MINIREVIEW

The T-Cell Receptor as Immunoglobulin: Paradigm Regained (44181)

JOHN J. MARCHALONIS,*,[1] SAMUEL F. SCHLUTER,* AND ALLEN B. EDMUNDSON†

Department of Microbiology and Immunology, College of Medicine, University of Arizona, Tucson, Arizona 85724; and Oklahoma Medical Research Foundation,† Oklahoma City, Oklahoma 73104*

Abstract. **The quest to determine the molecular nature of T-lymphocyte receptors for antigen was a "holy grail" to immunologists for over 25 years. This paper updates a review written 15 years ago (Marchalonis JJ, Hunt JC. Proc Soc Exp Biol Med 171:127–145, 1982), which proposed that "these molecules apparently do not bear determinants specified by the major histocompatibility complex, but express Ig-related variable regions and constant regions unique to T-cell products." We review subsequent contributions from molecular biology, protein chemistry, peptide immunochemistry, and structural biology establishing that T-cell receptors (TCRs) are members of the immunoglobulin family restricted to T cells that share 3-dimensional structural features, sequence homology, antigenic cross-reactivity, and common mechanisms of diversification with conventional immunoglobulins. These molecules and their light- and heavy-chain siblings appeared contemporaneously in vertebrate evolution with the emergence of sharks. We illustrate how extrapolation of concepts from immunoglobulin to T-cell receptors has aided in the understanding of these often enigmatic molecules, and, conversely, how concepts derived for T-cell receptors such as the role of "superantigens" can be directly applied to conventional immunoglobulins. A second precept that follows from the symmetry of the combining sites of Igs and TCRs is that MHC-restricted antibodies should exist. Such molecules have in fact been reported, and the x-ray crystallography for T-cell receptors suggests that the combining sites recognizing simultaneously MHC and peptide epitopes resemble the combining sites of antibodies directed against protein determinants. Additional immunoglobulin molecules of nonmammalian species have been detected and characterized based upon conserved homology to TCR and Igs, and it is anticipated that further study will enable the identification of more antigen-specific members of the family in mammals as well.** [P.S.E.B.M. 1997, Vol 216]

Original studies of the immunoglobulin-like-T-cell receptor were supported by grants to J.J.M. from the USPHS (NIAID and NCI); epitope mapping studies by the Arizona Disease Control Research Commission and Baxter Biotechnology/Hyland Division, Duarte, CA; and evolutionary studies by National Science Foundation (MCB9631846). A.B.E. acknowledges support from grants from National Cancer Institute (CA72803) and from the Oklahoma Medical Research Foundation to A.B.E.

[1] To whom requests for reprints should be addressed at Microbiology and Immunology, College of Medicine, University of Arizona, Tucson, AZ 85724-5049.

0037-9727/97/2163-0303$10.50/0

It was established more than 25 years ago that two separate universes of antigen-specific lymphocytes existed (1). The first set, termed B cells for bone marrow–derived lymphocytes, were the precursors of antibody-forming cells and expressed membrane-associated forms of immunoglobulin on their surfaces as receptors for antigen (2, 3). The second lymphocyte type was termed T cells for "thymus derived" and was responsible for cellular immunity and collaborative interaction with B cells. Although T cells, like B cells, showed exquisite specificity in the recognition of antigen, they had the apparently unique and

provocative feature of recognizing antigen in the context of products of the major histocompatibility complex (4, 5). The quest to determine the molecular nature and functional properties of the T-lymphocyte receptor for antigen was "a search for the holy grail" and "a great controversy" challenging immunologists for over 20 years.

In 1982, Marchalonis and Hunt (6) reviewed progress in the characterization of the antigen receptor of thymus-derived lymphocytes and proposed that "these molecules apparently do not bear determinants specified by the major histocompatibility complex (MHC), but express Ig-related variable regions and constant regions unique to T-cell products." This conclusion was based upon serological and biochemical analyses, which in general were limited in comparison to those for B cells because immunoglobulin-secreting plasmacytomas can produce large quantities of monoclonal Ig for study but T-cells express only membrane receptors and have not shown any capacity to secrete them in quantity. Consequently, a generally accepted solution to the T-cell receptor problem required the application of recombinant DNA technology. In 1984, two groups independently used subtractive hybridization approaches to generate cDNA clones from human (7) or mouse (8) T cells that were not expressed by B cells but showed clear homology to immunoglobulin λ light chains. Other workers rapidly joined the fray, and it was quickly established that humans and mice express two types of heterodimeric T-cell receptors with the predominant one consisting of α- and β-chains (9) and a minor one expressing γ- and δ-chains (10). It was then possible to use the derived amino acid sequences to construct computer models for the secondary and tertiary structure of these molecules and their interactive heterodimer (11–17). All of the approaches predicted an immunoglobulin Fab-like structure for the α/β heterodimer, with Novotny *et al.,* (12) stating that "a T-cell α/β receptor molecule probably has an antigen-specific binding site that is fundamentally no different than the conventional binding site of an antibody." Although T-cell receptors (TCRs) are firmly anchored to the cell membrane *via* a transmembrane structure, and have not yet been found to be secreted in any appreciable quantity by living cells, numerous workers have now produced recombinant constructs of T-cell receptor Fv fragments (18–23), free α- or β-chains (24–26), intact glycosylated constructs (27) in quantity for studies of antigen specificity (19), mapping of functional epitope determinants (16, 20, 22), and determination of 3-dimensional structure by x-ray crystallography (24–29). The conclusions of the crystallographic studies confirm the fundamental immunoglobulin-like nature of the T-cell receptor; for one: "The TCR resembles an antibody in the variable Vα and Vβ domains but deviates in the constant Cα domain and in the inter-domain pairing of Cα with Cβ" (27).

This minireview updates that of 1982 (6) in the light of recent discoveries in molecular biology and protein chemistry. There are many excellent recent reviews of T-cell receptor genetics and function. Here, we focus on the Ig characteristics of TCR and address four questions: (i) What is the relationship in an evolutionary sense to immunoglobulin light chains and heavy chains? (ii) How does the immunoglobulin structure of T-cell receptors contribute to our understanding of their unique recognition functions? Conversely, how does a knowledge of apparent T-cell properties such as reactivity with superantigens impact upon our understanding of recognition of B-cell superantigens by antibodies? (iii) How can products of different gene loci share antigenic markers such as idiotypes? (iv) Is there an expectation of further discoveries?

Gene Sequence Establishes TCRs as Immunoglobulin

A particularly vehement attack on the concept of the TCR as Ig, which was written in 1982 and entitled "The T lymphocyte antigen receptor—paradigm lost" (30), summarily dismissed all evidence for Ig, including idiotypic markers, on T cells. The authors argued that, because they had disqualified antibodies as the antigen-specific receptors on T cells and a larger family of possible cell-surface recognition molecules termed the Ig superfamily had recently been discovered, the T-cell receptors must be part of this superfamily. This group includes bona fide Igs and also marginally related molecules such as the Thy-1 alloantigen and cell adhesion molecules. Characterization of the genes specifying TCR, however, established immediately their similarities to Igs, particularly λ light chains (8, 9), and furthermore showed that they used the same mechanisms for the generation of diversity (GOD) that were used in the rearrangement of IgG segments (9, 10). The TCR segments, like those of Ig light and heavy chains, consisted of V, J, and sometimes D segments associated in translocon type organization with exons specifying constant domains. The recombination signal sequences and associated recombination activating genes were required for rearrangement of both immunoglobulin and TCR segments. Following the original characterization of TCR of humans and mice, explosive progress occurred with the extension of the studies to other mammals (31, 32), birds (33), and, most recently, amphibians (34, 35), bony fishes (36), and sharks (37, 38). In general, the combinatorial immune response that consists of bona fide rearranging immunoglobulins and the genetic mechanisms for V/D/J rearrangement is present in all jawed vertebrates from sharks to humans (39, 40). The phylogenetically early appearance of TCR coincident with that of Ig light and heavy chains establishes the fundamental importance to the system of both humoral immunity as represented by antibodies and cellular recognition as represented by TCR to the immune system. TCRs meet the rigorous definition for inclusion in the family of combinatorial Igs (40). Figure 1 is a dendogram constructed using the program CLUSTALW that illustrates the evolutionary clustering of variable domains of TCR α-, β-, γ-, and δ-chains and Ig κ- and λ-light chains and V_H domains. Firstly, the TCR V domains cluster with Ig V domains, particularly with light

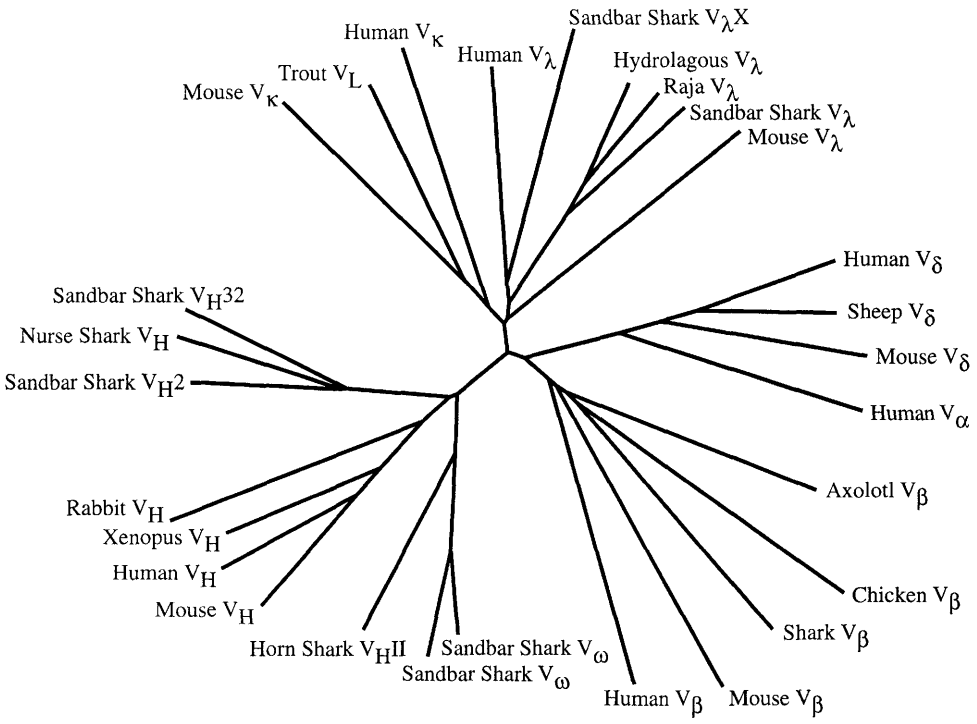

Figure 1. Diagram showing the relationships of immunoglobulin and T-cell receptor derived variable region domains. A phylogenetic analysis was performed using the computer program CLUSTALW (41), which uses the neighbor joining method (42). The radial tree was constructed from these results using the DRAWTREE program (43). Analyses were performed on amino acid sequences derived from gene sequence and are available from the Genbank database.

chains in accordance with previous cladistic analyses (39, 44) and general conclusions (7, 8, 12, 14). In the second place, the Vβ segments of diverse vertebrate species give a pattern consistent with phylogeny.

Figure 2 presents a similar analysis comparing constant (C) domains of TCRs and Igs with the outgroups here being the closely related membrane proximal domains of MHC molecules and β_2-microglobulin and the distantly related superfamily molecule of moths, hemolin (46). This molecule, which appears in hemolymph following bacterial infection, consists of Ig C–like domains. In several comparisons of this nature, using various methods for the construction of dendograms (39, 40, 44), the Cβ domains cluster rather closely with Cλ, but the quantitative similarity of Cα to the other immunoglobulin C domains is not so clear. The identification of the Vα domain with the other Ig V regions (see above) is unambiguous. The 3-dimensional models of Cα constructed from sequence data indicate a compact Ig C domain structure (16) and crystallography indicates some deviation from the canonical Ig fold (27).

The TCR Vα, Vβ, Vγ, and Vδ domains of humans and mice have been analyzed in great detail and found to be highly orthologous structures both in sequence and in arrangement of gene segments (48, 49). Figure 1, above, gen-

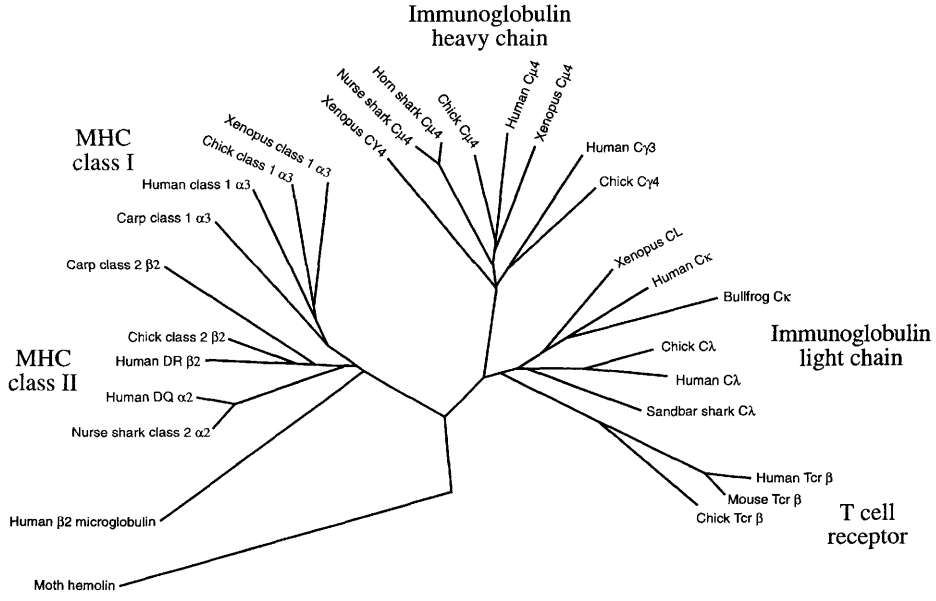

Figure 2. A rooted phylogenetic tree depicting the relationships of immunoglobulin constant type domains among family and superfamily members. The single constant domain of T-cell receptors and light chains was compared with the ultimate carboxyl terminal domain of heavy chains (e.g. Cμ4 for IgM, Cγ3 for IgG). Analyses of MHC products (39, 45) and hemolin (46) were restricted to the constant domain-like segments of these molecules. The tree was constructed using the progressive alignment procedure of Feng and Doolittle (47). Amino acid sequences derived from gene sequence were used in the analysis, and are available from the Genbank database.

eralizes the pattern of relationships for representatives of all gnathostomes. Figure 3 gives comparative alignments of representative Ig and TCR Vβ regions of mammals and sharks to emphasize maximum difference (or conservation) by comparing the two most distant living groups of jawed vertebrates. This figure illustrates the common features of Ig homology in evolution: (i) the molecules show >30% identity in sequence even though the ancestral divergence of humans and sharks was more than 450 million years ago (39), (ii) framework (Fr) and hypervariable (CDR) segments are readily apparent, (iii) strong conservation of sequence is maintained in segments forming β bands involved in inter- and intradomain contacts (27, 56), (iv) the fourth framework (Fr4), which is defined by the joining segment minigenes, is highly conserved and discriminates between TCR/light chain and V_H domain sets (57, 58). The general opening Fr4 motif of the TCR/light chain set is FGXGT(R/or/K)T, whereas that of V_H, including the recently described IgW heavy chain of sharks (59), is WGXGT (uncharged). The positioning of CDRs as loops between β bands is similar in TCR, light chains, and heavy chains, although there is individual variation within the length of the hypervariable loops. Moreover, the HV4 (fourth hypervariable) or CDR4 that lies in the Fr3 of Vβ has been identified as a major contributor to the binding site for superantigens (60). Furthermore, excess statistical variability and the formation of protuberances capable of binding superantigens also occur in comparable regions of Ig V_H domains (61–63) and V_L

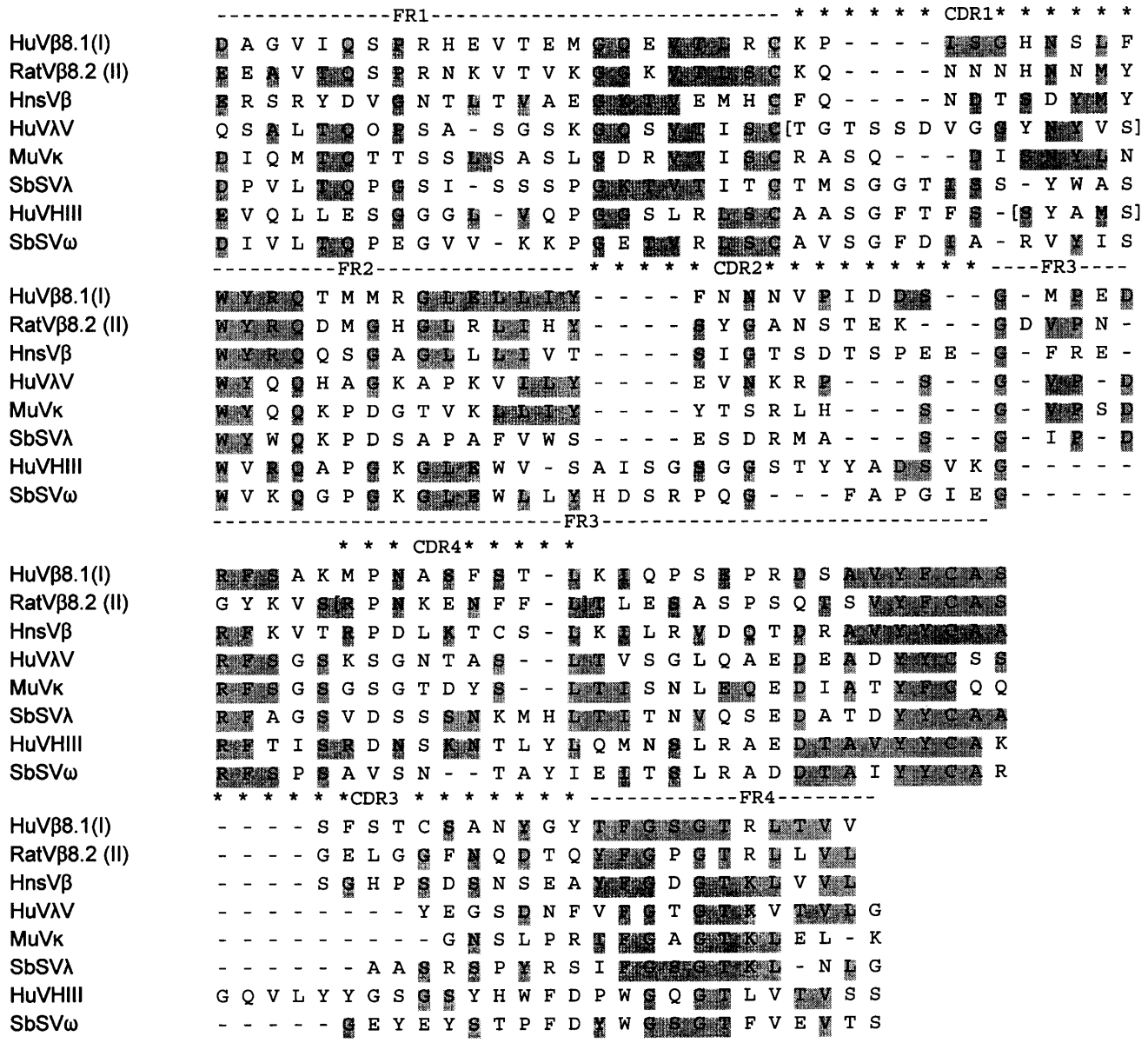

Figure 3. Comparative alignment of representative immunoglobulin and TCR β variable regions of mammals and sharks, assignment of framework (Fr) and complementarity determining regions (CDRs) is based upon Kabat *et al.* (50), as defined for V_H and V_L. HuVβ8.1 (I), human Vβ8.1, subgroup I (7, 50, 51); rat Vβ8.2 (II), rat Vβ8.2 subgroup II (52); HnSVβ, horned shark Vβ (37, 38); HuVλV, human λ-chain subgroup V (53); SbSVλ, sandbar shark λ-chain V region (54); MuVκ, murine Vκ subgroup V (50); HuVHIII, human V_H subgroup III (50); and SbSVω, sandbar shark V region of IgW chain (55). Shading indicates residues identical in Ig and TCRβ V domains.

domains (see below). This is an example of where we have learned about Igs from TCRS as opposed to the more common mode, where we have predicted TCR properties from those of Igs.

A comparative alignment of C_L-type domains including Cλ and Cβ of humans and sharks is given in Figure 4. This illustration includes the location of the β bands defined by x-ray crystallography of the human Cλ molecule Mcg (64). Ig and TCR constant domains consist of two β-pleated sheets, one made up of four β bands and the other comprising three. Both variable and constant domains have this basic structure, but the V domains often have two extra β bands defining CDR2. There is substantial homology within the β bands of the constant domains compared here. This is particularly evident in the 4-2(B) band that is involved in forming the contact surface with either CH or Cα respectively. In addition, the sentinel tryptophan (W) that is 14 residues distal to the first cysteine (C) is uniformly conserved. The constant domains of human and shark λ chains form extremely similar predicted 3-dimensional structures as illustrated by the similarity in β band size and sequence and length of intervening loops. Differences in length between the TCRs and Cλ, and also in comparison between the human and murine Cβ domains, are apparent in structures separating the β bands. The two most obvious are the extra length of the loops between β-bands 3-2(F) and 3-3(G) with much longer segments being present in the Cβ with respect to the light chains. An additional excess length occurs between β bands 4-4(D) and 4-3(E) where the human and horned shark Cβ have considerably longer stretches than do the Cλ domains. Furthermore, this segment distinguishes the human and murine Cβ, because the murine molecule is shorter in this region, and is more like the light chains in this regard. It is of interest that the peptide sequence in this human loop forms an autoantigenic epitope (15, 16). Thus, the sequences of TCR V and C domains predicted from gene sequence are consistent with the "IgT" concept where TCR were hypothesized to have Ig variable domains, but constant regions distinct from those of classical immunoglobulins (6, 65). Thus, it would be proper to conclude that TCRs are new Ig isotypes that are expressed by T cells, but not by B cells.

Three-Dimensional Structure of the TCR and Peptide-MHC Binding

Restriction in T-cell recognition by the MHC presented a formidable theoretical barrier to the early acceptance of Ig as the antigen-specific TCR (4, 5). The dichotomy between recognition of "nominal" antigen and self MHC led to the formulation of competing hypotheses of "dual receptors" for each molecule on T cells as opposed to a "single receptor" that bound both simultaneously as a "neoantigen" (4). Molecular modeling based upon sequence analysis and, most recently, the direct determination of the 3-dimensional structure of TCR showing MHC-restricted binding established that the combining site is very similar to that of Ig Fab and recognizes peptide bound to MHC using a combination of CDRs contributed by the Vα and Vβ domains (27, 28). Characterization of a human peptide MHC–specific molecule (27, 28) and a murine MHC-restricted molecule (28) indicated that the contact regions resembled the undulating surfaces of antibodies directed against protein antigens (66).

Garcia *et al.* (27), in their analysis of the recombinant murine TCR 2C (Vα3, Jα58, Cα; Vβ8.2, Dβ2, Jβ2.4, Cβ2) molecule binding to the dEV8 peptide presented by MHC

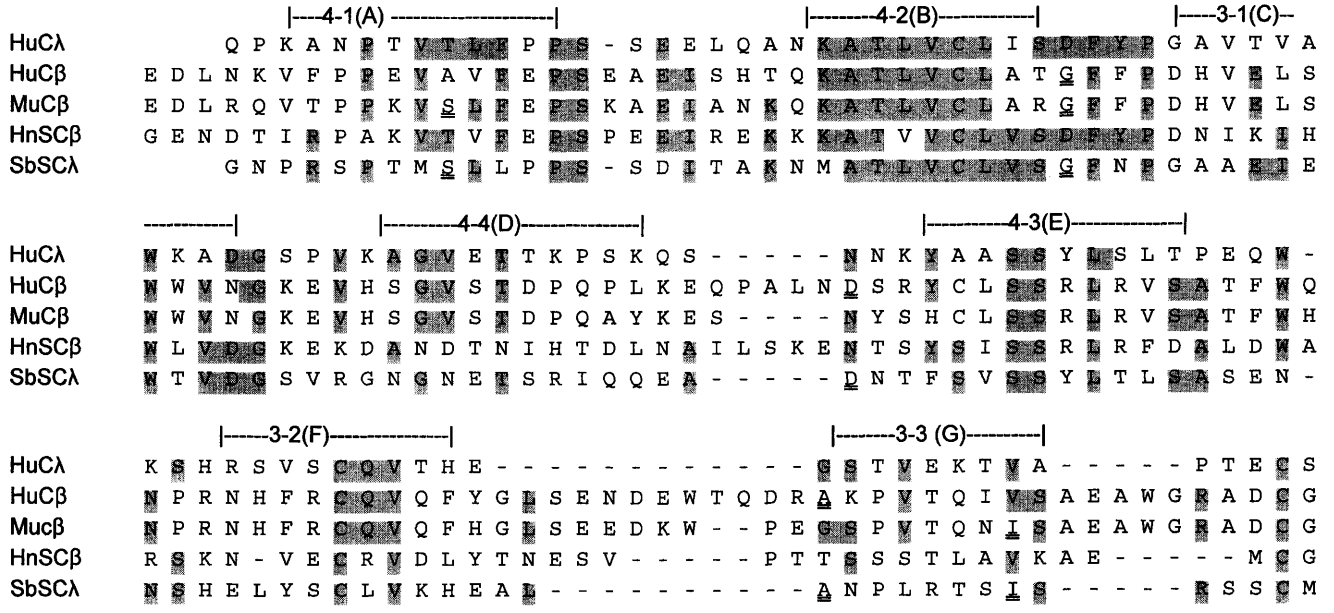

Figure 4. Comparative alignments of Cλ and Cβ domains of mammals and sharks. HuCλ, human Cλ of Mcg (53); HuCβ of YT35 (7, 50, 51); MuCβ, murine Cβ (50); HuS Cβ, horned shark Cβ (37, 38); SbSCλ sandbar shark Cλ (54). β bands are those established by x-ray crystallography for Mcgλ chain (53). Shading indicates residues shared between Cβ and Cλ.

class I, showed that the TCR covered the MHC binding groove with Vα CDR1 and CDR2 loops located over the N-terminal region of the bound peptide, and with the Vβ CDR1 and CDR2 positioned over the C-terminal segment of the peptide. The Vα and Vβ CDR3 segments straddled the central region of the peptide within the MHC α1 and α2 helices. The HV4 (CDR4) segments of both Vα and Vβ did not appear to be sufficiently close to the peptide MHC surface to participate in significant interatomic contacts.

In their crystallographic analysis at 2.5 Å resolution of the complex formed between the recombinant human αβ TCR (A6), the HTLV-1 tax peptide and HLA-A. Garboczi (28) reported that the TCR-binding surface fits diagonally across the MHC peptide binding groove and covers nearly all of the exposed nonimeric peptide and a much larger area of the MHC surface. The CDR3s of both Vα and Vβ chains meet at the center of the binding site over the central tyrosyl residue (Y). The CDR1 of Vα extends from the peptide N terminus to Y5. The Vβ CDR1 lies over the C-terminal segment of the peptide close to Y8. The CDR2 loops of both chains lie over the MHC molecule, with the Vα CDR2 above the α2 domain α helix and the Vβ CDR2 above the α1 MHC domain α helix. Overall, the A6 TCR like that of the 2C TCR above, has a flat contact surface formed by the CDR loops with a central pocket where the CDR3 loops converge. The structure resembles the contact surface of Fabs specific for protein antigens. Thus, the MHC restricted TCRS resemble a subset of antibodies in their combining sites. In the human A6 TCR, CDR1 loops 1 and 3 of both Vα and Vβ contact the target peptide (nominal antigen) with all three Vα CDRs in contact with HLA-A2 α helices. Only the CDR1 loop of Vβ contacts the MHC α helices. Thus, substantial agreement was found in the complexes formed by both human and murine MHC-restricted peptide-binding TCRs, but individual differences consistent with sequence and specificity distinctions were also apparent.

The mature TCR recognition repertoire is generated in the thymus during development by negative elimination of T cells that react strongly to self, and positive selection for T cells that show low affinity or avidity for self MHC (67). This process most probably results in the loss of the vast majority of potential α/β TCR, but enhances the probability of protective responses to viruses and other microbial pathogens located within cells. One would predict that the unselected TCR repertoire should resemble that of antibodies in having representatives that respond to conformational epitopes in the absence of MHC restriction. Consistent with this, examples of γδ TCR reactive with nonpeptide ligands (68), as well as non–MHC-restricted recognition (69) have been reported. Conversely, since αβ TCR that recognize peptide MHC complexes have structures resembling those of antibodies directed against proteins, it is legitimate to ask whether there are subsets of traditional antibodies that show MHC-restricted binding. The existence of antibodies recognizing antigen in the context of MHC was first shown by Ohno *et al.* (70) for antibodies directed against the H-Y antigen in mice. Klinman *et al.* (71) subsequently developed monoclonal antibodies specific for viral antigens presented in the context of either H-2b or d haplotypes. They estimated that the frequency of responding MHC restricted B cells is approximately $1/10^5$, and that MHC-restricted antibody responses can constitute a significant portion of the repertoire to influenza virus. Stryhn *et al.* (72) used recombinant phage display techniques to generate a monoclonal antibody Fab that binds an influenza hemagglutinin peptide in association with a K^k MHC. Comparison with two MHC-restricted T-cell hybridomas established that the antibody and the T cells showed similar fine specificity profiles. Furthermore, monoclonal antibodies specific to HLA/myelin basic protein complexes have been generated in multiple sclerosis (73–76). These will be considered in more detail below. Such antibody constructs may serve as useful additional models for understanding the contribution of CDR loops to MHC-restricted recognition of antigen.

Recognition of Superantigens

The concept of superantigens (SAg) developed from the finding of selective mitogens that could stimulate subsets of T cells expressing particular Vβ gene products (77). Studies involving site directed mutagenesis demonstrated that critical residues involved in the recognition of SAg lay outside of the CDR loops required for binding of nominal antigen. These residues were proposed to be located in segments lateral to the solvent-exposed face of the Vβ domain (60). In particular, amino acid residues in Vβ-Fr1 and Fr3 segments including the HV4 region were identified. A more recent study using site-directed mutagenesis of the Vβ10 domain in responses to Staphylococcal enterotoxins determined that residues in the CDR1 segment also were critical for recognition of SAg/MHC II complexes (78). Consistent with the involvement of CDR1, elimination of the glycosylation site at murine Vβ2Asn24 (N24) by mutation to Gln(Q) or His(H) confers reactivity to the MIs-1 retrovirus-derived endogenous SAg. Determination of the crystal structure of the mouse TCR β-chain 14.3d (Vβ8.2, Jβ2.1, Cβ1) complexed with either *Staphylococcus aureus* enterotoxins CII (SEC2) or C3 (SEC3) establish directly that residues in CDR2, and, to a lesser extent, in CDR1 and Fr3 (Hv4) bind in a cleft between the two domains of the SAgs. Model building including Vα indicated that the Vα domain would make no direct contacts with the SAg, a conclusion that was expected since β-chain alone can bind these SAgs (29). These results indicate that there can be considerable overlap between the SAg-binding and the peptide MHC-binding sites on TCRs. It is possible that SAg can act as a wedge between TCR and MHC to displace the antigenic peptide from the TCR-combining site (28), thereby eliminating specific peptide recognition.

The CDR1 loop is the least variable of the classical CDRs and has been considered a "public idiotype" serving as a marker for a particular gene product (16, 79). Likewise, the FR3 segment which includes the HV4 of TCR and V_H

domains is a marker for the product of an individual variable domain gene. These conclusions can be applied to TCR and Ig light and heavy chains, but are particularly appropriate for TCR, which show little, if any, capacity for somatic mutation (9). Virally specified SAg such as MIs-1 are endogenously incorporated into the mouse genome, but recent evidence indicates the existence of other endogenous SAgs. Kazatchkine's group (80), for example, reported recently that polyclonal human IgG immunoglobulin can synergize with bacterial SAgs in proliferation assays. Consistent with superantigen-type activity of endogenous antibodies, we found that monoclonal murine autoantibodies selected on synthetic Vβ CDR1 peptide epitopes that also reacted with intact TCR acted synergistically with appropriate *S. aureus* SAgs (81). Such autoantibodies may function in immunoregulation by modulating the relative numbers of T cells expressing particular Vβ gene products during normal immunity (15, 82), autoimmunity (16, 82, 83), and retroviral infections (83–86).

Serological Cross-Reactions between Immunoglobulins and TCRs

Characterization of TCR genes showed that the molecules were members of the Ig family, but represented new chains or isotypes that had not been found previously in serum or on B cells. This would not be a surprising finding today because unusual Igs or new isotypes are frequently discovered using recombinant DNA technology. For example, heavy chains having novel properties such as an inability to combine with light chains because of deletion of needed residues have been found in mammals such as the camel (87) and in sharks (88). Furthermore, a new class of potential B-cell receptors incorporating a distinct subset of V_H segments (59, 89) and a heavy chain with six constant domains distinct from those of μ-chain has been reported (59). However, at the height of the controversy (30, 90) the emphasis was placed on disputing the production of conventional rearranged Igs, particularly V_H and V_H-defined idiotypic markers by T cells. Scientific disputes invariably reflect the state of knowledge or ignorance at the particular time. Serological approaches were considered confusing because only some antisera against Igs reacted with T-cell products. Our analysis indicated that the successful antisera reacted with T-cell receptors because of minor cross-reactions associated with the V domains (6). Rigorously specific antisera for C domains of κ and λ light chains and γ and μ heavy chains did not react with T cells or their products in our hands. A molecule isolated from a monoclonal T lymphoma by affinity chromatography had an N-terminal sequence consistent with that of TCR Vβ (91), but it was not practical to isolate sufficient protein from monoclonal T cells grown *in vitro* to obtain extensive physical chemical characterization.

However, on the basis of gene sequence, TCR chains showed extensive sequence homology to Ig chains. The homology (see above) was clearly recognizable in V and C domains and was of the same order of magnitude as the identities found in comparing immunoglobulins of diverse vertebrate species with one another (39, 40). For example, it has long been known that antisera raised in rabbits will detect serological cross-reactions among Ig chains of many mammals (92) and even between homologous molecules of chickens and humans (93). TCR β-chains of humans and mice show extensive serological cross-reactions (94) consistent with high degrees of sequence identity, particularly in the constant domains and in direct comparison of orthologous variable domains. Certain human and shark λ-light chains show greater than 40% identity in sequence (95) and the μ-chains of the two show comparable identity (96). Consistent with this degree of sequence identity, serological cross-reactions mapping to defined peptide epitopes have been reported (97, 98). Thus, in retrospect it is not surprising for molecules such as T-cell receptor β-chains and light chains to cross-react serologically, because these molecules show on average greater than 30% identity to one another. We addressed this hypothesis directly by synthesizing nested hexadecameric peptides overlapping by five residues that duplicated the complete covalent sequence of the T-cell receptor β-chain specified by the *YT35* gene (14). As a calibration, we carried out a comparable synthesis duplicating the human λ5 light chain Mcg (99). The cross-reactivity between TCR β-chains and Ig light chains was assessed by determining the capacity of rabbit antisera to human or murine Igs to react with these peptides. The antisera were a large panel that included commercial diagnostic reagents as well as antibodies used in previously published studies. The reactivities observed were consistent with homologies to λ and κ light chains as illustrated in Figures 3 and 4 above. The strongest reactivity in enzyme-linked immunosorbent assay (ELISA) binding and competitive inhibition was with a peptide that corresponds to the "switch peptide" of light chains. The sequence is encoded by the C-terminal region of J segment (Fr4) and the N-terminus of the constant domain. Other regions reactive with anti-light chain sera corresponded respectively to CDR1 and Fr3 segments of the V region. In the overall evolutionary context, it is noteworthy that the peptide segments modeling Fr3 and the joining segment were also major epitopes shared between light chains of sharks and humans. A Cβ epitope corresponding to a segment predicted to loop out of the tight globular structure between β bands 4-4(D) and 4-3(E) was also observed.

Possibly the most striking example of a shared epitope between TCR and light chain is that defined by the sequence of the joining segment of the TCR β-chain (57, 58, 100, 101) because antibodies raised against this segment bind to intact T and B cells in immunocytofluorescence (102), and react with appropriate intact immunoglobulins, light chains, and T-cell receptor in ELISA and Western blot assays. The peptide sequence used in immunization was ANYGYTFGSGTRLTVV, which is shown in Table I aligned with a panel of synthetic peptides and corresponding sequences of

Table I. Summary Comparison of J Segment Sequences of Synthetic Peptides and Proteins Tested for Binding of Rabbit Antibodies to the Synthetic HuJβ1.2 Peptide

Synthetic peptide	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		Binding of anti-Jβ[a]	
	.... CDR3						- - - - - - - - - - - - Fr4 - - - - - - - - - -												
HuJβ1.2	A	N	Y	G	Y	T	F	G	S	G	T	R	L	T	V	V		- - -	strong
Jβ1.2 (12κ)	A	N	Y	G	Y	T	F	G	S	G	T	K	L	T	V	V		- - -	strong
Jβ1.2 (12T)	A	N	Y	G	Y	T	F	G	S	G	T	T	L	T	V	V		- - -	negative
Jλ (Mcg)						V	F	G	T	G	T	K	V	T	V	L	G	- - -	moderate
Jα (PY14)	S	A	S	K	I	I	F	G	S	G	T	R	L	S	I	R		- - -	moderate
Representative sequence																			
Chicken Jλ					G	I	F	G	A	G	T	T	L	T	V	L	G	- - -	negative
Mouse Jκ (S107)					L	T	F	G	A	G	T	K	L	E	—	L	K	- - -	moderate
SbS Jλ (lc5.1)					S	I	F	G	S	G	T	K	L	N	—	L	G	- - -	moderate
Hu Jκ (κIII)					R	T	F	G	Q	G	T	K	V	E	—	I	K	- - -	moderate
Mouse Jβ1.1		N	T	E	V	F	F	G	K	G	T	R	L	T	V	V		- - -	strong

[a] Binding to synthetic peptides and proteins was assessed by ELISA and Western blot analysis. Binding was specifically inhibitable by appropriate peptides. Based upon data of references 14, 20, 45, 57, 58, 98, 101. Hu, human; SbS, sandbar shark.

light chains and TCR that either react with rabbit antibody to the synthetic Jβ sequence or do not bind. Analysis of the reactions of affinity purified rabbit antibody raised against the Jβ peptide on various purified immunoglobulins and T-cell receptors as assessed by Western blot analysis is shown in Figure 5. The anti-Jβ antibodies were analyzed extensively using synthetic epitopes as well as immunoglobulin (101) and were found to show extremely high specificity for an epitope defined by the conserved Fr4 signature sequence FGXGT(RorK)VL. Substitutions at the variable position X had little effect on the reactivity. However, the nature of the residue at position six is critical and must be either of the two positively charged amino acids arginine (R) or lysine (K). Substitution with neutral or negatively charged residues drastically reduces reactivity with antibodies, even with peptides identical at all other positions to the immunizing Jβ synthetic peptide. Rabbits, the species that produce the most useful antibodies tend to have a negatively charged residue (glutamic acid [E]) at the homologous position. Consistent with the sequence dependency for antigenicity, immunoglobulins of chickens and turkey fail to react with antibody. The anti-Jβ bound the synthetic Jα peptide illustrated here, and reacted, as expected, with murine TCR β-chains as well as with light chains of humans and a variety of other species. In addition, a cross-reaction with immunoglobulin heavy chain of humans was observed and this is consistent with the crucial Jβ sequence being found in some human J_H segments. Since the preponderance of recent studies is carried out using molecular biology, it is essential to establish that the gene products are actually synthesized and expressed. We were able to use this antibody in Western blot analysis of a detergent extract of sandbar shark spleen cells (Fig. 5B) to show that the TCR β-chain as well as Ig light chain were actually generated.

Sharing of Idiotypic Markers between Immunoglobulins and TCR

An ingenious approach to characterizing the antigen specific T-cell receptor was to produce antibodies against V region defined idiotypes of immunoglobulins and use these reagents in functional studies and isolation of T-cell molecules (103). A good deal of success was reported initially in this area by McKern *et al.* (104), Eichman and Rajewsky (105), and Binz and Wigzell (103, 106). Although idiotypes requiring interaction between V_H and V_L such as the anti-arsonate idiotype were reported to be associated with T cells (107), the most extensively described studies involved V_H-linked (108–110) cross-reactive idiotypes (105, 106). These studies fell out of favor when it was established that V_H genes are not rearranged in functional T cells (111). This finding cannot refute the "IgT hypothesis" because gene clusters encoding distinct Ig chains are, in general, unlinked. However, in its simplest form this finding was taken as definitive evidence that conventional V_H structures could not form TCRs. Furthermore, its relevance to antigenic cross-reactivity was even less persuasive because peptide epitopes can be shared by products of unlinked genes that share a common evolutionary history as illustrated above. The question to be asked here is whether the recognition sites of distinct molecules, encoded by unlinked genes, can share antigenic cross-reactivity. This is an essential element of the internal image concept as recently reviewed by Bona (79). There are numerous examples of anti-idiotypic antibodies raised against high-affinity antibodies directed against hormone ligands that subsequently act as the ligand in physiological assays (112–114). A noteworthy example of idiotype cross-reactivity between the combining sites of two totally unrelated molecules was found by Volanakis and

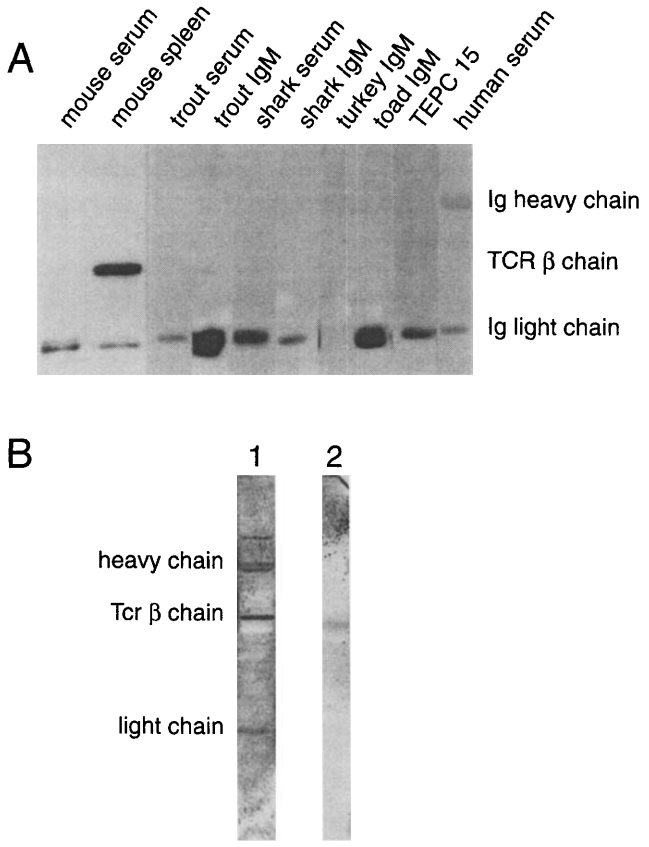

Figure 5. Immunoglobulin J segment cross-reactive epitopes are found on immunoglobulins and T-cell receptors throughout phylogeny. This is demonstrated here by the reactions of affinity purified rabbit antibody against the human Jβ peptide in Western blot analysis. (A) Reactions of whole serum, purified immunoglobulins, and tissue extracts as indicated. The mouse spleen sample was a Triton X-100 detergent extract of mouse spleen tissue. TEPC15 is a murine IgA/κ monoclonal. Although many components are visualized by a protein staining (not shown) in the serum and tissue samples, the affinity purified antibody to Jβ peptide reacts with either few components which correspond to immunoglobulin or TCR chains or does not react at all (e.g., turkey IgM). (B) Identification of a candidate TCR β-chain in shark spleen. Lane 1 shows the reactivity of anti-Jβ antibodies with a Triton X-100 detergent extract of spleen tissue. The Ig heavy and light chains were identified with specific rabbit anti-shark Ig sera which did not stain the putative TCR β band. Lane 2 shows that all the reactivities are specifically inhibited with the free Jβ peptide.

Kearny (115), who showed that C-reactive protein, an acute phase protein that binds phosphoryl choline, possesses a determinant which is cross-reactive with the V_H-defined idiotype of the phosphorylcholine binding myeloma protein TEPC-15. The conservation of the binding site geometry and idiotypy was found to be even more impressive because a lectin from the horseshoe crab *Limulus polyphemus,* which also binds phosphoryl choline, has definite sequence homology to C-reactive protein (116) and cross-reacts serologically with the human molecule (117, 118). Furthermore, certain monoclonal antibodies specific for TEPC-15 idiotopes show clear reactivity with both limulus lectin and C-reactive protein and will block the binding of the hapten. The cross-reactive idiotope was defined by peptide sequence corresponding to the juncture of the CDR2 and Fr3 segments of the TEPC-15 V_H domain. A more recent example in which a sequence correlation was found between a T-cell receptor and a monoclonal antibody followed from the discovery by Zhou and Whittaker that monoclonal anti-idiotypic antibodies directed against murine monoclonal antibodies specific for a myelin basic protein peptide (MBP)(acetyl-19) could modulate T cells and the development of murine experimental allergic encephalomyelitis (72). A monoclonal antibody (F28C4) was found that binds the MBP acetyl peptide in an extended confirmation with the central residues more tightly bound than the terminal residues in a situation resembling that for the binding of MBP to T-cell receptors (74). The mAb used an unusual Vλ light chain, VλX, that showed 75% overall sequence homology between the regions that contain the CDR3 of the light chain V_L and V_H and the VDJ junction of a TCR β-specific for myelin basic protein. Interestingly, a panel of VλX-containing monoclonal and polyclonal antibodies bound myelin basic protein, but were not polyreactive with other potential self antigens (75). The V_H domain apparently did not contribute to the cross-reactive Id because Igs containing the same heavy chain but distinct V_L usage were unreactive. Thus, both theoretical and biochemical data exist supporting combining site-related Ids shared among different molecules and between TCR and conventional Ig-V domains.

Modeling of T-Cell Receptors Based upon Immunoglobulin Homology

Igs usually consist of disulfide bonded heterodimers (and multiples thereof) comprising a short subunit containing one V- and one C-domain (light chains or TCR chains) and a longer segment containing one V- and two to six C-domains (the heavy chain). In the case of conventional Igs, the class or isotype is defined by the heavy chain involved. TCRs are heterodimers of short subunits α/β or γ/δ and have not been formally given isotypic designation, although they are Igs restricted in expression to T cells. It is noteworthy that the short V-C subunits show much greater diversity in evolution than do the heavy chains (40), although all meet the criterion of showing 30% or better identity to immunoglobulin domain prototypes (39). All are products of rearranging gene segments, V, J, and sometimes D in the formation of the intact variable domain. Another feature of the Ig family, as opposed to the superfamily, is that non-Ig domains are not covalently incorporated into either classical Igs or TCRs. A number of investigators have utilized the homology between TCR and immunoglobulin domains to construct models of secondary (11) and tertiary structures (12–14). Such models have been constructed based upon detailed comparisons with individual characterized immunoglobulin light chains (14–16) and also by alignments against a large panel of known light chain structures (13). In principle, the results are the same in providing

tentative models that T-cell receptor V regions are conventional V regions and that the constant domains of β-chains and α-chains resemble immunoglobulin C domains in sandwich β-pleated sheet structure, but show differences such as the presence of extra long loops directed towards the variable domain (14, 16, 119) and predicted compact size (α-chain). The crystallographic data for murine β-chains (120) confirms directly the presence of one of these loops, but the structure of Cα has not yet been determined because it is "disordered" in the recombinant Fab structure analyzed (28). Here, we will focus on the Ig structure of the TCR β-chain and its comparison with light chains because of its clear-cut nature and the larger amount of evidence presently available.

Figure 6 illustrates salient features of T-cell receptor structure based upon direct comparison with characterized λ light chains (A), a schematic alignment of a universal TCR/Ig V_H and V_L domain (B), and depicts a three dimensional representation of V_L and Vβ domains illustrating the relative positions of CDRs 1, 2, 3, and 4 (C). The overlap of the derived structure of the YT35 human β-chain (Vβ8.1/Dβ1.1/Jβ1.2/Cβ1) on the 3-dimensional structure of the human λ light chain Mcg (Vλ5) based upon x-ray crystallography (53) illustrates that the V domains both have the typical immunoglobulin domain structure consisting of one sheet of four β-pleated strands and the second containing three. The region corresponding to CDR2 lies between two structures which have certain characteristics of β bands in light chains, but are not completely characteristic. However, in heavy chains these tend to be the extra clearly defined β bands termed C' and C''. The conventional CDRs are in the same relative positions in Vλ and Vβ, but there are differences in length in comparison of individual molecules. Overall, the CDR1 Vβ domains tend to be shorter than those of Vλ domains; for instance, fourteen residues in Mcg as opposed to ten in the YT35 β-chain. The CDR1 is the least variable in T-cell receptors (7–9). The location of HV4 (CDR4) is in the loop connecting β bands 4-3 (E) and 3-2 (F). The location expanded in Figure 6C is indicated here by shading. Cβ and Cλ domains have the characteristic immunoglobulin constant domain 4-3 β-banded structure but differ in the size of the loops connecting the β bands. In particular, the large loop connecting bands 3-3 (G) and 3-2 (F) is considerably larger in the Cβ than it is in the Cλ and is large enough to interfere structurally with the rotation of the Vβ domain. A second extra large loop that can interfere with rotation of the Vβ connects β 4-4 (D) and 4-3 (E) in the human structure. This loop in the mouse would be roughly the size of that found in the light chain domain. This Cβ loop segment in humans is a site for N-linked glycosylation and is also a target for autoantibodies (16).

The line diagram in Figure 6B is a schematic representation of an intact variable segment formed by recombination of the germline V segment with appropriate D and J minigenes. It represents the position of frameworks and the four hypervariable segments in their relative positions in

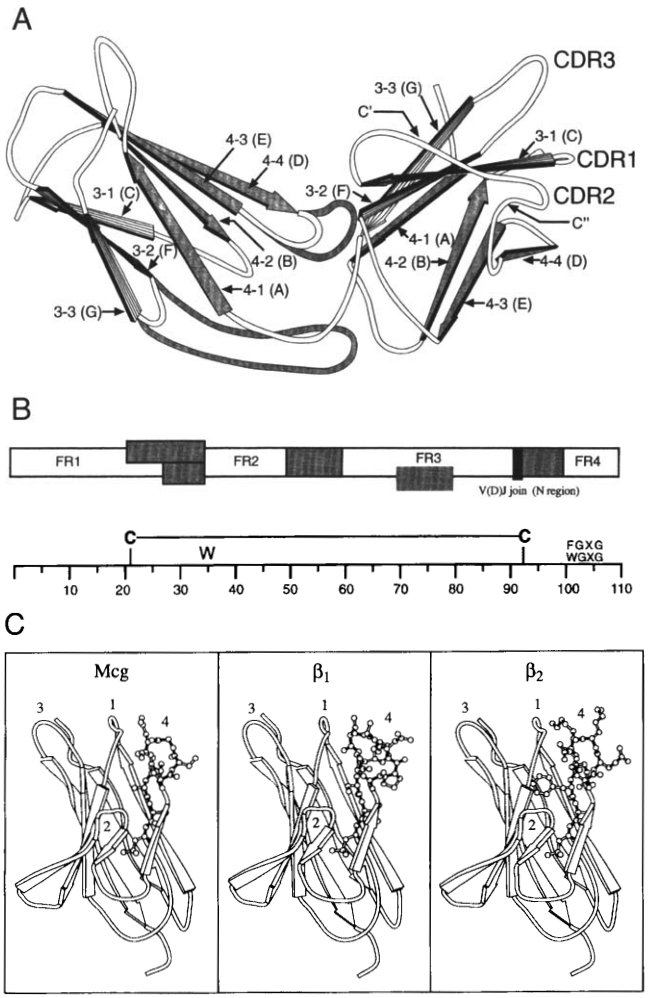

Figure 6. Models of TCR structure based upon comparison with Ig light chains. (A) Three-dimensional model of Ig domains of human TCR β superimposed onto the structure of the human λ light chain Mcg. Excess loops of the β-chain are indicated. (Based upon Refs. 14, 16, and 53.) (B) Schematic line diagram for a universal TCR, light-chain, and heavy-chain variable domain illustrating locations of framework segments (Frs), classical complementarity domains (CDRs), and the HV4 or CDR4 involved in binding of superantigens. (C) Left, MolScript model (121) of the V domain of the Mcg λ-type immunoglobulin light chains (53). Complementarity-determining regions (CDR) which contribute to an antigen-combining site of an antibody are labeled 1, 2, and 3. The loop labeled 4 is a ball-and-stick model of both polypeptide backbone (black) and side chain (white) atoms representing the sequence marked CDR4 in Table I. Middle, model of the Mcg V domain with the CDR4 sequence of a human β1 T-cell receptor grafted onto loop 4. This model was slightly altered by interactive model building and positional refinement to optimize the positioning of side chains. Right, Mcg V domain model with loop 4 modified to accommodate the CDR4 sequence of a human β2 T-cell receptor.

V_L, V_H, and Vβ structures. The alignments given in Figure 7 compare the framework 3 regions of TCR β-chains and immunoglobulin κ and λ light chains. The type 1 β-chains have many common structural features shared with the light chains; in particular, a recognizable Asp-Arg-Phe-Ser-Ser (DRFSS) motif four or five residues distal to the initial Gly, and an Asp 6 residues N-terminal from the Cys residues at the carboxyl terminus. This region contains regulatory idiotopes of light chains (122, 123) and superantigen binding

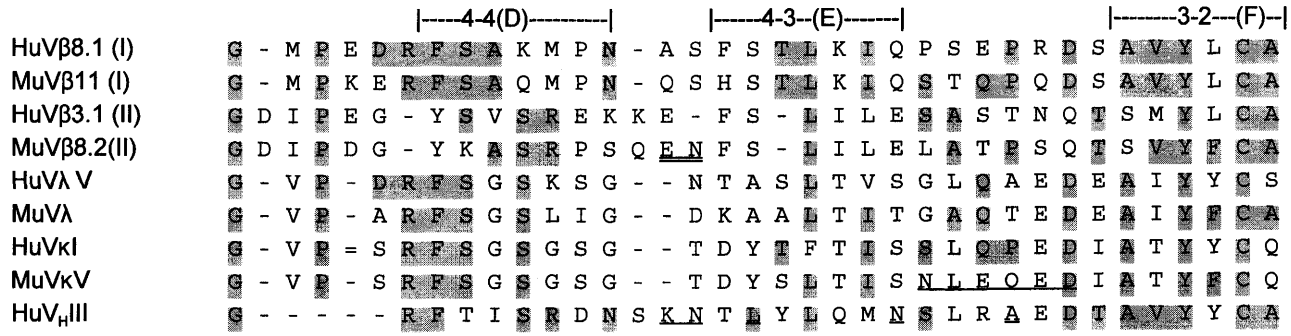

Figure 7. Comparative alignment of Fr3 segments of human and mouse TCR β-chains, κ and λ light chains, and a human V_H sequence (18/2 from Kabat *et al.* [50]). TCR β Type I and II chains are as defined by Kabat *et al.* HuVλ V is the human λ light chain Mcg, MuVλ is MOPC315, HuVκI is REI, and MuVκV is a Vκ10 sequence (122). The location of β bands 4-4 (D), 4-3 (E), and 3-2 (F) are based upon crystallographic studies of Mcg (53). The residues EN in the mouse Vβ8.2 sequence are those identified by Pullen *et al.* (60) as involved in recognition of Staphyloccocal superantigen. The underlined residues in the MuVκV sequence are implicated in the formation of the A48 regulatory idiotope (122). The underlined residues in the HuV_H III sequence are those identified by Hillson *et al.* (61) as implicated in the binding of Staphylococcal protein A. In the numbering system of Kabat *et al.* (50), the conserved RF sequence corresponds to residues 64 and 65 in the Vβ, 61 and 62 in the light chains, and 66 and 67 in the V_H. The universally conserved CYS corresponds to position 92 in Vβ, 88 in the light chains, and 92 in the V_H.

sites in Vβ chains (29, 60) and in V_H domains (124). Except for the human Vκ1 (Rei) used in this comparison, all of the sequences have a leucyl residue (L) 15 residues N-terminal to the Cys. This highly conserved residue lies in β band 4-3 (D) and is buried within the 3-dimensional structure of the domain. The assumption of a common root for Ig light chains and TCR chains can readily be tested by comparisons of both CDR and Fr segments within the V domains. One of the most interesting sets includes the residues between CDR2 and CDR3, referred to as Fr3 in immunoglobulin terminology (50).

Note that the carboxyl-terminal segments of Fr3 are extremely conserved around the cys residue participating in the intra-domain disulfide bond as illustrated here. At the beginning of Fr3, TCR molecules of the β1, but not the β2, subgroup, also share the canonical λ-chain sequence of DRFSS. These residues complete the turn leading to the outer strand (4-4) of the 4-chain β-pleated sheet. A conserved aspartic acid residue nine positions from the end of the Fr3 of the Bβ1 chain is repeatedly seen in immunoglobulin chains as part of a distorted helical turn at the distal end of the V domain. However, no specific functional role for this residue has been identified in a typical λ light chain like Mcg (53).

Within the boundaries assigned originally to Fr3 in light chains, a fourth hypervariable region was later defined for immunoglobulin heavy chains (125) and subsequently for TCR chains. This region corresponds to the last two residues of β strand 4-4 (D), the first five residues of β strand 4-3 (E), and the segment bridging the two. In the model of the polypeptide backbone shown for the Mcg backbone in Figure 6B (left panel), this region is the most lateral of the 4 CDR loops at the tip of the V domain. It is located well away from the conventional antigen combining sites of an antibody. In the latter, CDRs 1, 2, and 3 of the light chain are arranged around a pseudo 2-fold axis of rotation with their counterparts from a heavy-chain V domain to form active sites of highly diverse shapes and sizes. Two segments listed in Figure 2A illustrate the range of the diversity found in CDR4. In the human Vβ8.1 (type I), the CD4 nonapeptide has no formal electrical charges, while human Vβ3.1 (type II) contains three positively charged residues (Arg, Lys) and two negatively charged Glu residues. In the middle and right panels of Figure 6B, the side chains of representative β1 and β2 CDR4 segments are grafted onto a MOLScript model (121) of the V domain of the Mcg λ-chain (53). This model has been given an orientation to provide clear views of the CDR4 side chains. It is evident that the key side chains protrude into the solvent space and are readily accessible for binding to superantigen molecules. In the examples presented, the cognate antigens are likely to be polar but uncharged when attracted to the type I receptor. For interactions with the type II receptor, the antigen determinants are expected to include oppositely charged groups arranged in a pattern compatible with the distribution of the long side chains of the CDR4 loop. Most murine studies have involved the Vβ type II structure because products of Vβ8 genes dominate in expression in inbred mice (126). A similar protruding structure has been proposed for the superantigen binding site of V_H3 heavy-chain domains (62).

On the teleogical principle that Nature would be loathe to waste a good design, we often wondered in the early days of the x-ray analysis of Igs why the prominent and accessible lateral loop now called CDR4 was retained in the evolution of these molecules. That question was decisively answered when the CDR4 residues in T-cell receptors were identified as constituents of putative binding sites for superantigens (29, 60) and for microbial proteins in the case of V_H domains (61, 63). As Figure 6C clearly shows, it is straightforward to place β-chain sequences onto λ-chain scaffolding. One of the best markers to illustrate the simi-

larities in the three models is the Leu side chain which terminates the CDR4 loop just below and to the right of the CDR2 loop. Thus, the well-established crystal structure of immunoglobulin domains (53, 66) coupled with the sequence homologies to immunoglobulin provided very useful means of predicting general structural features and active sites in the products of T-cell receptor genes. Conversely, findings of reactivity to superantigens by products of individual Vβ genes provided a guidepost for reinterpreting immunoglobulin structures in defining a fourth hypervariable region that has proven to be functional as an idiotypic marker for products of particular V_L genes (123) and a recognition site for microbial proteins in heavy chains.

Conclusions and Unresolved Issues

The concept of a specific antigen receptor on lymphocytes was fundamental to Burnet's clonal selection theory (127) and to early theories of the regulation of specific immunity (128, 129), but this receptor could not be characterized in more detail than to consider it to be "antibody-like." This was essentially the "minimal hypothesis" (2), which became more sharply focused as knowledge of immunoglobulins grew to include the precept that distinct isotypes defined by individual heavy-chain constant domains could bear the same combining sites for antigen in terms of specificity and idiotypic cross-reactivity. A critical conceptual stumbling block facing the concept of the TCR as immunoglobulin was the propensity for TCR to recognize peptide antigens presented by the MHC. This property was not considered a defining characteristic of the antibody combining site. Moreover, some investigators found that antisera and isolation conditions eminently suitable for study of B cells surface Ig did not allow the isolation of the Ig-like product of T cells (3, 130). Furthermore, the association of combining sites with V_H-defined idiotypes was questioned when it was established that the V_H segments did not rearrange within T cells. A further complication was that T cells do not secrete large quantities of T-cell receptor and the few molecules associated with the plasma membrane (approximately 10^4 per cell) rendered it practically impossible to obtain enough material to carry out detailed studies. However, it was possible to obtain microgram quantities for immunization and partial sequence characterization (131–134). The isolation of cDNA products specifying TCR α, β, γ, and δ genes allowed direct comparison with the sequences of Ig chains and, through recombinant DNA technology, enabled the construction of TCR domains and intact structures for functional, serological and physicochemical analysis.

All of the latter approaches supported the hypothesis that the TCR was a novel form of Ig restricted to T-cells. This novel Ig showed characteristic features of members of the Ig "family" sequence homology, the requirement for the same rearrangement mechanisms found for other Ig chains, and the early evolutionary appearance coincident with that of Ig light and heavy chains. Furthermore, TCRs do not form covalent association with non-Ig domains which is a frequent occurrence among members of the broader "superfamily." Recent crystallographic studies, moreover, establish that the representative MHC-restricted αβ TCR of humans and mice have combining site surfaces resembling those of antibodies specific for protein antigens. The ability to recognize nominal antigen, here a peptide, as well as the surface α-helices of the MHC did not require the addition of special combining sites, but were carried out using traditional CDR1, CDR2 and CDR3 segments. Furthermore, crystallographic analysis of the binding of a murine TCR β chain to Staphylococcal superantigens indicates that the traditional CDRs 1 and 2 as well as the CDR4 located in Fr3 on the lateral face of the V domain participate in binding of these molecules reactive with individual germline gene products. It is worthwhile to note here that the concept of a fourth hypervariable region in this segment was first proposed by Capra and Kehoe in 1974 (125) based upon analysis of heavy chain variable region sequences. In this case, the concept of superantigens developed for T cells was directly applicable to understanding exposed loops in both Ig V_H and V_L domains with a number of recent studies demonstrating the existence of superantigen binding sites in V_H3 (61, 62) and V_H4 (63) domains. Consistent with the role of Fr3 segments in defining SA-reactive sites of V_H and Vβ, the segment of light chains is responsible for the definition of public idiotopes found in rheumatoid diseases (123).

The recent crystallographic studies of a few TCR have proven to be extremely encouraging to our understanding of TCR structures. However, parallels to the situation with conventional B-cell–derived Igs will no doubt hold. Foremost among these is the expectation that the individual monoclonal receptors generated by recombinant DNA technology will show considerable variation from one to another in combining site structure and possibly in other regions as a result of significant mutations elsewhere in the framework or CDR regions. This is the situation which has been found in detailed structural analyses of individual monoclonal Igs derived originally from multiple myeloma or subsequently, by forming hybridomas secreting monoclonal antibodies of defined specificity.

The breakthrough that enabled the isolation of cDNA copies of T-cell receptor genes was the application of subtractive hybridization (7, 8) in which mRNA specifying B-cell products were removed and message specifying proteins in a size range under 60 kDa were selected. Might it be possible that other receptors were missed because of these two considerations? It is probable that larger molecules would not be found, and this is a worthwhile consideration because products of approximately 90,000 that are serologically related to T-cell receptor α-chains have been reported (136). Do the Ig properties of T-cell receptors provide a means for isolating the TCR genes and proteins? Without rehashing early experiments, it can be concluded that antibodies cross-reactive between TCR and immunoglobulins

could be readily produced following alignment of sequences and synthesis of peptides corresponding to regions of strong identity. One of the most clear-cut of these is the joining segment peptide of light chains and TCR β-chains that show strong homology including the characteristic presence of a large positively charged arginine or lysine such that immunization leads to the production of antibodies reactive with light chains and TCR β-chains in vertebrate species ranging from sharks to humans. This segment was not suitable for the generation of DNA oligomers because of considerable degeneracy. However, a highly conserved stretch of sequence around the initial cys in the disulfide loop of the Cβ domain had a sequence also shared by λ-light chains and some heavy-chain constant domains (137). Construction of polymerase chain reaction primers from this region allowed the isolation of TCR β-chains and immunoglobulin light chains, and also the discovery of a new heavy chain consisting of one variable and six constant domains (55).

Thus, we suggest that the antigen-specific TCRs are ancient and well-established members of the family of rearranging Igs and that their binding and functional properties can now be analyzed in great detail. Some surprises may still be found in the discovery of new antigen receptors built according to this general design plan.

We thank Ms. Diana Humphreys for expert assistance in preparation of this manuscript. This manuscript is dedicated to the memory of Alan F. Williams.

1. Miller JFAP, Mitchell GF. Thymus and antigen-reactive cells. Transplant Rev **1:**3–42, 1969.
2. Marchalonis JJ, Cone RE. Biochemical and physiological properties of lymphocyte surface immunoglobulin. Transplant Rev **14:**3–49, 1973.
3. Vitetta ES, UHR JW. Synthesis, transport, dynamics and fate of cell surface Ig and alloantigens in murine thymocytes. Transplant Rev **14:**50–75, 1973.
4. Doherty PC, Gotze D, Trinichiri IG, Zinkernagel RM. Models for recognition of virally modified cells by immune thymus derived lymphocytes. Immunogenetics **3:**517–524, 1976.
5. Benacerraf B, McDevitt HO. Histocompatibility-linked immune response genes. Science **175:**273–279, 1972.
6. Marchalonis JJ, Hunt JC. The antigen receptor of thymus-derived lymphocytes: Progress in the characterization of an elusive molecule. Proc Soc Exp Biol Med **171:**127–145, 1982.
7. Yanagi T, Yoshikai Y, Leggett K, Clark SP, Aleksander I, Mark TW. A human T cell–specific cDNA clone encodes a protein having extensive homology to immunoglobulin chains. Nature **308:**145–149, 1984.
8. Hedrick SM, Neilsen EA, Cavalier J, Cohen DI, Davis MM. Sequence relationships between putative T-cell receptor polypeptides and immunoglobulins. Nature **308:**153–158, 1984.
9. Kronenberg M, Siu G, Hood LE, Shastri N. The molecular genetics of the T-cell antigen receptor and T-cell antigen recognition. Annu Rev Immunol **4:**529–591, 1986.
10. Saito H, Kranz D, Takagaki Y, Hayday A, Eisen H, Tonegawa S. A third rearranged and expressed gene in a clone of cytotoxic T-lymphocytes. Nature **312:**36–39, 1984.
11. Beale D, Coadwell J. Unusual features of the T-cell receptor C domains are revealed by structural comparisons with other members of the immunoglobulin superfamily. Comp Biochem Physiol **85B:**205–215, 1986.
12. Novotny J, Tonegawa S, Saito H, Kranz DM, Eisen HN. Secondary, tertiary and quaternary structure of T-cell specific immunoglobulin like polypeptide chains. Proc Natl Acad Sci U S A **83:**742–746, 1986.
13. Chothia C, Boswell DR, Lesk AM. The outline structure of the T-cell αβ receptor. EMBO J **7:**3745–3755, 1988.
14. Kaymaz H, Dedeoglu F, Schluter SF, Edmundson AB, Marchalonis JJ. Reactions of anti-immunoglobulin sera with synthetic T-cell receptor peptides: Implications for the three-dimensional structure and function of the Tcr β chain. Int Immunol **5:**491–502, 1993.
15. Marchalonis JJ, Kaymaz H, Dedeoglu F, Schluter SF, Yocum DE, Edmundson AB. Human autoantibodies reactive with synthetic autoantigens from T-cell receptor β chain. Proc Natl Acad Sci U S A **89:**3325–3329, 1992.
16. Marchalonis JJ, Schluter SF, Wang E, Dehghanpisheh K, Lake D, Yocum DE, Edmundson AB, Winfield JB. Synthetic autoantigens of immunoglobulins and T-cell receptors: Their recognition in aging, infection and autoimmunity. Proc Soc Med Exp Biol Med **207:**129–147, 1994.
17. Claverie J-M, Prochnicka-Chalufour A, Bougueleret L. Implications of a Fab-like structure for the T-cell receptor. Immunol Today **10:**10–14, 1989.
18. Ward ES. Secretion of T-cell receptor fragments from recombinant *Escherchia coli* cells. J Mol Biol **224:**885–890, 1992.
19. Novotny J, Ganju RK, Smiley ST, Hussey RE, Luther MA, Recny MA, Siliciano RF, Reinherz EL. A soluble single chain T-cell receptor fragment endowed with antigen combining properties. Proc Natl Acad Sci U S A **88:**8646–8650, 1991.
20. Lake DF, Bernstein RM, Hersh EM, Kaymaz H, Schluter SF, Marchalonis JJ. Construction and serological characterization of a recombinant human single chain T-cell receptor (scTcr). Biochem Biophys Res Commun **201:**1502–1509, 1994.
21. Lake DF, Bernstein RM, Schluter SF, Marchalonis JJ. A method for generating diverse single chain proteins using a universal $(Gly_4\text{-}ser)_3$ encoding oligonucleotide. Biotechniques **19:**700–702, 1995.
22. Lake DF, Helgerson S, Landsperger WJ, Marchalonis JJ. Physical and epitope analysis of a recombinant human TCR Vα/Vβ construct support the similarity to immunoglobulin. J Protein Chem **16:**309–320, 1997.
23. Soo Hoo WF, Lacey MJ, Denzin LK, Voss EWJ, Hardman KD, Kranz DM. Characterization of a single chain T-cell receptor expressed in *Escherichia coli.* Proc Natl Acad Sci U S A **89:**4759–4763, 1992.
24. Fields BA, Ysern X, Poljak RJ, Shao X, Ward ES, Mariuzza RA. Crystallization and preliminary x-ray diffraction study of a bacterially produced T-cell antigen receptor Vα domain. J Mol Biol **239:**339–341, 1994.

24a. Fields BA, Ober B, Malchiodi EL, Lebedeva MI, Braden BC, Ysern X, Kim J-K, Xuguang S, Ward ES, Mariuzza RA. Crystal structure of the Vα domain of a T cell antigen receptor. Science **270:**1821–1824, 1995.

25. Malchiodi EL, Eisenstein E, Fields BA, Ohlendorf DH, Schlievert PM, Karjalainen K, Mariuzza RA. Superantigen binding to a T cell receptor β chain of known three-dimensional structure. J Exp Med **182:**1833–1845, 1995.
26. Bentley GA, Boulot G, Karjalainen K, Mariuzza RA. Crystal structure of the β chain of a T cell antigen receptor. Science **267:**1984–1987, 1995.
27. Garcia KC, Degano NM, Stanfield RL, Brunmark A, Jackson MR, Peterson PA, Teyton L, Wilson IA. An αβ T cell receptor structure at 2.5 Å and its orientation in the TCR-MHC complex. Science **274:**209–219, 1996.
28. Garboczi DN, Ghosh P, Utz U, Fan QR, Biddison WE, Wiley DC. Structure of the complex between human T-cell receptor, viral peptide and HLA-A. Nature **384:**134–141, 1996.
29. Fields BA, Malchiodi EL, Hongmin L, Ysern X, Stauffachers CV, Schlievert PM, Karjalainen K, Mariuzza RA. Crystal structure of a T-cell receptor β-chain complexed with a superantigen. Nature **384:**188–192, 1996.
30. Jensenius JC, Williams AF. The T lymphocyte antigen receptor-paradigm lost. Nature **300:**583–588, 1982.
31. Lamoyi E, Angiolillo AL, Mage RG. An evolutionary conserved rabbit T-cell receptor β chain variable region. Immunogenetics **23:**266–270, 1986.
32. Ishiguro N, Tanaka A, Shinagawa AM. Sequence analysis of bovine T-cell receptor α chain. Immunogenetics **31:**57–60, 1990.

33. Cooper MD, Chen C-HL, Lucy RP, Thompson CB. Avian T-cell ontogeny. Adv Immunol **50:**87–116, 1991.
34. Fellah JS, Kerfourn F, Charlemagne J. Evolution of the T cell receptor genes: Extensive diversity of Vβ families in the Mexican Axolotl. J Immunol **153:**4539–4545, 1994.
35. Chretien I, Marcuz A, Fellah J, Charlemagne J, Du Pasquier L. The T cell receptor β genes of *Xenopus*. Eur J Immunol **27:**763–771, 1997.
36. Partula S, De Guerra A, Fellah JS, Charlemagne J. Structure and diversity of the T cell antigen receptor β-chain in a teleost fish. J Immunol **155:**699–706, 1995.
37. Rast JP, Haire RN, Litman RT, Pross S, Litman GW. Identification and characterization of T-cell antigen receptor related genes in phylogenetically diverse vertebrate species. Immunogenetics **42:**204–212, 1995.
38. Rast JP, Litman GW. T cell receptor gene homologs are present in the most primitive jawed vertebrates. Proc Natl Acad Sci U S A **91:**9248–9252, 1994.
39. Marchalonis JJ, Schluter SF. Evolution of variable and constant domains and joining segments of rearranging immunoglobulins. FASEB J **3:**2469–2479, 1989.
40. Marchalonis JJ, Bernstein RM, Shen SX, Schluter SF. Emergence of the immunoglobulin family: Conservation in protein sequence and plasticity in gene organization. Glycobiology **6:**657–663, 1996.
41. Thompson JD, Higgins DG, Gibson TJ. CLUSTALW: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acid Res **22:**4673–4680, 1994.
42. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol **4:**406–425, 1987.
43. Felsenstein J. PHYLIP (Phylogeny Inference Package) version 3.572c. Distributed by author. Seattle, WA: Department of Genetics, University of Washington, 1996.
44. Marchalonis JJ. Lymphocyte membrane glycoprotein: Molecular and functional properties. In: Marchalonis, JJ, ed. The Lymphocyte: Molecular and Functional Properties. New York: Marcel Dekker, pp307–390, 1987.
45. Marchalonis JJ, Hohman VS, Kaymaz H, Schluter SF. Shared antigenic determinants of immunoglobulins in phylogeny and in comparison with T-cell receptors. Comp Biochem Physiol **105B:**423–441, 1993.
46. Sun SC, Lindstrom I, Boman HG, Faye I, Schmidt O. Hemolin: An insect immune protein belonging to the immunoglobulin superfamily. Science **250:**1729–1732, 1990.
47. Feng DF, Doolittle RF. Progressive alignment and phylogenetic tree construction of protein sequences. Methods Enzymol **183:**375–387, 1990.
48. Hood L, Rowen L, Koop BF. Human and mouse T-cell receptor loci: Genomics, evolution, diversity and serendipity. Ann N Y Acad Sci **758:**390–412, 1995.
49. Clark SP, Arden B, Kabeliz D, Mak TW. Comparison of human and mouse T-cell receptor variable gene segment subfamilies. Immunogenetics **42:**531–540, 1995.
50. Kabat EA, Wu TT, Perry HM, Gottesman KS, Foeller C. Sequences of Proteins of Immunological Interest (5th ed). Bethesda, MD: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, 1991.
51. Toyonaga B, Mak TW. Genes of the T-cell antigen receptor in normal and malignant T-cells. Annu Rev Immunol **5:**585–620, 1987.
52. Herrmann T, Hochgrebe T, Torres-Nagel NE, Huber B, Hunig T. Control of the rat T-cell response to retroviral and bacterial superantigens by class II MHC products and TCRβ-Vβ8.2 alleles. J Immunol **152:**4300–4309, 1994.
53. Edmundson AB, Ely KR, Abola EE, Schiffer M, Pagniotopoulos N. Rotational allomerision and divergent evolution of domains in immunoglobulin light chains. Biochemistry **14:**3933–3936, 1975.
54. Hohman VS, Schluter SF, Marchalonis JJ. Complete sequence of a cDNA clone specifying sandbar shark immunoglobulin light chain: Gene organization and implications for the evolution of light chains. Proc Natl Acad Sci U S A **89:**276–280, 1992.
55. Bernstein RM, Schluter SF, Shen S, Marchalonis JJ. A new high molecular weight immunoglobulin class from the carcharhine shark: Implications for the properties of the primordial immunoglobulin. Proc Natl Acad Sci U S A **93:**3289–3293, 1996.
56. Chothia C, Lesk AM, Ghirardi E, Tomilson IM, Walter G, Marks JD, Llewellyn MB, Winter G. Structural repertoire of the human VH segments. J Mol Biol **227:**799–817, 1992.
57. Schluter SF, Marchalonis JJ. Antibodies to synthetic joining segment peptide of the T-cell receptor β-chain: Serological cross-reaction between products of T-cell receptor genes, antigen binding T-cell receptors and immunoglobulins. Proc Natl Acad Sci U S A **83:**1872–1876, 1986.
58. Marchalonis JJ, Schluter SF, Hubbard RA, Diamanduros A, Barker WC, Pumphrey RSH. Conservation of immunoglobulin variable and joining region structure and the design of universal anti-immunoglobulin antibodies reactive with antigen-binding T cell receptors. Int Rev Immunol **3:**241–273, 1988.
59. Bernstein RM, Schluter SF, Shen S, Marchalonis JJ. A new high molecular weight immunoglobulin class from the carcharhine shark: Implications for the properties of the primordial immunoglobulin. Proc Natl Acad Sci U S A **93:**3289–3293, 1996.
60. Pullen AM, Bill J, Kubo RT, Marrack P, Kappler JW. Analysis of the interaction suite for the self superantigen MIs-1^a on T cell receptor Vβ. J Exp Med **173:**1183–1192, 1991.
61. Hillson JL, Karr NS, Oppliger IR, Mannik M, Sasso EH. The structural basis of germline-encoded VH3 immunoglobulin binding to staphylococcal protein A. J Exp Med **178:**331–336, 1993.
62. Silverman GJ. Human antibody responses to bacterial antigens: Studies of a model conventional antigen and a proposed model B cell superantigen. Int Rev Immunol **9:**57–78, 1992.
63. Domiati-Saad R, Attrep JF, Brezinschek H-P, Cherrie AH, Karp DR, Lipsky PE. Staphylococcal-enterotoxin D functions as a human B cell superantigen by rescuing VH4-expressing B cells from Apoptosis. J Immunol **156:**3608–3620, 1996.
64. Edmundson AB, Ely KR, Abola EE, Schiffer M, Pagniotopoulos N. Rotational allomerision and divergent evolution of domains in immunoglobulin light chains. Biochemistry **14:**3933–3936, 1975.
65. Graves M. Antigen receptors on T-lymphocytes: A solution in sight? Nature **256:**92–93, 1975.
66. Padlan EA. Anatomy of the antibody molecule. Mol Immunol **31:**169–217, 1994.
67. Jameson SC, Hogquist KA, Bevan MJ. Positive selection of thymocytes. Annu Rev Immunol **13:**93–126, 1995.
68. DeLibero G. Sentinel function of broadly reactive human γδ cells. Immunol Today **18:**22–26, 1997.
69. Kaufmann SHE. γ/δ and other unconventional T lymphocytes: What do they see and what do they do? Proc Natl Acad Sci U S A **93:**2272–2279, 1996.
70. Ohno S, Matsunaga T, Epplen JT, Hozumi T. Interaction of viruses and lymphocytes in evolution, differentiation and oncogenesis. In: Fougereau M, Dausset J, Eds. Immunology 80—Progress in Immunology IV. London: Academic Press, pp577–598, 1980.
71. Klinman NR, Froscher BG, Cruise KM, Sherman LA. The antigen receptor and MHC gene families. Antibodies that are MHC restricted. In: Kelsoe G, Shulze DH, Eds. Evolution and Vertebrate Immunity. Austin: University of Texas Press, pp247–259, 1987.
72. Stryhn A, Andersen PS, Pedersen LO, Svejgaard A, Holm A, Thorpe CJ, Fugger L, Buus S, Engberg J. Shared fine specificity between T-cell receptors and an antibody recognizing a peptide/major histocompatibility class I complex. Proc Natl Acad Sci U S A **93:**10338–10342, 1996.
73. Zhou S-R, Whitaker JN. Specific modulation of T cells and murine experimental allergic encephalomyelitis by monoclonal anti-idiotypic antibodies. J Immunol **150:**1629–1642, 1993.
74. Maier CC, Galin FS, Jarpe MA, Jackson P, Krishna NR, Gautam AM, Zhou S-R, Whitaker JN, Blalock JE. A $V\lambda_X$-bearing monoclonal antibody with similar specificity and sequence to encephalitogenic T cell receptors. J Immunol **153:**1132–1140, 1994.
75. Galin FS, Maier CC, Zhou S-R, Whitaker JN, Blalock JE. Murine $V\lambda_X$ and $V\lambda_X$-containing antibodies bind human myelin basic protein. J Clin Invest **97:**486–492, 1996.
76. Puri J, Arnon R, Gurevich E, Teitelbaum D. Modulation of the immune response in multiple sclerosis. J Immunol **158:**2471–2476, 1997.
77. Johnson HM, Russell JK, Pontzer CH. Staphylococcal enterotoxin superantigens. Proc Soc Exp Biol Med **198:**765–771, 1991.
78. Bellio M, Lone Y-C, de la Calle-Martin O, Malissne B, Abastado J-P, Korilsky P. The Vβ complementarity determining region 1 of a major

histocompatibility complex (MHC) class I–restricted T cell receptor is involved in the recognition of peptide/MHC I and superantigen/MHC II complex. J Exp Med **179:**1087–1097, 1994.
79. Bona CA. Internal image concept revisited. Proc Soc Exp Biol Med **213:**32–42, 1996.
80. Baudet V, Hurez V, Lapeyre C, Kaveri SV, Kazatchkine MD. Intravenous immunoglobulin (IVIg) modulates the expansion of Vβ3+ and Vβ17+ T cells induced by Staphylococcal enterotoxin B superantigen *in vitro.* Scand J Immunol **43:**277–282, 1996.
81. Dehghanpisheh K, Marchalonis JJ. Retrovirally induced mouse anti-TCR monoclonals can synergize the *in vitro* proliferative T cell response to bacterial superantigens. Scand J Immunol **45:**645–654, 1997.
82. Marchalonis JJ, Schluter SF, Wilson L, Yocum DE, Boyer JR, Kay MMB. Natural human antibodies to synthetic peptide autoantigens: Correlations with age and autoimmune disease. Gerontology **39:**65–79, 1993.
83. Lake DF, Schluter SF, Wang E, Bernstein RM, Edmundson AB, Marchalonis JJ. Autoantibodies to the α/β T-cell receptors in human immunodeficiency virus (HIV) infection: Dysregulation and mimicry. Proc Natl Acad Sci U S A **91:**10849–10853, 1994.
84. Dehghanpisheh K, Huang D, Schluter SF, Watson RR, Marchalonis JJ. Production of IgG autoantibodies to T-cell receptors in mice infected with the retrovirus LP-BM5. Int Immunol **7:**31–36, 1995.
85. Marchalonis JJ, Ampel NM, Schluter SF, Garza A, Lake DF, Galgiani JN, Landsperger WJ. Analysis of autoantibodies to T-cell receptors among HIV-infected individuals: Epitope analysis and time course. Clin Immunol Immunopathol **82:**174–189, 1997.
86. Watson RR, Dehghanpisheh K, Huang DS, Wood S, Ardestani SK, Liang B, Marchalonis JJ, Wang Y. T-cell receptor Vβ CDR1 peptide administration moderates immune dysfunction and cytokine dysregulation induced by murine retrovirus infection. J Immunol **155:**2282–2291, 1995.
87. Muyldermans ST, Atarhouch J, Saldanha J, Barbosa JA, Hamers R. Sequence and structure of VH domain from naturally occurring camel heavy chain immunoglobulins lacking light chains. Protein Eng **7:**1129–1135, 1994.
88. Greenberg AS, Avila D, Hughes M, Hughes A, McKinney EC, Flajnik MF. A new antigen receptor gene family that undergoes rearrangement and extensive somatic diversification in sharks. Nature **374:**168–173, 1995.
89. Greenberg AS, Hughes AL, Guo J, Avila D, McKinney EC, Flajnik MF. A novel "chimeric" antibody class in cartilaginous fish: IgM may not be the primordial immunoglobulin. Eur J Immunol **26:**1123–1129, 1996.
90. Cotinho A, Meo T. Immunoglobulin gene expression by T lymphocytes (editorial review). Scand J Immunol **18:**70–100, 1983.
91. Mackel-Vandersteenhoven A, Vasta GR, Waxdal MJ, Marchalonis JJ. Segmental homology between T cell receptors and immunoglobulin variable regions: Evidence that antisera to synthetic J_H1 peptide react with murine and human T cell products. Proc Soc Exp Biol Med **178:**476–485, 1985.
92. Nash DR, Mach JP. Immunoglobulin classes in aquatic mammals. Characterization by serologic cross-reactivity, molecular size and binding of human free secretory component. J Immunol **107:**1424–1430, 1971.
93. Mehta PD, Reichlin M, Tomasi TB Jr. Comparative studies of vertebrate immunoglobulins. J Immunol **109:**1272–1277, 1972.
94. Dedeoglu F, Hubbard RA, Schluter SF, Marchalonis JJ. T-cell receptors of man and mouse studied with antibodies against synthetic peptides. Exp Clin Immunogenet **9:**95–108, 1991.
95. Hohman VS, Schluter SF, Marchalonis JJ. Diversity of immunoglobulin light chain clusters in the sandbar shark (*Carcharhinus plumbeus*). J Immunol **155:**3922–3928, 1995.
96. Shen SY, Bernstein RM, Schluter SF, Marchalonis JJ. Heavy chain variable regions in carcharhine sharks: Development of a comprehensive model for the evolution of V_H domains among the gnathanstomes. Immunol Cell Biol **74:**357–364, 1996.
97. Marchalonis JJ, Hohman VS, Kaymaz H, Schluter SF. Shared antigenic determinants of immunoglobulins in phylogeny and in comparison with T-cell receptors. Comp Biochem Physiol **105B:**423–441, 1993.
98. Marchalonis JJ, Schluter SF, Yang H-Y, Hohman VS, McGee K, Yeaton L. Antigenic cross-reactions among immunoglobulin of diverse vertebrates (Elasmobranchs to man) detected using Xenoantisera. Comp Biochem Physiol **101A:**675–687, 1992.
99. Marchalonis JJ, Dedeoglu F, Kaymaz H, Schluter SF, Edmundson AB. Antigenic mapping of a human λ light chain: Correlation with 3-dimensional structure. J Protein Chem **11:**129–137, 1992.
100. Schluter SF, Rosenshein IL, Hubbard RA, Marchalonis JJ. Conservation among vertebrate immunoglobulin chains detected by antibodies to a synthetic joining segment peptide. Biochem Biophys Res Commun **145:**699–705, 1987.
101. Marchalonis JJ, Schluter SF, Hubbard RA, McCabe C, Allen RC. Immunoglobulin epitopes defined by synthetic peptides corresponding to joining-region sequence: Conservation of determinants and dependence upon the presence of an arginyl or lysyl residue for cross-reaction between light chains and T cell receptor chains. Mol Immunol **25:**771–784, 1988.
102. Shankey TV, Schluter SF, Marchalonis JJ. Flow cytometric analysis of human lymphocytes using affinity purified antibody to T cell receptors synthetic J region. Cell Immunol **118:**526–531, 1989.
103. Binz H, Wigzell H. Shared idiotypic determinants on B and T lymphocytes reactive against the same antigen determinants. V. Biochemical and serological characteristics of naturally occurring soluble antigen-binding T-lymphocyte derived molecules. Scand J Immunol **5:**559–571, 1976.
104. McKearn TJ, Stuart FP, Fitch FW. Anti-idiotypic antibody in rat transplantation immunity. I. Production of anti-idiotypic antibody in animals repeatedly immunized with alloantigens. J Immunol **113:**1874–1882, 1974.
105. Rajewsky K, Eichmann K. Antigen receptors of T helper cells. Contemp Top Immunobiol **7:**69–112, 1977.
106. Binz H, Wigzell H. T cell receptors with allo-major histocompatability complex specificity from rat and mouse. Similarity of size, plasmin susceptibility and localization of antigen-binding region. J Exp Med **154:**1261–1278, 1981.
107. DeLuca D, Decker JM, Marchalonis JJ. Surface expression and partial characterization of an arsonate hapten-specific idiotype-bearing T-cell receptor. Cell Immunol **90:**514–530, 1985.
108. Owen FL, Spurll GM, Panageas E. Thy^d, a new thymocyte alloantigen linked to IgH-1. Implication for a switch mechanism for T cell antigen receptors. J Exp Med **155:**52–60, 1982.
109. Tokuhisa T, Taniguchi M. Two distinct allotypic determinants on the antigen-specific suppressor and enhancing T cell factors that are encoded by genes linked to the immunoglobulin heavy chain locus. J Exp Med **155:**126–139, 1982.
110. Suzan M, Bored A, Lieberkind J, Valsted F, Rubin B. The 59,36-Ig-idiotypes: Genetic linkage to Ig-C_H loci, T cell dependent of synthesis and possible specificities. Scand J Immunol **14:**673–685, 1981.
111. Kraig E, Kronenberg M, Kapp JA, Pierce CW, Samuelson LE, Schwartz RH, Hood L. T and B cells that recognize the same antigen do not transcribe similar heavy chain variable region gene segments. J Exp Med **158:**192–198, 1983.
112. Lombes M, Edelman IS, Erlanger BF. Internal image properties of a monoclonal autoantiidiotypic antibody and its binding to aldosterone receptors. J Biol Chem **268:**2528–2536, 1989.
113. Leu JG, Chen BX, Diamanduros AW, Erlanger BF. Idiotype mimicry and the assembly of a supramolecular structure: An anti-idiotypic antibody that mimics taxols in its tubulin-microtubule interaction. Proc Natl Acad Sci U S A **91:**10690–10694, 1994.
114. Izadyar L, Friboulet A, Remy MH, Roseto A, Thomas D. Monoclonal anti-idiotypic antibodies as functional internal images of enzyme active sites: Production of a catalytic antibody with a cholinesterase activity. Proc Natl Acad Sci U S A **90:**8876–8880, 1993.
115. Volanakis JE, Kearney JF. Cross-reactivity between C-reactive protein and idiotype determinants on a phosphocholine-binding murine myeloma protein. J Exp Med **153:**1604–1614, 1981.
116. Robey FA, Liu T-Y. Limulin: A C-reactive protein from *Limulus polyphemus.* J Biol Chem **256:**969–975, 1981.
117. Vasta GR, Marchalonis JJ, Kohler H. Invertebrate recognition protein cross-reacts with an immunoglobulin idiotype. J Exp Med **159:**1270–1276, 1984.
118. Ying S-C, Marchalonis JJ, Gewurz AT, Siegel JN, Jiang H, Gewurz BE, Gewurz H. Reactivity of anti-human CRP and SAP monoclonal antibodies with Limulin and Pentraxins of other species. Immunology **76:**324–330, 1992.
119. Rubin B, Arnaud J, Caspar-Bauguil S, Conte F, Huchenq A. Biologi-

cal function of the extracellular domain of the T-cell receptor constant region. Scand J Immunol **39:**517–525, 1994.
120. Bentley GA, Boulot G, Karjalainen K, Mariuzza RA. Crystal structure of the beta chain of a T cell antigen receptor. Science **267:**1984–1987, 1995.
121. Kraulis PJ. MolScript: A program to produce both detailed and schematic plots of protein structures. J Appl Crystallogr **24:**946–950, 1991.
122. Zaghouani H, Bonilla FA, Meek K, Bona C. Molecular basis for expression of the A48 regulatory idiotope on antibodies encoded by immunoglobulin variable-region genes from various families. Proc Natl Acad Sci U S A **86:**2341–2345, 1989.
123. Potter KN, Li Y, Abraham GN, Capra D. The MoAb-VκIIIb cross-reactive idiotope on A27a (Humkv325) encoded kappa chains maps to framework region 3. Scand J Immunol **44:**305–313, 1996.
124. Roben PW, Salem AN, Silverman GJ. V_H3 family antibodies bind domain D of staphylococcal protein A. J Immunol **154:**6437–6445, 1995.
125. Capra JD, Kehoe JM. Variable region sequences of five human immunoglobulin heavy chains of the V_HIII subgroup: Definitive indication of four heavy chain hypervariable regions. Proc Natl Acad Sci U S A **71:**845–848, 1974.
126. Kurt RA, Park JA, Panelli MC, Schluter SF, Marchalonis JJ, Carolus B, Akporiaye ET. T lymphocytes infiltrating sites of tumor rejection and progression display identical Vβ usage but different cytototoxic activities. J Immunol **154:**3969–3974, 1995.
127. Burnet FM. The Clonal Selection Theory of Acquired Immunity. Nashville, TN: Vanderbilt University Press, 1959.
128. Marchalonis JJ, Gledhill VX. An elementary stochastic model for the induction of antibody production and tolerance. Nature **220:**608–611, 1968.
129. Bretscher P, Cohn M. A theory of self-non-self discrimination. Paralysis and induction involve the recognition of one and two determinants on an antigen, respectively. Science **169:**1042–1049, 1970.
130. Cone RE, Rosenstein RW. Isolation of T cell membrane proteins with antisera to non-isotypic determinants of immunoglobulins. Evidence that IgT "light" chains are not identical to B cell κ chains. Mol Immunol **18:**67–77, 1981.
131. Kubo RT, Roehm N. Preparation and characterization of a "pan-reactive" rabbit anti-mouse T-cell receptor antiserum. Mol Immunol **23:**869–878, 1986.
132. Marchalonis JJ, Vasta GR, Hunt JC, Wang AC, Minowada J. Some T-cell leukemia lines express surface markers related to a restricted set of human V_H-determinants. Cell Immunol **77:**161–175, 1983.
133. Cone RE, Marchalonis JJ. Partial amino acid sequence of monoclonal extracellular antigen-specific T-cell proteins. Immunol Invest **22:**541–552, 1993.
134. Mackel AM, Craddock GR, Warr GW, DeLuca D, Marchalonis JJ. Xenoanti-sera against a murine T cell tumor product cross-react with immunoglobulin Fab determinants. J Immunol **131:**1582–1590, 1983.
135. Acuto O, Fabbi M, Smart J, Poole CB, Protentis J, Royer HD, Schlossman SF, Reinherz EL. Purification and NH2-terminal amino acid sequencing of the β subunit of a human T-cell antigen receptor. Proc Natl Acad Sci U S A **81:**3851–3855, 1984.
136. Cone RE. Soluble extracellular antigen-specific T-cell immunoproteins. J Leukoc Biol **59:**605–612, 1996.
137. Schluter SF, Hohman VS, Edmundson AB, Marchalonis JJ. Evolution of immunoglobulin light chains: Complementary DNA clones specifying sandbar shark constant regions. Proc Natl Acad Sci U S A **86:**9961–9965, 1990.