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Not just another Fab: the crystal structure of a TcR–MHC–peptide complex

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The structure of a ternary complex formed between a T-cell receptor, a major histocompatibility complex (MHC) protein and a viral peptide provides new insights into the cellular immune response. The results provide a molecular basis for understanding the development of T cells and the reactions leading to transplant rejection and autoimmunity.

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Electronic identifier: **0969-2126-005-00159**

Structure 15 February 1997, **5**:159–163

© Current Biology Ltd ISSN 0969-2126

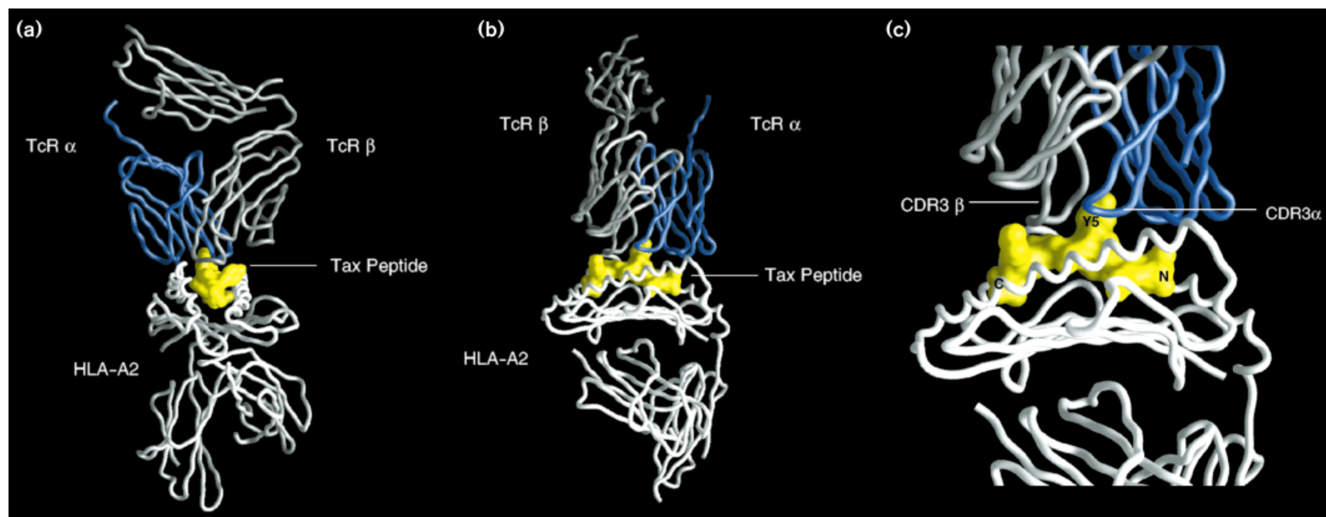
The development and regulation of the adaptive immune response in complex organisms has required the evolution of intriguing mechanisms that can dynamically respond to and resolve problems of molecular recognition. The emergence of the clonal selection theory and the demonstration of the combinatorial diversity of antibody and T-cell receptor (TcR) molecules have clarified how the immune system generates an immense repertoire of antigen-recognition sites. We now know that one cell type, the B cell in the case of antibodies and the T cell for the TcR, is dedicated to the production of a unique antigen-combining site that is formed at the interface of two immunoglobulin (Ig) variable domains [1]. The total repertoire of these combining sites is defined by the total number of different T or B cells that have undergone unique gene rearrangements. The specificity of the antigen-combining site is determined primarily by the sequence of hypervariable loops at the surface of the variable Ig domains. For antibody molecules, the repertoire of binding sites covers a range of antigens including small molecules, carbohydrates, DNA, and large protein surfaces. The problem of fighting many pathogens is resolved by generating a nearly infinite repertoire of receptor-binding sites by genetic recombination.

The repertoire of mature TcRs does not cover as broad an array of potential targets as antibodies, but is instead restricted to the recognition of peptides that are bound to major histocompatibility complex (MHC) glycoproteins [2,3]. The shaping of the T cell repertoire occurs during T cell development in the thymus, where the majority of initial T cells are eliminated and only a small percentage survive to make up the mature pool of cells. As Niels Jerne pointed out over 25 years ago [4], this means that a preselected and 'functional population of lymphocytes is generated before environmental antigens start to impinge on the system'. This mechanism allows for the deletion [5] of many T cells that react strongly against normal self molecules (negative selection). In addition, lower affinity (or avidity) interactions between the TcR and self MHC molecules [5,6] are required for T cells to mature properly (positive selection), skewing the specificity of final T cell population towards self MHC molecules. These processes of positive and negative selection somehow ensure enough diversity in the TcR repertoire so that T cells can respond to almost any potential pathogen. Here we can begin to appreciate the enigma and fascination of the T cell receptor, as it is a molecule that functionally must incorporate the ability to recognize at least two distinct targets: the first to survive T cell development in the thymus and the second to initiate an immune response to a specific antigen. How this duality is fostered by the immune system is beginning to be understood at the molecular level, with many insights provided by the crystal structure of a TcR–MHC–peptide ternary complex [7]. This crystal structure has provided the first atomic resolution view of how a human TcR (A6-TcR) recognizes a human MHC class I molecule human leukocyte antigen A2 (HLA-A2) with bound antigenic peptide (Tax peptide from the human T-cell lymphotropic virus (HTLV-1)). General lessons for the TcR–MHC–peptide interaction may be at hand, given the similarity of a mouse TcR–MHC crystallographic complex that is based on initial molecular replacement models [8].

The structure of the T cell receptor

The TcR is a membrane-bound protein complex comprised of a number of subunits. Two subunits (α and β) are involved in recognition of the peptide–MHC complex and additional subunits (γ, δ, ε, and ζ/η proteins) are involved in the signal transduction mechanism. The extracellular domains of the α and β chains are similar to the Fab portion of an Ig molecule; each chain is composed of one variable and one constant Ig domain with the two variable domains (Vα and Vβ) forming the binding site for a peptide–MHC complex [1]. The crystal structures of the β chain and the variable domain of an α chain of the TcR have been solved independently [9,10] and the structure of the extracellular domains of an intact αβ heterodimer has been solved to high resolution [8]. This work has confirmed the Ig-domain structure of the TcR and uncovered new variations that are unique to the TcR. The Vα domain defines a new class of variable-domain folds, where one of the β strands (c″) that typically forms a sheet

Figure 1



The ternary complex of the A6-TcR–HLA-A2–Tax peptide. Cα traces of the TcR and HLA-A2 are shown together with the molecular surface of the Tax peptide (yellow). **(a)** The end view of the complex; the HLA-A2 α1 helix is on the right and the α2 helix is on the left. The α (blue) and β (grey) chains of the TcR are labeled; the constant domain of the TcR α chain is omitted due to poor definition in the electron-density maps [7]. **(b)** Side view of the ternary complex. The HLA-A2 α1 helix is positioned in front of the Tax peptide. **(c)** Close-up view of the ternary complex in the same orientation as (b). The N and C termini of the peptide are labeled along with Tyr5 (Y5) of the Tax peptide and the TcR CDR3 loops.

with four additional β strands (cc′fg) switches over to the other β sheet (formed by strands abde). The homodimer of Vα domains observed in crystals is very similar to the packing arrangement observed for the αβ heterodimer of the A6-TcR, leaving the c″ strand exposed at the TcR surface with the potential to form interactions between two αβ heterodimers [10] or other accessory molecules. The Cα domain is even more divergent from the canonical Ig fold [8], showing only one sheet of the typical Ig-domain β sandwich. In the typical fold, the β sheet is formed by strands cfg, however, in the TcR Cα domain these strands lie too far apart to form hydrogen bonds and strand f even forms a short helical region. This novel fold may fill a function unique to the T-cell receptor, such as the proposed interaction with another TcR subunit [8]. The structural variations in both the Vα and Cα domains suggest that the TcR α chain in particular may have evolved new functional roles yet to be elucidated.

Like antibodies, TcR sequence comparisons have revealed regions of hypervariability (complementarity determining regions or CDRs) that correspond to surface loops of the Ig fold [1,11]. In the TcR, two of these loops (CDR1 and CDR2) are encoded entirely by the germline V_α and V_β genes, while the DNA encoding a third loop (CDR3) is only fully formed during recombination and shows greater sequence diversity. In contrast to antibodies, there are fewer germline encoded V regions for the TcR and these do not undergo somatic mutation during an immune response, leading to reduced variability in the CDR1 and CDR2 regions of the TcR. The TcR CDR3 loops, however, are more variable than in antibodies, due to a greater number of germline joining segments and the addition of non-template encoded nucleotides (N nucleotides) in both the α and β chains. Prior to the recent crystallographic studies, models for the formation of TcR–MHC complexes have positioned the less diverse CDR1 and CDR2 loops in contact with the MHC molecule, with the CDR3 loops positioned to interact with MHC-bound peptide [7]. In addition to the CDR loops, TcRs have another region of polymorphism (HV4).

The structure of the ternary complex

In the HLA-A2–Tax-peptide–A6-TcR crystal structure, the TcR binds to the composite MHC–peptide molecular surface with the TcR αβ dimer axis aligned diagonally, relative to the MHC α helices (Figs 1a,b). This orientation is similar to the one deduced from initial molecular replacement models for a murine MHC–peptide–TcR complex [8]. The CDR1 of the TcR α chain (CDR1α) covers the N-terminal region of the Tax peptide, while CDR1 of the β chain (CDR1β) is near the C-terminal region of the peptide (Fig. 1c). Both CDR1 loops interact directly with the peptide, in spite of their reduced variability relative to the CDR3 loops (Table 1). By far the largest percentage of the MHC–peptide surface area is buried by the β chain of CDR3 (CDR3β), amounting to more than 50% of the total buried surface. Over two thirds of the total buried HLA-A2–Tax surface is actually contributed by residues from HLA-A2 (Fig. 2a); this buried HLA-A2 surface might provide a basal level of TcR binding affinity derived from

Table 1

Contacts of the A6-TcR hypervariable loops.

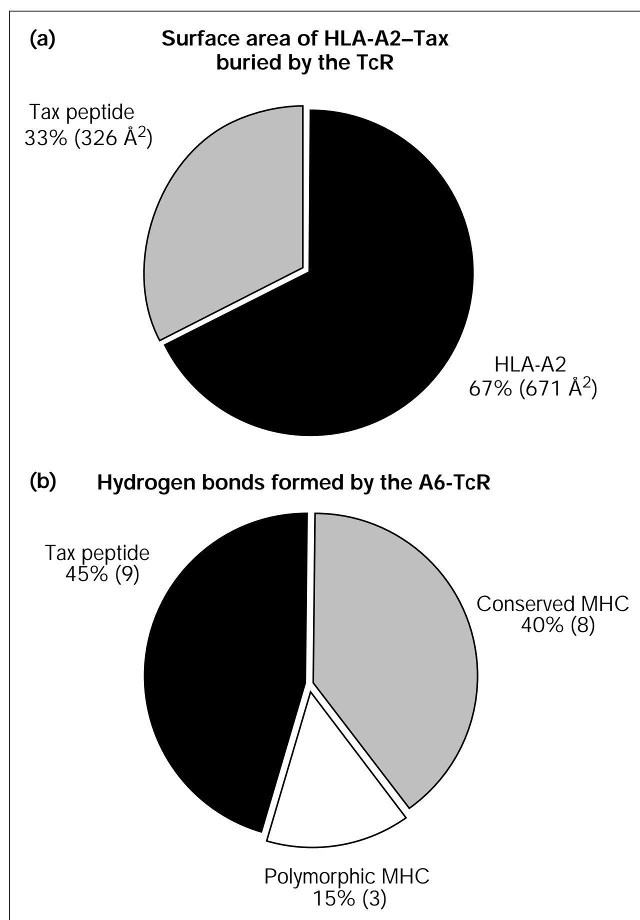
CDR loop	Contacts
α1	Peptide and MHC, α1 and α2 helices
α2	MHC, α2 helix
α3	Peptide and MHC, α1 helix
β1	Peptide
β2	-
β3	Peptide and MHC, α1 and α2 helices

thymic selection that is then modulated by a specific peptide. The authors [7] note that the majority of the potential hydrogen bonds between the TcR and the MHC molecule are made to residues that are conserved in many MHC alleles (Fig. 2b). However, a substantial fraction of the total hydrogen bonds formed by the TcR (~45%) are directly to the Tax peptide (Fig. 2b). One of the peptide residues (Tyr5) protrudes out of the center of the MHC-binding site and is bound in a prominent TcR pocket formed between the CDR3α and CDR3β loops (Fig. 1c).

Various mechanisms have been proposed to explain signaling through the TcR. Some of these rely on the aggregation or clustering of the TcR while others envisage TcR conformational changes that could be induced upon binding to MHC–peptide complexes. The A6-TcR does not apparently undergo any large conformational changes upon binding the HLA-A2–Tax complex, as judged by comparison with other known TcR structures [7]. This observation is consistent with models of T cell activation based on the clustering of TcRs. However, TcR binding to the MHC does induce a conformational adjustment of the Tax peptide within the HLA-A2-binding site, as well as a small shift in one of the HLA-A2 α helices, as compared with the structure of HLA-A2–Tax without bound TcR [12]. The Tax peptide conformational change occurs within the central amino acids, where direct contacts with the MHC molecule are minimal. The structures of five different peptides bound to HLA-A2 have shown substantial variation in the conformation in this region [12]. Peptide conformational changes, such as those observed in the A6-TcR–HLA-A2–Tax structure, might help explain the effects of amino acid changes in either peptide or MHC residues that are not directly in contact with the TcR. For example, Tax peptide variants that either prevent or favor this conformational change could be expected to influence TcR-binding affinity. However, conformational changes of this magnitude are unlikely to be a general feature of TcR binding, as other MHC molecules are known to interact more directly with the central residues of bound peptides [13].

In summary, the TcR interacts directly with exposed residues of both the MHC and the peptide, laying to rest

Figure 2



Relative contributions of peptide and MHC to the interaction with the A6-TcR. **(a)** The water accessible surface area of the HLA-A2–Tax complex that is buried by the binding of the A6-TcR. The absolute buried surface area and the fraction of the total surface area for the Tax peptide and HLA-A2 are indicated. **(b)** The number of potential hydrogen bonds formed between the A6-TcR and HLA-A2–Tax. The interactions between HLA-A2 and the MHC are divided into two categories depending on whether the HLA-A2 contact residue is conserved among many MHC proteins (grey) or whether it corresponds to an amino acid that varies among MHC molecules (polymorphic; white). The A6-TcR interactions with the Tax peptide form the final category of potential hydrogen bonds (black). The percentage of A6-TcR hydrogen bonds (20 bonds in total) for each category is indicated, the number of hydrogen bonds is shown in parentheses.

models in which antigenic peptides are 'detected' indirectly through conformational changes in the MHC, although conformational adjustments during ternary complex formation are observed. The MHC–peptide surface is continuous and peptide interactions are not limited to the TcR CDR3 loops, but include direct interactions with the CDR1 loops as well. The same relative orientation of the TcR to the MHC molecule has been observed in two distinct ternary complexes, suggesting that this may be a conserved structural feature [7,8].

Implications for T cell development, alloreactivity and autoimmunity

The high resolution TcR–MHC–peptide crystal structure makes it possible to address the molecular basis for some of the functional mysteries surrounding the TcR. As mentioned above, prior to encountering its eventual target (antigenic-peptide–MHC complex), the TcR must recognize a self-peptide–MHC complex in the thymus in order for the T cell to mature properly into a functional lymphocyte. In addition, some mature T cells not only react with antigenic peptides bound to self-MHC molecules, but can also react against non-self MHC molecules in a process known as allorecognition. For example, the 2C-TcR [8] binds to a self-MHC molecule complexed with a self-peptide (H2-K^b bound to the self-peptide EV-8) but also to a complex formed between non-self MHC and a mitochondrial peptide (H2-L^d bound to the mitochondrial peptide p2Ca). In other words, although thymic selection biases the TcR repertoire towards self-MHC molecules, this selection mechanism also retains a significant cross-reactivity to MHC molecules that are not present in the thymus. This cross-reactivity forms the molecular basis for recognition events that govern transplant rejection and may explain how a T cell response to an infectious agent could lead to the self-reactivity observed in autoimmune diseases [14].

The answer to this puzzling cross-reactivity may lie in a conserved mode of binding between the TcR and the MHC, as proposed by Garboczi *et al.* [7]. More importantly, many of the MHC surface residues that contact the TcR are conserved among many MHC alleles (11 out of 16 residues). There are therefore potentially conserved contacts that could form between a TcR and different MHC molecules or the same MHC molecule complexed with different peptides. As the MHC molecule provides approximately two thirds of the total buried surface area within the TcR–MHC–peptide complex (Fig. 2a), this establishes a plausible basis for TcR cross-reactivity. In this scenario, the peptide could provide the extra binding energy that is necessary to trigger the T cell activation process. Each TcR may be poised to react to some small additional binding energy that is contributed by specific peptide–TcR contacts. TcR affinities are typically in the 1–100 μM range and the peptide contribution to overall binding affinity could be in the 1–5 kcal mol^{-1} range; this additional binding energy would be enough to take a non-reactive, low-affinity interaction (e.g. ~10^{-2}–10^{-3} M) with the MHC molecule, into the appropriate affinity range. It is clearly important to understand the kinetics of this interaction, as the efficiency of TcR signaling is sensitive to the dissociation rate of the ternary complex [15]. This aspect of TcR binding raises a number of questions. Could the formation of a couple of hydrogen bonds between the TcR and peptide trigger the specific recognition? How many different ways could a single TcR surface be complemented at the level of a few good additional non-covalent interactions? In the case of the Tax peptide, if the tyrosine bound in the TcR CDR3 pocket (Fig. 1c) is changed to alanine, the TcR still binds, forms crystals, and partially activates T cells [7]. Presumably full reactivity could be restored to this alanine mutant if the TcR–MHC interaction was enhanced at another site: for example a substitution at one of the polymorphic MHC positions. This interplay between peptide sequence variability and MHC surface polymorphism seems to be poised at the edge of the binding energy required for T cell activation.

It may be interesting to recall the lessons learnt from studies of growth hormone receptor binding, which illustrate two relevant features of protein–protein interactions [16,17]. Firstly, the same receptor-binding site can bind to two different protein surfaces (growth hormone sites 1 and 2): the problem of finding a protein surface complementary to a given target site clearly has more than one solution. Secondly, a small percentage of residues (~20–30%) in a large protein–protein interface may carry the majority of the binding energy. In the case of TcR binding, we may also find that different peptide–MHC combinations can satisfy a single TcR surface with a distinct set of key energetic interactions, in the context of a conserved set of TcR–MHC contacts. There are three classes of TcR cross-reactive interactions that are of fundamental interest to understand: thymic selection/antigen recognition; allorecognition; and pathogen-triggered autoimmunity. In each of these cases a single TcR is thought to interact with at least two different MHC–peptide complexes. For each TcR, we can now expect a certain fraction of its interactions with MHC molecules to be conserved, but this leaves a set of interactions that confers specificity on the TcR to be explained. Are these always necessarily direct interactions with peptides? Must cross-reacting complexes have homologous peptides bound to the MHC molecule? We now find ourselves in a position where we are able to further probe the structural basis for specificity and affinity in TcR–MHC recognition and eventually reveal the answers to these interesting questions.

Acknowledgements
The author would like to thank Jon Widom, Eric Sundberg and Scott Garman for helpful comments on the manuscript.

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