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Immunology

Secondary, tertiary, and quaternary structure of T-cell-specific immunoglobulin-like polypeptide chains*

(antibody binding site/x-ray structure/β-sheet/T-cell receptor/T8 antigen)

JIŘÍ NOVOTNÝ†, SUSUMU TONEGAWA‡, HARUO SAITO‡§, DAVID M. KRANZ‡, AND HERMAN N. EISEN‡

†Molecular and Cellular Research Laboratory, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114; and ‡Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

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ABSTRACT **To explore the possibility that the difference in antigen recognition between B and T cells derives from a structural difference in their respective antigen-specific receptors (immunoglobulins on B cells and immunoglobulin-like molecules on T cells), we compared the extracellular segments of the T-cell receptor α, β, and γ polypeptide chains and the N-terminal segment of the T-cell T8 (Lyt-2) antigen chain with the corresponding regions of immunoglobulins whose three-dimensional structures are known. The results indicate that the four T-cell polypeptide chains are organized into immunoglobulin-like domains consisting of multistranded antiparallel β-sheet bilayers. Invariant amino acid side chains that are conserved in diverse immunoglobulins, including those that mediate domain–domain interactions and form a constant scaffold for antibody binding sites, are also conserved in the chains encoded by the T-cell receptor genes and in the N-terminal domain of T8 (Lyt-2). It appears that the binding sites of the antigen-specific T-cell αβ-chain receptors and of antibodies are very similar in their overall dimensions and geometry: a T-cell αβ receptor molecule probably has an antigen-specific binding site that is fundamentally no different than the conventional binding site of an antibody.**

The capacity of the immune system to distinguish among an enormous number of different antigenic structures is based on a correspondingly great diversity within its constituent B- and T-lymphocyte populations. Both populations are made of many clones, each able to recognize only one or a few similar antigens. Extensive studies of the specific responses elicited by antigens have made it clear, however, that B and T cells differ profoundly in the way they recognize antigens. B cells, via the surface Igs that serve as their antigen-specific receptors, recognize antigens alone, whereas T cells, via their Ig-like antigen-specific receptors (T-cell receptors), characteristically recognize antigens only on other cells and in conjunction with the other cell's major histocompatibility complex (MHC)-encoded surface glycoproteins (1). The difference in antigen recognition suggests that there may be an underlying structural difference between the antigen-specific receptors of B and T cells.

Several Ig-like genes are expressed in T cells. The genes for receptor α and β chains exhibit pronounced clonal variability in their N terminus-encoding domains, and the polypeptide chains they encode form the disulfide-bonded αβ heterodimer present on virtually all mature T cells (2–4). A considerable body of evidence, all of it still indirect, strongly suggests that the αβ molecules are antigen-specific receptors; here we refer to them as αβ receptors (5–9). γ-Gene transcripts, detectable only in certain T cells, exhibit less sequence diversity than do α- and β-chain genes (10) and, although the γ-gene sequence clearly encodes an integral membrane protein, this protein and its function have not yet been identified. The gene for T8 antigen of human T cells (the Lyt-2 of murine T cells) also encodes an integral membrane protein with two extracellular domains (11, 12), but only the N-terminal domain of the protein is Ig-like, and it is not clonally variable.

The particular question we focus on here is whether the antigen-specific αβ receptor is likely to have two distinct antibody-like binding sites per heterodimeric receptor molecule, one for antigen and the other for the restricting MHC glycoprotein. The T8 antigen and γ chains are included in this study to broaden our analysis, but some of the other T-cell surface proteins with Ig-like sequences (e.g., Thy-1, OX-2) are not considered. To answer the question, we used sequences of cDNA clones representing transcripts of these genes to study their homology in sequence and similarity in three-dimensional structure with Igs based on x-ray crystallographic analyses of mouse and human Fab fragments, light (L) chain variable (V) domain dimers (V_L–V_L), and human Fc fragment (13–18). Justification for this approach rests with the general rule that in the evolution of multigene families, such as Igs, protein folding is even more conserved than are amino acid sequences (19).

METHODS OF ANALYSIS

The amino acid sequences of the α (7, 8), β (5, 6, 9), γ (9), and T8 (11, 12) chains were aligned with homologous sequences of Igs whose three-dimensional structures are known (13–18). Using the alignment, we examined the structural roles of invariant side chains. Structural manipulations and stereo drawings were carried out with the use of the program CHARMM (20) and previously described graphics facilities and software (21). Amino acid sequence profiles of hydrophobicity, charge, and secondary structure propensities were computed as described (22). To avoid conveying a sense of precision that is neither warranted nor intended, we focus our discussion only on gross structural features.

RESULTS AND DISCUSSION

Amino Acid Alignments and Secondary Structure Profiles. Sequences of the N-terminal domains were aligned (Fig. 1) with a mouse myeloma V_L domain (MCPC 603; ref. 23). Sequences of the membrane-proximal domains of the α, β, and γ chains were also aligned with constant (C) domains of

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Abbreviations: MHC, major histocompatibility complex; V, variable; C, constant; L, light; H, heavy.

*A preliminary report of this study was presented at the 69th Annual Meeting of the Federation of American Societies for Experimental Biology, Anaheim, CA, April 21–26, 1985 (39).

§Present address: Division of Tumor Immunology, Dana–Farber Institute, Boston, MA 02115.

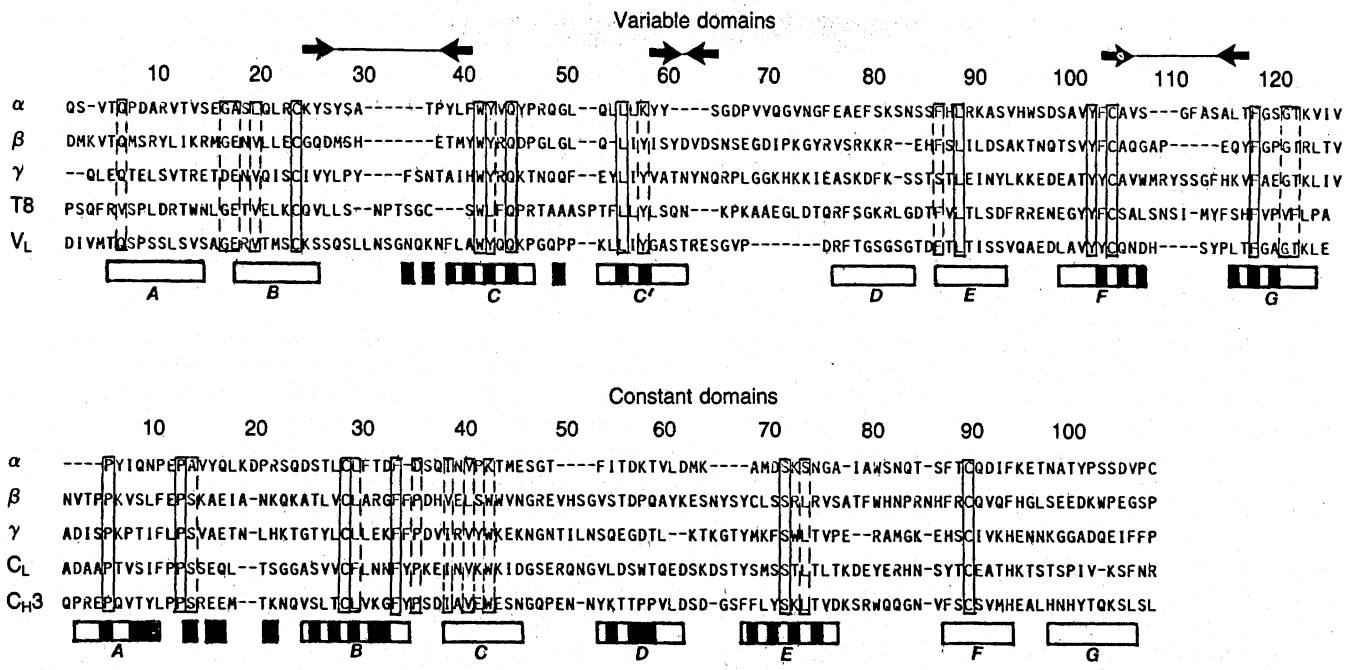

FIG. 1. Amino acid sequence alignments. The sequences are given in the one-letter code, and homology is emphasized by vertical boxes. Full boxes denote residues identical in all of the sequences compared; dashed boxes indicate positions with four of five residues identical. (*Upper*) V domains of T-cell receptor α and β chains, the T-cell γ chain, N-terminal domain of T8 antigen chain, and the V_L domain of mouse myeloma MCPC 603. The three antibody L chain hypervariable loops (23) are indicated by double-headed arrows. The α, β, and γ sequences are cDNA clones pHDS 58, 11, and 4/203, respectively, from the T-cell line 2C (8, 9). The β sequence shown may not correspond to the β subunit of the $\alpha\beta$ receptor of this cell line; a second β sequence for clone 2C will be presented elsewhere. (*Lower*) C domains of T-cell receptor α and β chains, the T-cell γ chain, the C_L domain of human cryoglobulin KOL, and the C_H3 domain of crystallized pooled human Fc fragment. Segments corresponding to β-strands in Ig crystallographic structures (13, 14, 16) are indicated by horizontal boxes labeled A–G; residues involved in interdomain contacts (13, 14, 16, 29) are denoted by black rectangles.

known tertiary structure—i.e., mouse MCPC 603 C_L domain (23), and human C_H3 domain (23).

All Ig domains share the same folding scheme. Lesk and Chothia (24) described this "Ig fold" as a consecutive sequence of seven β-strands, A to G, arranged in two antiparallel β-sheets packed face-to-face: A–B–D–E and C–F–G (cf. Fig. 1). Each of the β-strands has characteristic amino acid residues at positions where the side chains point inside the sheet. Fig. 1 shows that these structurally important residues (positions 19, 23, 41, 86, 88, 101, and 103 in the V domain alignment and 28, 38, 40, 42, 71, 73, and 89 in the C domain alignment) are often conserved in the T-cell α, β, γ, and T8 chains as well. There are seven β-strands in Ig C domains and eight or nine in Ig V domains. The amino acid profiles we compute for the T-cell receptor and T8 domains predict β-strands in positions homologous to Ig β-strands (cf. Fig. 2) and also indicate eight β-strands in all of the T-cell N-terminal domains and seven in the α, β, and γ membrane-proximal domains. The membrane-proximal domain of T8 differs from the others and lacks sequences in common with Ig domains.

That the N-terminal domains of the T-cell α, β, γ, and the T8 chains all have the Ig fold is also supported by the following findings: (*i*) α-, β-, γ-, and T8-chain segments that correspond to β-strands show a higher degree of conservation than those corresponding to loops (cf. Fig. 1); (*ii*) all of the insertions/deletions occur at positions that in Ig chains belong to surface loops or "hypervariable regions" (which are especially variable surface loops); (*iii*) the hydrophobicity of the putative β-strands is similar to that found in the corresponding Ig β-strands, with the mid-sheet β-strands (e.g., C, C', and F in the V-type domains) usually more hydrophobic than the edge β-strands (e.g., A and D in the V-type domains; cf. Fig. 2).

The residues often found to be conserved among Igs and α, β, γ, and T8 chains are primarily of three kinds: (*i*) those buried in the domain core (mostly hydrophobic residues, such as residues 19, 23, 41, 86, 88, 101, and 103 of the V domain alignments in Fig. 1 and residues 29, 38, 40, 42, 73, 87, and 89 of the C domain alignments in Fig. 1); (*ii*) those important in the architecture of loops (Gly-16 of the V domains and Pro-12 and -35 of the C domain alignments in Fig. 1); (*iii*) and those occupying key positions in domain–domain contacts (42, 44, 55, 102, 104, and 117 of the V domains and 13, 16, 29, and 74 of the C domain alignments in Fig. 1). Moreover, side chain replacements in these positions often involve either chemically homologous residues (e.g., tyrosine for phenylalanine in position 102 of the V domain alignment) or residues with similar structural propensities (e.g., the turn-promoting glycine and aspartic acid residues in position 16 of the V domain alignment). Similar patterns of side-chain invariance have been noted in comparisons of tertiary structures of other functionally and evolutionarily related proteins (cf. e.g., refs. 17 and 25–27).

Noncovalent Association of T-Cell Receptor C Domains. In the antibody molecule, the association of homologous domain pairs from L and H (heavy) polypeptide chains, such as V_L–V_H (containing the antibody-combining site), C_L–C_H1 and C_H3–C_H3, are stabilized by noncovalent interactions between closely packed, complementary β-sheet surfaces. The primary structure of the β-sheets involved in these domain–domain interactions (C–C'–F–G in the V domains and A–B–E–D in the C domains) are less variable than those of the solvent-facing sheets (28), and the same is true for the α, β, and γ chains. As shown in Fig. 1, 29% of the positions in the C–C'–F–G β-sheet of the V domains are boxed (i.e., often conserved) compared to only 15% of the positions in the solvent-facing A–B–D–E sheet. In the C domains, the two sheets have comparable numbers of boxed positions (18%

and 17%, respectively), but in pairwise comparisons with the Ig C_L and C_H3 domains, the α-, β-, and γ-chain A–B–D–E sheets indicate a higher degree of conservation (41% on the average) than do their solvent-facing C–F–G β-sheets (33% on the average). Thus, the pattern of side-chain conservation in the N-terminal and membrane-proximal domains of the T-cell αβ receptor is consistent with dimeric α1–β1 (V_L–V_H-like) and α2–β2 (C_L–C_H-like) domain modules. However, the interface of the C domains in the T-cell receptor most resembles the corresponding interface in Ig C_H3 domains, which is relatively rich in electrically charged residues (Table 1). Stable β-sheet–β-sheet contacts involving buried charges require formation of ion pairs that neutralize each other. Table 1 indicates that electrostatic interaction between the α2–β2 domains is favorable (net electrostatic charge 2 compared to the net charge 4 in isolated domains), whereas some other domain–domain interactions are less favorable. For example, in the absence of compensatory features, a γ2–γ2 homodimer is not likely to be stable (net charge +6).

Similar Architecture of Antibody and T-Cell Receptor Antigen-Combining Sites. The geometry of the interface between Ig V_L and V_H domains, which dimerize via close-packing of their respective C–C′–F–G β-sheets, has been shown to correspond to a nine-stranded, twisted, "β-barrel" (29). In all Igs studied to date, conservation of side chains inside and between the V_L and V_H domains preserves the geometry of V_L–V_H domain interface with its included antigen-combining region. Buried residues containing polar atoms (such as positions 6, 41, and 121 of Fig. 1) form a conserved hydrogen-bonding network. Two hydrogen bonds contributed by invariant glutamine side chains (position 44 of Fig. 1) extend across the V_L–V_H interface to anchor the V_L and V_H β-sheets in their relative orientation (Fig. 3 *Upper*). Six aromatic residues (positions 42, 102, and 117 in each V domain aligned in Fig. 1) come into contact at the bottom of the interface, forming a cluster of perpendicularly oriented ring planes mutually stabilized by "herringbone" interactions (Fig. 3 *Lower*; ref. 29).

It is highly significant that all of these 10 side chains are conserved in the T-cell α and β subunits and often in the γ and T8 polypeptides as well (Fig. 1). While conservation of an isolated amino acid residue might be considered fortuitous, invariance of all these residues, widely scattered through the linear amino acid sequence but proximal to each other in three dimensions, strongly suggests that T-cell receptor and antibody binding sites have very similar overall geometry.

Differences Between the Ig and T-Cell Receptor Binding Sites. The key structural features of the V_L and V_H contact surfaces are related by a pseudo-2-fold symmetry. Because of this symmetry, V_L–V_L dimers can associate in the same manner as the V_L–V_H dimers, creating a domain interface that resembles the antigen-combining site (15, 18) and pos-

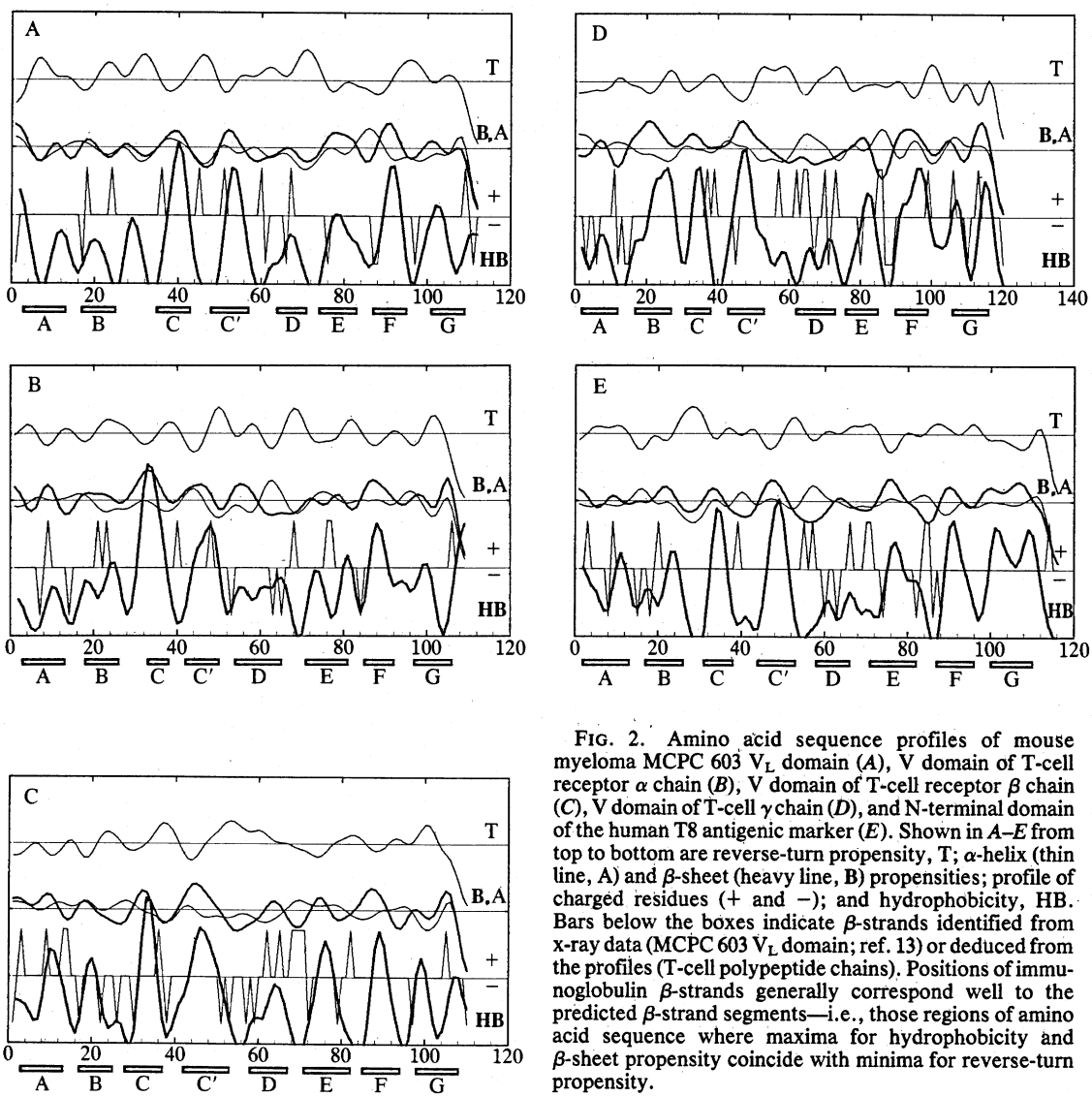

FIG. 2. Amino acid sequence profiles of mouse myeloma MCPC 603 V_L domain (*A*), V domain of T-cell receptor α chain (*B*), V domain of T-cell receptor β chain (*C*), V domain of T-cell γ chain (*D*), and N-terminal domain of the human T8 antigenic marker (*E*). Shown in *A–E* from top to bottom are reverse-turn propensity, T; α-helix (thin line, A) and β-sheet (heavy line, B) propensities; profile of charged residues (+ and −); and hydrophobicity, HB. Bars below the boxes indicate β-strands identified from x-ray data (MCPC 603 V_L domain; ref. 13) or deduced from the profiles (T-cell polypeptide chains). Positions of immunoglobulin β-strands generally correspond well to the predicted β-strand segments—i.e., those regions of amino acid sequence where maxima for hydrophobicity and β-sheet propensity coincide with minima for reverse-turn propensity.

Table 1. Types of side chains found in β-sheets that mediate domain–domain contacts

Domains	Number of contact positions	Aromatic side chains, %	Charged side chains, %	Net charge
		Ig		
C_L	27	11	7	−2
C_H1	27	11	15	+2
C_H3	20	11	26	−1
V_L	17	35	6	+1
V_H	27	30	11	−1
		T-cell receptor		
C_α	20	11	33	−3
C_β	20	11	33	+1
C_γ	20	7	33	+3
V_α	17	56	12	+1
V_β	17	31	6	−1
V_γ	17	38	6	0

Amino acid residues involved in Ig domain–domain contacts were identified by accessibility calculations; those of T-cell receptor contacts were inferred from primary structure alignments (cf. Fig. 1). The numbers in the third and fourth columns represent the percentage of individual residue types that occur in contact positions of interface-forming β-sheets. "Net charge" gives the algebraic sum of charged residues (Lys, +1; Arg, +1; Asp, −1; Glu, −1) buried at interdomain contact surfaces.

sesses antigen-binding capacity (18, 30–32). Both the α- and β-chain V domains are slightly more homologous in sequence to V_L than to V_H domains. Therefore, the V_α–V_β interface may be more akin to that of V_L–V_L dimers, as in the crystallographic structure REI (15), than of V_L–V_H dimers.

Although *V* gene segments in the T-cell receptor β-chain gene family seem to differ more from each other than do those of any of the Ig *V* gene families (33), our cumulative amino acid sequence profiles indicate that the degree of secondary structure conservation is the same in β chains as in Ig chains (Fig. 4). Unlike Igs, however, most of the clonal variability in T-cell receptor β chains seems to be concentrated between residues 46 and 66 (Fig. 4*B*)—i.e., in a segment homologous to the second hypervariable region of Igs. The significance of this localization is unclear. The variability does not correlate with MHC specificity; therefore, it is unlikely that it has any connection with the phenomenon of MHC restriction (34).

CONCLUDING REMARKS

The approach used in this study is based on the rule that, within a protein family, three-dimensional folding is more highly conserved than is amino acid sequence homology (19, 25–27). For example, the β-sheet α-carbon atoms of β_2-microglobulin and the human C_H3 domain can be superimposed with an average root-mean-square distance of 0.9 Å (35), although their amino acid sequences have only 22% identical residues.

The available crystallographic data indicate that antibody binding sites correspond to surface concavities (grooves or pockets) formed at the interface of β-sheets contributed by the two V domains. Because Igs exhibit great diversity in binding affinities for very many different antigens, the shapes of their binding sites must also vary greatly in fine structure. Nonetheless, it has been possible to identify invariant amino acids that define the overall boundaries and geometry of all the known sites (29). Interactions among these residues form a scaffold that can accommodate a virtually limitless amount

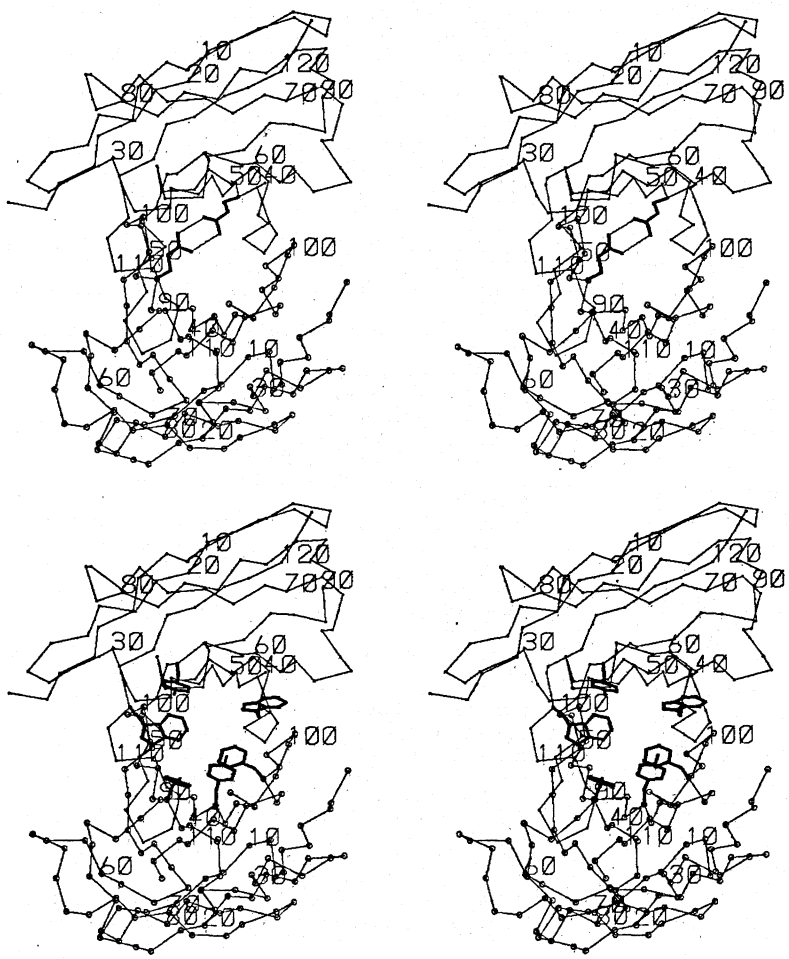

FIG. 3. The invariant side chains of the V_L–V_H interface. The stereo plots show α-carbon tracings of polypeptide chain backbones of V_L and V_H domains in mouse myeloma MCPC 603 as determined by x-ray crystallography (13). The L chain domain α carbon atoms are circled. (*Upper*) In Igs, the invariant glutamine residues (position 44 in Fig. 1 V domain alignments) form hydrogen bonds across the V_L–V_H interface (antigen-combining region). These residues are conserved in T-cell polypeptide chains as well. (*Lower*) The unique geometry of the V_L–V_H interface in Igs results from the perpendicular "herringbone" arrangement of aromatic side chains at the domain–domain contact area (29). There are six invariant aromatic rings involved in the herringbone cluster (positions 42, 101, and 117 of the V domain alignments of Fig. 1) as well as several other aromatic residues contributed by the hypervariable regions (not shown). All of the three invariant Ig polypeptide side chains are conserved in the T-cell polypeptide chains.

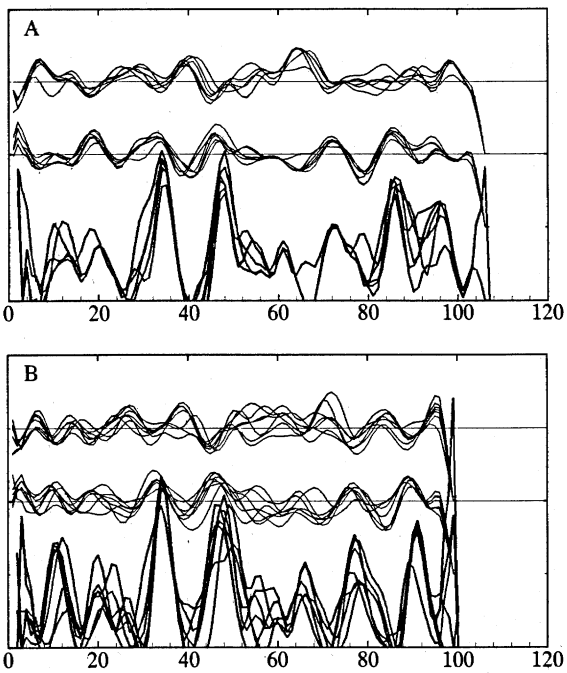

FIG. 4. Overlay of sequence profiles from seven Ig κ chains (23) (*A*) and T-cell receptor β chains (33) (*B*). Reverse-turn propensity, β-sheet propensity, and hydrophobicity are plotted from top to bottom. Although the β-chain sequences are known to be more variable than, for instance, Ig κ chains, the profiles suggest that the degree of structural conservation is comparable in both types of chains. Unlike Igs, however, most of the structural variability in T-cell receptor β chains is concentrated between residues 46 and 66–i.e., in the segment homologous to the second hypervariable region of Igs.

of sequence variation that occurs in parts of the "hypervariable loops" that delineate the surface of the binding site.

We have shown here that the invariant side chains defining the scaffold for antibody binding sites are shared with the T-cell Ig-like chains, most notably the antigen-specific $\alpha\beta$ receptor. Since these invariant features also define the dimensions of the T-cell receptor binding sites, they set limits on the size range of the antigenic epitopes that can occupy these sites (36). Hence, $\alpha\beta$ receptors and Igs are likely to accommodate, in their respective binding sites, antigens of the same size range. It follows from this conclusion that the $\alpha\beta$ receptor is no more likely than an antibody to harbor two discrete V-region binding sites for independent epitopes, i.e., one for the antigen and the other for a restricting MHC element.

If there is a single antigen-specific T-cell receptor and if, as we suggest, the antigen-binding sites of T-cell receptors and Igs are fundamentally no different, what is the structural basis for the difference in antigen recognition between B and T cells? It is possible that the answer lies in the structure of the antigen recognized, rather than in the binding sites themselves. Thus, while the epitopes that occupy the binding sites of Igs are derived entirely from antigen, those recognized by T-cell receptors may be derived in part from the nominal antigen and in part from the restricting MHC element. This view would correspond to various versions of the "altered-self" hypothesis (e.g., refs. 1 and 37) and seems to be supported by recent evidence for the reversible formation of a complex between an immunogenic peptide and an MHC class II molecule (38). Advances in the characterization of T-cell receptors and MHC-restricting elements should provide the purified substances needed to test these and other possibilities.

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