unclear. It is not required for antigen specificity but may be necessary to enhance L-H gene interactions or to facilitate some

unknown function. In T-cell receptors the very presence of the V regions strongly suggests that these regions have a primary role in determining antigen and MHC specificity. An interesting question is whether the CTL and T-helper cell receptors carry distinct C regions each of which has a unique role in the activation or execution of the cell's effector function. An alternative view is that the receptors of the two types of T cells have the same C regions and that while the $\alpha\beta$ heterodimer is specific with regard to ligand binding it has only a nonspecific role in triggering the cell's effector functions.

Comparison of the β cDNA (clone pHDS11) from CTL clone 2C and the β cDNA (clone 2B4.71) from T-helper cell clone 2B4 indicates that at least these CTL and T-helper cells use the same C_β gene segment (clone 2C and clone 2B4 are from the BALB.B and B10.A strains, respectively, and the single base pair differences between their cDNAs may be due to a strain difference.) This conclusion is supported by two observations. First, the near identity of the two cDNA sequences occur not only in the C regions but also in the 3' untranslated regions. Second, the J segments in the two cDNAs are different but belong to the same cluster linked to a single C gene segment²¹. While sequencing of more β genes is required before a firm conclusion can be drawn, these results already suggest strongly that the same C gene segments are used for β chains of CTL and T-helper cells.

Concerning the V regions, several earlier observations suggested that CTL and T-helper cell receptors use non-overlapping sets of gene segments that have evolved separately under different selective pressure: first, the antigen repertoires seem to be quite different; second, different classes of MHC gene products restrict CTL and T-helper cell responses; third, no clearly demonstrated case of T-cell reactivity that crosses the MHC class barrier has been reported (D. Raulat, personal communication). An alternative view is that CTL and T-helper cell receptors share a common pool of germ-line gene segments for V regions and that the distinction between function arises by somatic selection.

The amino acid sequences predicted by the 2C β cDNA (clone pHDS11) and 2B4 β cDNA (clone 2B4.71) are only similar at 21% of residues in the V (or V+D) segment-encoded regions (Fig. 5a). This is substantially lower than the similarity observed between any pair of immunoglobulin V_k segments (40–98%)²³ and is even less than that observed between the C_β region and the immunoglobulin C_k or $C_{\lambda 1}$ domain (22% and 31%, respectively)¹⁶. While characterization of more V_β regions is required before a firm conclusion can be drawn, the weakness of the similarity between the CTL V_β and T-helper cell V_β regions is striking and may reflect independent pools of V and/or D gene segments.

Finally, it will be extremely interesting to compare in similar fashion the V_α and C_α regions of CTL and T-helper cells. It is possible that, as in immunoglobulin molecules, the C region of only one of the two types of chains in these antigen-recognition molecules is involved in effector function.

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