Specificity on a knife-edge: the $\alpha\beta$ T cell receptor
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The interaction between the $\alpha\beta$ T cell receptor (TCR) and the peptide bound to the major histocompatibility complex class I molecule (pMHC-I) constitutes a central interaction in adaptive immunity. How these receptors interact with such low affinity while maintaining exquisite specificity for peptide antigen and host MHC (MHC-I restriction) remains a challenge to be explained by structural immunologists. Moreover, how this extracellular interaction is transmitted as an intracellular signal via the CD3 complex remains unresolved. Nevertheless, several structures of TCRs, non-liganded and ligated to a defined pMHC-I, combined with detailed biophysical analyses, have provided insight of the structural basis of MHC-I restriction. In addition, structures of isolated CD3 components have enabled T cell signalling mechanisms to be postulated. Recent findings in this area, which include seven distinct TCR/pMHC-I complexes, have fundamental implications in adaptive immunity as well as therapeutic applications to modulate the adaptive immune response.

Introduction

This review is focussed on structural correlates underlying the cytotoxic T lymphocyte (CTL) response directed towards major histocompatibility complex class I (MHC-I) molecules bound to peptide antigens (pMHC-I). The pMHC-I complexes are expressed on infected cells where they are specifically recognized by $\alpha\beta$ T cell receptors (TCR). The formation of this TCR/pMHC-I complex is the central event of antigen recognition in the adaptive immune response (Figure 1). This exquisite corecognition event means that CTLs are highly specific and genetically restricted to recognizing the MHC-I molecules of the individual from which they were derived; this concept is known as MHC-I restriction [1].

The antigen-specific chains of the TCR do not possess intracellular signalling domains but, instead, are coupled to the conserved multisubunit signalling apparatus, CD3. The mechanism by which TCR ligation is communicated directly to the signalling apparatus remains unclear. The $\epsilon$, $\gamma$, $\delta$ and $\zeta$ subunits of the CD3 complex associate to form CD3$\gamma\delta$ and CD3$\epsilon\zeta$ heterodimers and a CD3$\gamma\delta$ homodimer. However, the exact molecular arrangement of this TCR/CD3 complex is not fully elucidated (Figure 1).

Here, we focus on recent (2003 onwards) structural insights gained from the study of different TCR/pMHC-I complexes and the CD3 complex. Within this timeframe, the structures of seven distinct TCR/pMHC-I complexes have been determined, some of which have been complemented by biophysical and mutagenesis approaches. Collectively these studies have provided insight into TCR selection and MHC-I restriction. The structures of isolated components of the CD3 complex have been determined, enabling several models of T cell signalling to be postulated (for a more comprehensive review on this subject, see [2**]).

Overview of the TCR/pMHC-I complex

The TCR resembles an antibody Fab fragment, being comprised of two Ig-like chains ($\alpha$ and $\beta$ chains), each consisting of a variable (V) and constant (C) domain. The antigen-recognition site of the TCR is made up of six complementarity-determining regions (CDRs), three each from the $\alpha$ and $\beta$ domain (Figure 2).

The TCR interacts with pMHC-I, which are highly polymorphic molecules. MHC polymorphism (in humans, termed human leukocyte antigen [HLA]) controls the size and diversity of the peptide repertoire presented by any given individual. This polymorphism is generally concentrated in the antigen-binding cleft [3,4], which comprises a $\beta$-sheet floor bounded by two long $\alpha$ helices, with peptide binding being mediated via six pockets within this cleft (Figure 1.2). HLA alleles can code for MHC-I proteins that can differ from each other

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by only a single amino acid or by >30 amino acids, thus creating different MHC-I allotypes [5].

What is remarkable about the TCR/pMHC-I interaction is its exquisite specificity, as even one amino acid difference between MHC allotypes can have a profound impact on TCR recognition [6]. Many such allotypic differences have only a subtle effect upon the substructure of the pMHC-I complex [7]. The interaction is of very weak affinity (see Table 1) and is characterized by a
slow association and fast dissociation rate. A slow association rate is consistent with remodelling of the CDR loops upon ligation. From the initial suite of important TCR/pMHC-I studies [8,9], several generalizations were made regarding the nature of this interaction [10]:


(ii) Relative to the peptide, the MHC-I dominates the interaction with the TCR [10].

(iii) The CDR1 and CDR2 loops and the CDR3 loops interact with the MHC-I and peptide, respectively, in a two-step manner [11].

(iv) The interaction is characterized by induced fit, where the CDR3 loops mould around the pMHC-I; the pMHC-I moves minimally upon ligation.
The interaction is enthalpically driven, consistent with the CDR loops being stabilized upon ligation [12]. Below we describe the recent structural studies of TCR/pMHC-I complexes, highlighting how some complexes deviate from the generalizations outlined above, and what they inform us about the nature of the TCR/pMHC-I interaction (Table 1).

### ‘Vanilla’ complexes

MHC-I molecules generally present short peptide fragments (8–10 amino acids), providing up to three sidechains for TCR interaction. In extreme cases, these peptides might possess only one (or none) upward-facing sidechains, and these are considered as ‘vanilla’ peptides that present variable challenges for TCR recognition [13,14].

In achieving this recognition, the immune response sometimes selects a limited repertoire of ‘immunodominant’ TCRs that interact with the bland pMHC-I complexes. Immunodominant TCRs are so termed because they display restricted αβ TCR usage (i.e. identical TCRs are used from unrelated individuals to interact with a pMHC-I), which is surprising given that the TCR repertoire is enormous [15]. Two structures have provided insights into the structural basis of immunodominant TCRs, revealing a multitude of specificity-governing interactions between the TCR and the pMHC-I. Stewart-Jones et al. [16] reported the structure of a TCR (termed JM22) that docked orthogonally onto HLA-A2 bound to an influenza matrix peptide, MP (Table 1, Figure 3a) that had no upward-facing side-chains. Instead, a residue from the CDR3α loop protruded into the MHC-I cleft, thereby providing a key foothold for this interaction.

Kjer-Nielsen et al. [17,18,19] reported the structure of LC13 TCR in complex with HLA-B8 bound to the Epstein–Barr virus determinant, FLR (Table 1, Figure 3b). Here, the position 7 (P7)-tyrosine of the peptide was the focal point, being engulfed by the CDR loops, including the CDR1α loop. Importantly, the CDR3α loop was shown to have a major role in interacting with the MHC-I. To enable pMHC-I ligation, the LC13 TCR underwent significant conformational change, which included deformation of the CDR1α.

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**Table 1**

<p>| TCR pMHC-I complexes published since 2003. |
|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th><strong>TCR</strong></th>
<th><strong>pMHC-I (MHC-I/peptide)</strong></th>
<th><strong>Unliganded TCR structure</strong></th>
<th><strong>Biophysical data:</strong></th>
<th><strong>General comments</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>JM22</td>
<td>HLA-A2/MP</td>
<td>No</td>
<td>– 6.6 μM</td>
<td>Immunodominant TCR complex: orthogonal docking; CDR3α, CDR3β and CDR3γ interact with peptide and MHC-I; CDR1α interacts with peptide.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>– ΔG = -7.1, ΔH = -23</td>
<td></td>
</tr>
<tr>
<td>LC13</td>
<td>HLA-B8/FLR</td>
<td>Yes</td>
<td>–12.5 μM</td>
<td>Immunodominant TCR complex; CDR3α interacts mainly with MHC-I; CDR3 loops dictate energetics of interaction; entropically favoured interaction; movement in constant domain upon pMHC-I ligation.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>– ΔG = -6.7, ΔH = -2.4</td>
<td></td>
</tr>
<tr>
<td>SB27</td>
<td>HLA-B35/LPEP</td>
<td>No</td>
<td>– 9.9 μM</td>
<td>Super-bulged complex: immunodominant TCR; CDR3α interacts with peptide and MHC-I; peptide dominant interactions, in which CDR1β plays prominent role.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>– N/D</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1G4</td>
<td>HLA-A2/NY-ESO</td>
<td>Yes</td>
<td>– 13.3 μM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>– N/D</td>
<td></td>
</tr>
<tr>
<td></td>
<td>KK50.4</td>
<td>HLA-E/UL40</td>
<td>No</td>
<td>– 30.2 μM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>– N/D</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BM3.3</td>
<td>H-2Kβ/HSV8</td>
<td>No</td>
<td>– 114 μM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>– N/D</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AHIII12.2</td>
<td>HLA-A2/p1049</td>
<td>No</td>
<td>– N/D</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>– N/D</td>
<td></td>
</tr>
</tbody>
</table>

*a As determined by surface plasmon resonance.Key: N/D, not determined.*

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(v) The interaction is enthalpically driven, consistent with the CDR loops being stabilized upon ligation [12].
and CDR2α loops. Nevertheless, despite these conformational changes, the interaction was entropically favoured, as was recently demonstrated for another TCR/pMHC-I interaction [20*,21]. Despite the extensive LC13/HLA-B8/FLR interface, an alanine scanning study revealed that the CDR3 loops represented the energetic ‘hot spots’ of recognition, whereas the CDR1 and CDR2 loops were shown to be important in stabilizing the conformation of the ligated CDR3 loops, and not in directly contacting the antigen [22**].

The super-bulged complex

The antithesis to the vanilla peptides are the so-called ‘hot-chilli’ or super-bulged peptides that can bind to MHC-I and provide an abundance of peptide sidechains for the TCR. These peptides, which can be up to 14 amino acids in length, bulge centrally from the groove and were previously considered to represent an obstacle for TCR ligation [23]. These unusual pMHC-I complexes can also select immunodominant TCRs, as observed for the vanilla pMHC-I complexes [24,25]. Given that TCRs
typically ‘survive’ off a starvation diet of peptide structure, it was unclear how the TCR would adapt to a smorgasbord of available peptide residues. Recently, the structure of a TCR (SB27) bound to a 13-mer epitope complexed to HLA-B35 partly addressed these questions [26**,27]. In this complex, the TCR interactions (including the CDR1β loop) were dominated by the peptide, LPEP (Figure 3c). The SB27 TCR was orthogonally perched atop the centrally-bulged pMHC-I, interacting with seven sidechains, and made relatively fewer contacts with the MHC-I compared with other TCR/pMHC-I complexes (Figure 3). Hence, only limited contacts between the TCR and MHC-I appeared essential for mediating MHC-I restriction. These observations prompted a closer analysis of all TCR/pMHC-I structures, and revealed that positions 65, 69 and 155 of the MHC-I are invariably involved in contacting the TCR. This conservation suggests that this ‘restriction triad’, which corresponded to crucial interaction sites [22**,27], has a major role in shaping MHC-I restriction (Figure 2).

**Improved TCR recognition of a pMHC-I complex with altered anchor sites**

Recently, the structure of a TCR complexed to a 9-mer tumour-derived epitope (termed NY-ESO) bound to HLA-A2 was reported (Figure 3d) [28]. This peptide was more typical of pMHC-I complexes, being a ‘mild salsa complex’ with a bulky Trp and Met available to mediate TCR contacts [29]. Although TCRs cannot interact directly with the buried residues that anchor the peptide in the binding cleft, it was shown that these residues can influence TCR binding indirectly. The conservative substitution of residues at P9 of the peptide caused conformational changes that propagated down the length of the peptide leading to an alteration in the angle of binding of the TCR, and resulted in a better ‘fit’, and increased affinity, for the TCR [28].

**A non-classical MHC-I complex**

HLA-E, a member of the class of non-classical (Class 1b) MHC-I, exhibits limited polymorphism and typically has a role in innate immunity by binding almost exclusively peptides of the MHC-I leader sequence [30]. Recently, however, a viral mimic (from cytomegalovirus, CMV) of the MHC-I leader sequence was shown to stimulate an HLA-E-mediated adaptive immune response. The structure of this TCR/HLA-E complex has highlighted the finely tuned specificity of the interaction, where TCR specificity is controlled by a single peptide residue at P8 (Figure 3e). In the CMV-derived peptide this residue is an Ile, whereas in Class I leader sequences, this residue is a Val or Leu. Mutation of P8-Ile to P8-Val abrogates TCR binding. This heightened specificity is achieved by all three CDRβ loops converging onto position 8 of the peptide [31].

**Mistaken identity in TCR recognition**

Despite the constraints of MHC-I restriction, up to 10% of an individual’s T cell population can crossreact with other MHC-I allotypes, so called T cell allorecognition. T cell crossreactivity manifests as T cell-mediated transplant rejection, making it an issue of clinical importance. The structures of a murine TCR, named BM3,3, in complex with disparate pMHC-I peptides have provided some insight into the paradox of TCR crossreactivity, whereby the degeneracy of recognition is partly attributable to conformational plasticity of the CDR3α loop (Figure 3f) [32,33⁎]. Nevertheless, the structural basis of direct T cell alloreactivity remains unresolved.

The structure of a xenoreactive TCR/pMHC-I complex, in which the murine TCR docked orthogonally to the peptide-binding groove of the human pMHC-I was reported (Figure 3g) [34]. Given that this TCR is not selected on the xenogeneic human MHC-I, the conformational restraints that generally impose diagonal docking were attributed to the hard-wired components such as CD8. However, as mentioned above, orthogonal docking has been observed in the SB27/HLA-B35/ LPEP (Figure 3c) and JM22/HLA-A2/MP (Figure 3a) structures. Nevertheless, other studies also suggest that CD8 might have a greater role in guiding MHC-I restriction than previously appreciated [21].

**T cell signalling**

TCR/pMHC-I post-engagement is transmitted as a signal via components of the CD3 complex [35]. The stoichiometry and basic arrangement comprises an αβ TCR heterodimer, a CD3εγ heterodimer, a CD3ζδ heterodimer and a CD3ζζ homodimer, interacting within the transmembrane regions [36]. How signalling is achieved through this complex is unknown because structural changes upon TCR/pMHC-I engagement appear localized to this interface, with no changes observed in the constant domains of the TCR. A caveat to this is that LC13 undergoes a ligand-induced conformational change in the AB loop of its Cα domain (Figure 1) [19], which might potentially reflect one mode of TCR signalling.

Recently, the structures of the murine and human CD3εγ and CD3ζδ heterodimers have been determined [37–40]. These studies reveal that the ectodomains of CD3εγ and CD3ζδ is comprised of Ig domains that interact with each other to form an unusual side-to-side dimer configuration (Figure 4). Several points can be made by the comparison of all four structures: (i) there is little variation in the structure of the CD3ζε chain when bound to either CD3γ or CD3ζ; (ii) in the two human CD3 structures the antibody epitopes are overlapping; and (iii) there is a prominent acidic patch on CD3ζ. It is speculated that CD3ζ is located under the FG loop of Cβ and adjacent to the AB loop of Cα [41]. Collectively, these factors permit
one to infer the assembly of the TCR/CD3 complex (Figure 1).

Further to the model of the TCR/CD3 assembly, two different models of TCR signalling have been proposed. The rigid pairing of the CD3εγ subunits, together with the putative docking of the TCR/CD3εγ complex is considered to favour a piston-like displacement of CD3 in the cell membrane upon TCR ligation [39,42,43]. However, the position of the antibody (OKT3) bound to CD3ε argues against the piston-like movement and supports either a rotational or lateral movement of the CD3ε relative to the TCR [44]. Such physical changes could render the cytoplasmic components of these molecules available for interaction with second messenger mediators, such as kinases and cellular signalling components.

**Concluding remarks**

The recent series of TCR/pMHC-I structures has prompted a re-evaluation of the generalities pertaining to this interaction. It appears that there is no common diagonal docking mode, and that the individual contributions of the CDR loops vary to such a degree that it is difficult to reconcile this with a two-step binding model. The peptide has been shown to dominate the interaction in one study; furthermore, a common thermodynamic signature does not correlate with the observed plasticity of recognition. Nevertheless, from the handful of unique TCR/pMHC-I structures determined, a rough docking mode is preserved, in which the Vα domain is positioned over the α2 helix and the N-terminal end of the peptide, whereas the Vβ domain is positioned over the α1 helix and C-terminal end of the peptide. Within this approximate framework, three positions on the MHC-I molecule have surfaced to be repeatedly used, and this might represent the minimal requirements of MHC-I restriction. The continued pursuit of novel TCR/pMHC-I complexes, accompanied by biophysical analyses, is required to build a comprehensive collection of structures that enable further understanding of the interaction. Important areas to pursue include crossreactive TCRs, atypical epitopes, immunodominant TCRs and HLA allotype-specific TCRs. In this regard, it is important to formally ascertain how small shifts in pMHC-I structure, caused by MHC-I polymorphism, can have such profound effects on TCR recognition.

The structures of the individual components of the CD3 complex are known, but a burning question in the field is
the structural basis of the TCR/CD3 interaction. As structural biologists tackle larger macromolecular assemblies, the ultimate challenge will be to examine the ensemble of molecules within the immunological synapse. Undertaking such studies, in conjunction with spectroscopic measurements and other imaging techniques might provide an opportunity to understand the dynamics of how pMHC-I engagement leads to T cell signalling. Ultimately, this body of information might then be used to modulate the immune response for therapeutic applications.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


The authors demonstrate how CDR3 loop flexibility contributes to the crossreactivity of a TCR.


