Structural Aspects and Chemistry of T Cell Receptor Recognition of Antigen-MHC Complexes

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Modern immunology developed from two divergent and contentious perspectives. Elie Metchnikoff proposed in 1884 that leukocytes (white blood cells) might be important contributors to both natural and acquired immunity, primarily via their capacity to phagocytose foreign material.[1] This *cellular* view of immunity was challenged by Paul Ehrlich, who proposed a *humoral* paradigm in which preexisting receptors on the surface of cells could react with foreign invaders on the basis of their chemical complementarity;[2] ultimately, excess receptors would be released from the cell as antibodies. Despite Metchnikoff and Ehrlich sharing the 1908 Nobel Prize in Medicine, the humoral paradigm has dominated immunology for most of the 20th century. Yet as is the case in so many other worldly conflicts, both views were in fact correct and complementary.

Self-nonself discrimination refers to the process by which the vertebrate immune system identifies and reacts specifically against "foreign" entities such as infectious disease agents, tumors, or tissue transplanted from a genetically nonidentical individual. Specificity is engendered by B and T lymphocytes, which are similar morphologically and develop from common stem cell precursors. B cells express antibodies either as cell-surface receptors or secreted molecules (immunoglobulins, Igs). Immunoglobulins bind to a virtually infinite array of antigenic surfaces. T cells, on the other hand, bear a surface T cell receptor (TCR) which typically is specific for a composite of "foreign" peptide antigen bound to a major histocompatibility complex (MHC) molecule. These two recognition systems are intimately linked by the requirement of most B cell responses (secreted Ig) for "T cell help" in the form of specific growth and differentiation factors known as lymphokines. Subsets of T cells may also act directly to kill virally infected or transformed cells.

T cell receptors occur in two forms, $\alpha\beta$ and $\gamma\delta$, which are on distinct cells. $\alpha\beta$ T cells predominate in the blood and lymphoid organs such as lymph nodes and spleen. $\gamma\delta$ T cells occur as a minor species in these environments (5-15%) and in some species occupy unique niches in the skin and on the mucosal surfaces of the gastrointestinal and urogenital tracts. Furthermore, while peptide-MHC complexes are clearly the major targets of $\alpha\beta$ TCR, the antigenic ligands recognized by $\gamma\delta$ TCR are likely to be different (Y. Chien and H. Schild, manuscript submitted) and appear to include microbial antigens commonly encountered at epithelial boundaries.

Here we present a comparison of immunoglobulins and T cell receptors. The emphasis is on peptide-MHC recognition by the $\alpha\beta$ T cell receptor. By highlighting similarities and differences between them, our goal is to provide a framework in which to view similar structures having distinct functions. In addition, we present an overview of recent developments in the field which are likely to yield new insights into the structure and function of antigen-specific T cell receptors.

Immunoglobulins have been well characterized both structurally and functionally. By contrast and in keeping with the humoral/cellular dichotomy over the past 100 years, molecular data regarding T cell receptors have until recently been sparse. Aside from obstacles (now overcome) in cloning TCR genes, this disparity is due largely to difficulties in studying both the structure of cell-surface TCR and its interaction with membrane-bound MHC molecules.
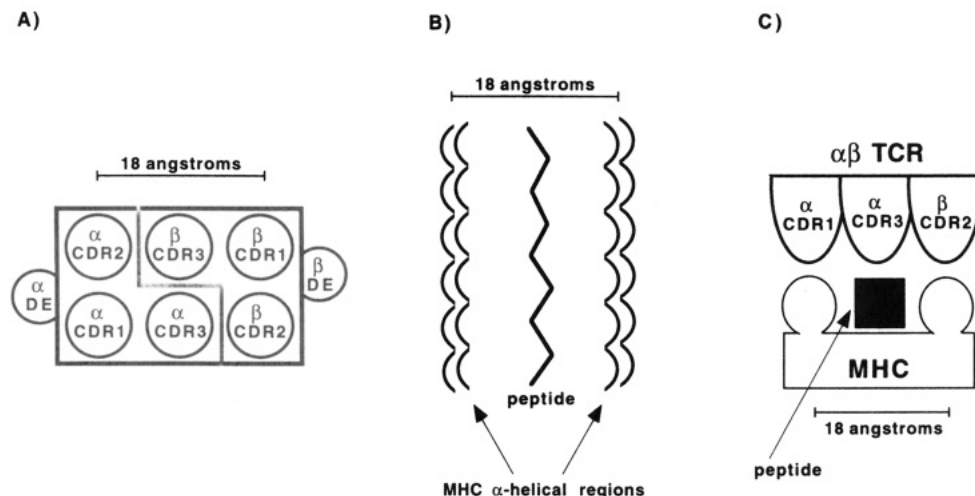
Structure and Genetics: The Ig Paradigm

The antigen binding sites of both immunoglobulins and T cell receptors are formed from two disulfide-linked protein chains: heavy (H) and light (L) in Ig and either α and β or γ and δ in TCR. As is commonly the case in large proteins, these molecules are composed of independently folding domains, each of roughly 100 amino acids. The N-terminal domains of Ig and TCR chains together form an antigen binding site and are quite variable (V) relative to the C-terminal constant (C) domains which are common to particular receptor groups.

Ig structure has been well characterized by X-ray crystallography (see the Account in this issue by Davies and Chacko). Each Ig domain is essentially a sandwich of two β-pleated sheets. In V domains these sheets support three loops called complementarity determining regions (CDRs). Thus a given H-L heterodimer possesses six CDRs which confer that antibody's specificity. Rigidity conferred by Ig domain pairing and CDR loop stabilization by buried, hydrogen-bonded asparagines permit surface expression of aromatic residues on CDRs that would otherwise be energetically unfavorable, e.g., tyrosine and tryptophan.[3] In turn, aromatic residues on the receptor surface contribute substantially to the energy of antigen binding.

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A)   B)   C)

18 angstroms

18 angstroms   αβ TCR

α   β   β   α   α   β
CDR2   CDR3   CDR1   CDR1   CDR3   CDR2
α   β
DE   DE
α   α   β   MHC
CDR1   CDR3   CDR2
peptide   18 angstroms

peptide

MHC α-helical regions

Figure 1. Schematic model of peptide recognition by the αβ T cell receptor. (A) Top view of CDR loop topology on the αβ TCR by analogy with Ig. α chain CDRs and DE loop are on the left, β chain on the right; (B) top view of a peptide–MHC complex in which the central peptide is flanked by α-helical regions of MHC; (C) side view of interaction between αβ TCR and a peptide–MHC complex. α-Helical regions of MHC are represented as ovals, and the peptide is represented as a central black box. Although the TCR and peptide–MHC complex are of the same width (18 Å), the former is much shorter than the latter, implying that the same antigenic complex could be recognized by different TCRs, each of which might bind in a distinct position along the axis of the peptide.

On the basis of sequence homology, it has been proposed that T cell receptors and immunoglobulins are likely to have similar tertiary and quaternary structures.[4,5] In particular, examination of residues in the Ig framework revealed that at 40 positions amino acids are identical or highly conserved between V regions of Ig and TCR α or β chains.[6] Many of these residues are thought to be important either in maintenance of Ig domain structure or for pairing of V domains.[7] Furthermore, in the putative CDRs of TCR, there is a relative preponderance of asparagine,[8] as is the case in Ig CDRs.[3]

In contrast to this overall similarity in primary structure are sequence variability analyses which chart the relative variation of amino acids at each numbered position in a group of aligned protein sequences. Such analyses reveal an additional region of pronounced variation beyond that of Ig in TCR β chains,[4,9,10] corresponding to the DE loop of immunoglobulins (Figure 1A). This has raised the possibility that the DE loops of T cell receptors, in close proximity to Ig

CDR homologs, might also participate in peptide–MHC specificity.

Such a possibility has been addressed by recent mutagenesis experiments on T cell receptor Vα, Vβ, or both[11-13] (and Rock et al., in preparation), which show that mutations in CDR1, -2, and -3 equivalents can disrupt αβ T cell receptor recognition. On the other hand, DE mutants of both Vβ and Vα failed to inhibit recognition[13] (Rock et al., op. cit.). These data both support the idea that TCR uses the same antigen binding loops as Ig in conferring peptide specificity and argue against the involvement of either the α or β chain DE loop in peptide recognition.

Concentration of Genetic Diversity in the TCR CDR3 Loop

The many possible specificities of Igs and TCRs derive from relatively random recombination of subgenic coding segments in each B or T cell to produce a variable domain exon.[14,15] Ig H, TCR β, and TCR δ chain families use variable (V), diversity (D), and joining (J) subgenes, while Ig L, TCR α, and TCR γ families use V and J segments alone. The vicinity of DNA rearrangement in all immune receptor chains corresponds to the CDR3 loop in an individual V domain (Figure 1A).

Gene rearrangement greatly expands the potential repertoire of antigen-specific immune receptor chains by combinatorial and junctional mechanisms. Com-

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Table I. Potential Amino Acid Sequence Diversity in Immunoglobulin and T Cell Receptor Genes without Allowance for Somatic Mutation[a]

	Ig		αβ TCR		γδ TCR	
	H	κ	α	β	γ	δ
variable segments	250–1000	250	100	25	7	10
diversity segments	10	0	0	2	0	2
D's read in all frames	rarely			often		often
N-region addition	V–D, D–J	none	V–J	V–D, D–J	V–J	V–D, D–D, D–J
joining segments	4	4	50	12	2	2
variable region combinations	62 500–250 000		~2500		70	
log of junctional combinations	~11		~15		~18	

[a] The approximate number of variable (V) gene segments for Ig heavy (H) and light (κ) are contrasted with TCR α, β, γ, and δ. Random pairing of V segments produces the combinatorial diversity listed as "variable region combinations"; V segments encode complementarity determining regions 1 and 2 (CDR1 and CDR2). CDR3 is encoded almost entirely by the D and/or J segments (with a negligible contribution from the V elements which is not included in these calculations). Junctional diversity stems from use of different D and J gene segments, N-region nucleotide addition (up to six bases at each junction), variation in the joining position of V, D, and J segments, and translation of D segments in different reading frames. Numbers are corrected for N-region mimicry of germ-line sequences and out-of-frame joining codon redundancy as detailed in ref 17. Adapted from ref 15.

binatorial diversity entails the rearrangement and domain pairing in one heterodimeric receptor of five different gene elements, each of which is drawn from multiple copies in germ-line DNA. Junctional diversity refers to both the imprecise joining of gene elements and the relatively random addition of nucleotides to the cut ends of coding segments before joining. The latter process, catalyzed by terminal deoxynucleotidyltransferase, is called N-region nucleotide addition.

Despite similarities of gene rearrangement in the genesis of Ig and TCR V domain exons, the major means of generating diversity is clearly different in each receptor family (Table I). Both αβ and γδ T cell receptors employ significantly fewer V gene elements than do either L or H chains.[15] Since each V domain of a heterodimer could potentially associate with any V domain of the paired chain, this sparsity of TCR V gene elements also reduces the number of TCR heterodimer frameworks that can be produced by combinatorial association.

In contrast to the relatively meager extent of V element derived diversity in TCR, V–J and V–D–J junctional diversity (encoding CDR3) of TCR is markedly greater than that of Ig. T cell receptor genes utilize N-region nucleotide addition in all four chain families, whereas in Igs only H chains do so. In addition, TCR α and β chains use a relatively large number of J_α and J_β gene segments (Table I), while δ chains employ two to three different D regions simultaneously.[16,17] There is thus likely to be 10 000-fold more genetic variation of CDR3 in αβ TCRs relative to Igs and 1000-fold more in γδ relative to αβ TCRs.[15] On the other hand, TCR genes do not undergo somatic mutation, a mechanism for the generation of Ig diversity in B cells following specific activation.

We also characterized CDR3 size variation for Ig H and L and TCR α, β, γ, and δ families from the Kabat database[10] of antigen-specific immune receptor chains (Rock et al., submitted). Ig H and TCR δ CDR3s are the most variable in size and are significantly longer than γ and L chains, respectively. By contrast, α and β chain distributions are constrained and nearly iden-

tical in distribution. CDR3 length profiles suggest that γδ TCRs are in some respects more similar to Ig antigen receptors than to αβ TCRs and that T cells bearing γδ TCRs may be fundamentally different from αβ T cells in their recognition properties. Thymic selection does not appear to play a significant role in constricting either α or β chain CDR3 length; rather, the initial sizes of D and J elements alone appear to account for virtually all of the length variation observed.

Topology of TCR–Peptide–MHC Interaction

The most significant functional difference between TCR and Ig concerns the nature of ligands recognized. Ig epitopes on protein molecules comprise relatively large areas of 15–22 amino acids on one or more surface loops.[18-21] Buried surface area between antigen and antibody in solved cocrystal structures ranges from 650 to 900 Å^2. Recognition is conformationally dependent, and most if not all of the protein's surface is antigenic (capable of eliciting a specific immune response).

In contrast, the TCR typically binds to peptide antigen only when the latter is displayed by the major histocompatibility complex glycoprotein. Unlike the discontinuous, unprocessed epitopes of Ig, TCR epitopes are linear and generally produced from native proteins by intracellular proteolytic digestion. In addition, the antigenic peptide presented by an MHC molecule is likely to have a much smaller area of direct contact with the TCR than does an unprocessed protein antigen with Ig.

Given the skewing of genetic diversity toward the CDR3 region of T cell receptors,[15] the presumed similarity between TCR and Ig protein structures,[4-6] and the crystal structure of a class I MHC molecule,[22,23]

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a model of $\alpha\beta$ TCR recognition has been proposed[6,15,24] in which the CDR3 regions of a receptor are positioned over the peptide binding cleft of MHC while CDR1 and CDR2 loops would contact the α-helical regions of the MHC (Figure 1B,C).[6,15,24] The large number of possible peptides bound in the cleft of MHC molecules could thus be complemented by the extraordinary genetic diversity observed in CDR3 loops of T cell receptors. Correspondingly, the less diverse CDR1 and CDR2 regions (encoded by V gene elements) would bind the α-helical regions of MHC which, while polymorphic, are much less so than the possible universe of antigenic peptides in the MHC cleft.

This model also makes sense from a structural perspective as shown in Figure 1. The distance between α-helical regions of MHC that flank the antigenic peptide binding site is roughly 18 Å, very similar to the distance separating the CDR1 and CDR2 loops of an Ig heavy chain from those of a light chain (and thus by inference a TCR). Since the area on a peptide–MHC complex to which the TCR might bind is roughly 3 times the antigen binding area of Ig (and presumably TCR), the proposed model of interaction would still be compatible with TCR binding in many different registers on MHC.

A direct test of this model was attempted by Patten et al.,[25] who transferred CDR3 loops of different T cell receptors onto each other in hopes of transferring peptide specificity (in situations where the MHC molecule was the same). Similar strategies have been successful in immunoglobulins[26–29] although the affinity of antigen–antibody complex in such cases is generally decreased. In spite of numerous attempts to transfer TCR specificity between closely related TCRs, no transfer of reactivity was observed. We now know (see affinity section) that TCRs have much lower affinities for peptide–MHC complexes than do antibodies for protein antigens and that the latter class of receptor may thus be more tolerant of local structural disruptions.

Identification of Direct CDR3–Peptide Contacts

Ultimately, Jorgensen et al.[30] developed a novel strategy to test the above model of TCR recognition. Their approach is analogous to classical genetic second-

site suppression in which disruption of protein–protein contacts produced by mutants in one molecule is compensated for by a change in the second, interacting molecule. Such a strategy was made possible by the wealth of information concerning interaction of $\alpha\beta$ TCRs with a complex of moth cytochrome c (88–103) (MCC) presented by the mouse class II MHC molecule I-E^k [31,32] (P. Reay et al., submitted).

In the MCC/I-E^k complex, the minimal peptide determinant of MCC is from positions 95–103, a 9-mer.[31,33] Three positions are critical for MCC binding (95I, 100Q, 103K); three appear to contact the TCR directly (97Y, 99K, 102T); and three are likely to control precise spatial arrangement of T cell receptor contact points, perhaps via induced effects on MHC α-helical regions (96A, 98L, 101A) (P. Reay et al., submitted). 5C.C7 is a T cell line which recognizes the MCC/I-E^k complex via use of the V$_{\alpha 11.1}$ and V$_{\beta 3}$ gene elements. Single-chain transgenic mice have been created that express either the 5C.C7 α or β chain on 90% or 98% of their peripheral blood T cells, respectively. These animals use the transgenic TCR chain in heterodimeric receptors with a wide variety of paired endogenous TCR chains.

Mice were immunized with either MCC or one of four variant peptides which could bind to I-E^k but failed to stimulate 5C.C7. These peptides were designed to substitute amino acids which contact the TCR directly with other residues that would presumably disrupt the wild-type 5C.C7/MCC/I-E^k complex. Variant peptide antigens include 99E (lysine to glutamate at position 99), 99Q (lysine to glutamine), 102E (threonine to glutamate at position 102), and 102K (threonine to lysine). By holding one TCR chain constant in each immunization of single chain transgenic mice, these workers were able to constrain the interaction of TCR with the peptide–MHC complex.

While the wild-type peptide induced a strong response from both sets of mice, peptides substituted at position 99 elicited a much lower response from 5C.C7 α transgenic mice, and peptides altered at position 102 elicited a negligible response in β transgenic mice. Given the transgene-imposed constraints, this result indicated that 5C.C7's V$_\alpha$ chain determines recognition of peptide position 99 in this complex while the V$_\beta$ chain confers specificity at position 102.

Hybridomas which reacted specifically with the immunizing peptide were generated from β chain transgenic mice injected with the 99E or MCC peptides. Sequence analysis of endogenous α chains (summarized in Figure 2) revealed that, of six hybridomas reactive to 99K (wild-type) MCC, all retained a negatively charged residue at position 93 in the CDR3 homolog. This amino acid was either a germ line-encoded glutamate (E) or in one case an N-region-encoded aspartate (D). Yet of eight hybridomas reactive to 99E, all endogenous α chains bore an N-region-determined,

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A)

Cell	Peptide	Vα	VJα junctional sequence	Jα
5C.C7	99K	11.1	CAAEASNTNKVVF	C7
111	99K	11.1	CAAEPSNTNKVVF	C7
116	99K	11.1	CAAEASAGNKLTF	AC25
102	99K	11.1	CAAD GNNRIFF	MD13
202	99E	11.1	CAAKSSGSWQLIF	28

B)

Cell	Peptide	Vβ	VDJβ junctional sequence	Jβ
5C.C7	102T	3	CASSLNNANSDYTF	1.2
99 K6	102T	3	CASSLNNANSDYTF	1.2
99 K3	102T	3	CASSLNSANSDYTF	1.2
99 K2	102T	3	CASSKNWGQDTQYF	2.5
E3	102E	8.1	CASSGRTGVSDYTF	1.2
E7	102E	8.1	CASSDRTGASDYTF	1.2
E18	102E	8.1	CASSKRTGVSDYTF	1.2
E35	102E	8.1	CASSERTGVSDYTF	1.2
E22.1	102E	8.1	CASSERAGASDYTF	1.2
E21	102E	8.2	CASGENRSGNTLYF	1.3
E34	102E	6	CASSIWTGKRDFSNERLFF	1.4
E24	102E	6	CASSTYTGKG NQAPLF	1.5
E12	102E	6	CASSRTQGKG NQAPLF	1.5
E22.2	102E	6	CASSNHLGRHN NQAPLF	1.5
E13	102E	6	CASSIGRLP SQNTLYF	2.4
E10	102E	6	CASS__SWTGK__ SAETLYF	2.3
K19	102K	6	CASS__PNWGGN__ SAETLYF	2.3
E20	102E	1	CASSQGQGRGTEVFF	1.1
E16	102E	1	CASS N NSDYTF	1.2
E28	102E	1	CASSQGQGVGSDYTF	1.2
E30	102E	1	CASSPGQGT SDYTF	1.2
E4	102E/K	1	CAS GQGNNSPLYF	1.6
E34	102E	4	CASSGTT NSDYTF	1.2

Figure 2. T cell receptor V(D)J junctional sequences from hybridomas generated against peptide analogs of moth cytochrome *c* (88–103) (MCC). Cell: Cell line from which receptor genes were sequenced. Peptide: Immunizing peptide from which listed cell lines and clones were derived. (A) TCR α chain sequences from TCR β chain transgenic mice immunized with MCC (88–103) (99K) or the variant 99E peptide (in which negatively charged glutamate, E, has been substituted for positively charged lysine, K, at position 99). V$_α$: TCR α variable gene element. VJ$_α$ junctional sequence: Rearranged gene sequence corresponding to CDR3 of the α chain protein. J$_α$: TCR α joining gene element. (B) β chain sequences from α chain transgenic mice immunized with MCC (88–103) (102T) or variant peptides in which the wild-type threonine at position 102 of the peptide has been replaced with glutamate (102E) or lysine (102K). V$_β$: TCR β variable gene element. VJ$_β$ junctional sequence: Rearranged gene sequence corresponding to CDR3 of the β chain protein. J$_β$: TCR β joining gene element. Conserved junctional residues proposed to contact peptide are in bold. Underlined residues are the only TCR differences between hybridomas E10 (peptide 102E-specific) and K19 (peptide 102K-specific). Hybridoma E4 is cross-reactive for 102E and 102K peptides. From ref 30.

positively charged lysine (K) at the same position. Since all 14 of these hybridomas employed identical transgenic β chains and the same V$_α$ subgene (V$_{α11.1}$), the reciprocal charge reversals at α93 (CDR3) in response to charged amino acid side chains on the immunizing peptides indicated a salt bridge between these residues and thus direct contact between the TCR α chain CDR3 homolog and antigenic peptide.

Analogously, Jorgensen et al. found that position 100 of the 5C.C7 β chain is likely in close proximity to
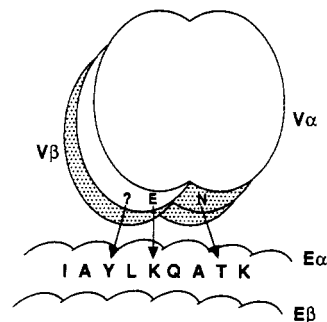
Figure 3. Proposed model for the 5C.C7 TCR–MCC (88–103)–I-E^k interaction. V$_α$ (white and V$_β$ (hatched) refer to the α and β chain variable domains of the 5C.C7 T cell receptor. TCR complementarity determining regions (CDRs) are represented by oval lines at bottom of V$_α$ and V$_β$ domains (corresponding to circles in Figure 1A); the middle layer of ovals represents the CDR3 loops, while the CDR1 and CDR2 loops of each domain are pictured either to the front or to the rear. E$_α$ and E$_β$ refer to the α-helical regions of the mouse class II MHC molecule, I-E^k. A glutamate (E) at position 93 in the V–J junction of the V$_α$ domain is in contact with a lysine (K) at position 99 of the moth cytochrome *c* peptide. In addition, an asparagine (N) at position 100 of the V$_β$ domain is located in the vicinity of a threonine (T) residue at position 102 of the peptide. Adapted from ref 30. Although it has been determined that the V$_α$ CDR3 loop changes in response to peptides substituted at position 97 (tyrosine, Y), a predominant TCR contact residue is yet to be determined.

position 102 of the peptide, although in this case a change in the V$_β$ germ line sequence seems mandatory as well. Recent data also show that residue 97 of the peptide is also determined by the V$_α$ CDR3 (Jorgensen et al., in preparation). Such reciprocal charge motifs constitute the strongest evidence to date of direct TCR–peptide contact and bolster the concept that a major role in peptide recognition by T cell receptors is played by the CDR3 loop homologs. A model summarizing this data is presented in Figure 3.

The pronounced role of antigenic peptides in the determination of TCR specificity is paradoxical in the sense that the exposed peptide surface is so small relative to the α-helical regions of MHC. This paradox is resolved by two countervailing factors. First, a majority of the polymorphic residues of MHC are known by X-ray crystallography to be oriented into the peptide binding groove rather than being exposed on the surface of the complex. Second, the data on substitutions of the cytochrome *c* peptide show that peptide residues having negligible solvent accessibility are still of substantial importance in specific T cell recognition.[33] Thus while the TCR interacts directly with only a few amino acid side chains of an antigenic peptide, it is sensitive to their spatial conformation, as well as to the conformation of juxtaposed MHC α helices.

Induced Fit of TCR–Peptide–MHC Interactions?

Although recent evidence indicates direct contact between TCR CDR3 loops and antigenic peptides, the nature of other TCR contacts has been less clear. Ehrich *et al.*[34] developed an experimental system to evaluate the extent to which antigenic ligands affect the overall

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TCR–MHC interaction. Responses were compared of T cells bearing structurally related TCR[30] to MCC peptide variants presented by 13 mutant antigen presenting cell (APC) lines. Each APC expresses the mouse class II I-E^k molecule with a single substitution of an amino acid side chain in the MHC α-helical region predicted to point "up" toward the TCR. Substituted residues are similar in hydrophobicity to the wild-type amino acid but either reverse charges or increase side chain size to promote steric hindrance. Analysis of sets of hybridomas differing by as little as one amino acid in their TCRs allowed these investigators to evaluate the effect of small defined changes in TCR CDR3 or peptide on the overall MHC–TCR interaction.

The results were surprising. TCRs cross-reactive to different peptide analogs make different MHC contacts depending on the peptide being recognized, indicating that the same TCR can contact the same MHC in more than one way. Use of a particular V_α or V_β gene segment does not seem to predispose the TCR to contact MHC in any particular way. In addition, the extent of ternary complex changes brought about by small alterations of an apparent CDR3–peptide contact can be as profound as those between unrelated TCR recognizing unrelated peptide antigens. These results suggest an optimization of contacts between TCR and peptide–MHC for each ternary complex. Such optimization of side-chain packing at the protein–protein interface may involve main-chain conformational changes of the α and β CDR3 regions[35] and/or use of water as a spacer molecule to optimize hydrogen bonding.[36] Induced fit of ligand-binding surfaces on α and β TCR chains might thus be a general component of specific recognition by the TCR.

Low Affinity of TCR Interaction with Peptide–MHC

Two technical obstacles have until recently stood in the way of obtaining reliable data on affinity of the TCR for peptide–MHC ligands. First, neither TCR nor MHC is soluble in its typical natural state. Second, deriving affinity measurements for macromolecules having K_D's greater than 10^{-7} M has, until recently, been a very arduous task. Sedimentation analysis, for example, often requires high protein concentrations, e.g., in the millimolar range. Equilibrium dialysis is also impractical because the two ligands have similar molecular weights.

The first impediment has been removed successfully by a variety of strategies which have been developed to engineer soluble forms of TCR or MHC heterodimers. One involves expressing the two chains in a glycolipid form which can be cleaved from the cell surface by a specific enzyme.[37,38] Another creates a secreted form

of TCR via linkage to antibody constant domains.[39,40] Third, TCR V dimers can be produced in *Escherichia coli* analogous to single-chain antibodies; the V domains are connected on the same protein chain via a linker polypeptide.[41,42] Finally, T cell receptor chains have been fused with an intervening protease cleavage site to CD3 ζ chains and expressed in a basophil cell line, enabling specific activation to be assessed by basophil degranulation.[43]

Recently published experiments using reagents produced by two of these methods have produced reliable estimates of T cell receptor affinity. Matsui *et al.* used soluble class II heterodimers complexed to different peptides to inhibit the binding of a labeled V_β-specific monoclonal antibody to the TCR expressed on a T cell line.[44] Using 5C.C7 and hybridoma 228 (specific for the 99E variant of MCC with I-E^k), inhibition was shown to be both peptide- and TCR-specific. Dissociation constants were derived of from 4×10^{-5} to 6×10^{-5} M.

Weber *et al.*[45] calculated a similar K_D of 10^{-5} M for the interaction of another TCR with a complex of hemagglutinin peptide and I-E^d. The latter experiments utilized a soluble TCR to inhibit responses by several hybridomas to peptide–MHC on presenting cells.

Both sets of early affinity experiments measure TCR binding indirectly and are thus subject to possible artifacts. For example, if the soluble peptide–MHC complex used by Matsui does not completely block antibody binding to the T cell receptors, an artificially low-affinity estimate would be obtained. Furthermore, results on inhibition of T cell activation depend on soluble TCR concentrations of up to 1–2 mg/mL, which could produce secondary effects on cell reactivity of an unknown nature. Finally, TCR constructs engineered for solubility could have subtle differences in protein structure relative to the mature, cell-bound receptor.

Some of these objections can be overcome by recent data utilizing surface plasmon resonance (SPR) to measure T cell receptor peptide–MHC interactions directly. SPR is an optical phenomenon arising in thin metal films under conditions of total internal reflection.[46,47] A sharp dip in the intensity of reflected light occurs at a specific resonance angle, which varies with refractive index of the medium close to the nonilluminated side of the metal film. Thus the concentration

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of dissolved material close to the metal film, which correlates with refractive index, can be determined by monitoring resonance angle position. A biosensor instrument has been developed which combines a transducer based on surface plasmon resonance with a biological recognition mechanism (BIAcore, Pharmacia). One interacting ligand is linked to a solid-state surface (carboxymethylated dextrose), and the binding of a second moiety in solution to the one on the surface is detected. Continuous flow technology enables real time measurements and thus derivation of kinetic information.

The BIAcore instrument has recently been used to confirm some of the initial TCR affinity results[44] using soluble T cell receptors coupled to the sensor chip (K. Matsui, Jay Boniface, P. Steffner, and M. M. Davis, in preparation), as well as to obtain kinetic parameters. Matsui et al. find that the cytochrome *c*-specific T cell receptors have very slow on-rates ($\sim 10^3$ M^{-1} s^{-1}) and very fast off-rates [$(1-10) \times 10^{-2}$ s^{-1}]. Thus, although only a few TCRs have been characterized thus far, it seems likely that the affinity of binding to peptide–MHC ligands will be very weak. While most familiar antibodies have much higher affinities, more in the $10^{-8}–10^{-10}$ M range, this is likely due to a selection for utility, as surface IgM antibodies have been characterized with affinities down to $10^{-5}–10^{-3}$ M[48-50] Why might TCRs have such low affinities? As discussed in more detail elsewhere,[44,54] other surface molecules on T cells (which have cell surface ligands) have higher affinities, in the 10^{-7} M range. It would seem that these molecules then would drive the cell adhesions of T cells, with TCR engagement a secondary event. This might be a way of "steering" T cells toward appropriate target cells (and away from inappropriate cells and tissues). Also interesting in the light of these measurements were

earlier studies indicating that T cells specific for hapten–MHC complexes could bind hapten directly.[51,52] There is also at least one reported case in which a peptide could bind specific T cells directly.[53] Without a measurement involving an MHC molecule those reports were difficult to credit, but now that such systems have been described, and particularly in light of Ehrich et al.'s[34] finding that much of TCR specificity must center on the bound peptide, it is reasonable to speculate that they may indicate that most of the binding energy of TCR recognition derives from peptide recognition.[54] Most peptides would not bind directly as their conformation (in a complex with MHC) is too dependent on MHC binding, but rigid haptens such as arsonate[51] or fluorescein[52] would not change nearly as much.

Conclusions

In contrast to recognition by immunoglobulins, which have long been known to exhibit broad affinities of interaction with a vast universe of antigens, peptide recognition by T cell receptors is of low affinity but precise. Specific TCR interaction is sensitive to subtle changes in either peptide or MHC. Even slight perturbations of noncontact peptide residues can abrogate recognition (Reay *et al.*, submitted; Ehrich *et al.*[34]), presumably due to induced conformational changes on the rest of the peptide or MHC α helices.

The primacy of TCR CDR3 loops in peptide–MHC specificity is reflected by skewing of genetic diversity in T cell receptor chains to the region of DNA rearrangement which encodes CDR3.[15] Given the apparent plasticity of contacts between T cell receptors and peptide–MHC,[34] the demonstrated flexibility of an Ig H chain CDR3 loop,[35] and greater length of T cell receptor CDR3 regions relative to immunoglobulins (Rock, E.; Sibbald, P.; Davis, M.; Chien, Y. Submitted), we suspect that induced fit of CDR3 main-chain conformations may be a general feature of TCR recognition.

A century ago the cellular theory of immunity fell into disfavor in part because its mechanisms could not be discerned in a manner that compared to the pictures of antibodies drawn by Ehrlich.[2] It is ironic that these complementary arms of specific immunity employ antigen recognition molecules that subserve markedly different functions yet at the same time have so much in common structurally.

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