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T cell receptor engagement of peptide-major histocompatibility complex class I does not modify CD8 binding

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Abstract

Activation of cytotoxic T cells is initiated by engagement of the T-cell receptor (TCR) with peptide-major histocompatibility class I complexes (pMHCI). The CD8 co-receptor also binds to pMHCI, but at a distinct site, and allows the potential for tripartite TCR/pMHCI/CD8 interactions, which can increase T cell antigen sensitivity. There has been a substantial interest in the effect of the pMHCI/CD8 interaction upon TCR/pMHCI engagement, and several conflicting studies have examined this event, using the soluble extracellular domains of CD8 and the TCR, by surface plasmon resonance. However, the evidence to date suggests that the TCR engages cognate pMHCI before CD8 recruitment, so the question of whether TCR engagement alters CD8 binding is likely to be more relevant to the biological order of T cell antigen encounter. Here, we have examined the binding of CD8 to several variants of the HLA A2-restricted telomerase$_{540–548}$ antigen (ILAKFLHWL) and the HLA A2-restricted NY-ESO-1$_{157–165}$ antigen (SLLMWITQC) that bind to their cognate TCRs with distinct affinities and kinetics. These interactions represent a range of agonists that exhibit different CD8 dependency for activation of their respective T cells. By using engineered affinity enhanced TCRs to these ligands, which have extended off-rates of ∼1 h compared to seconds for the wildtype TCRs, we have examined pMHCI/CD8 binding before and during TCR-engagement. Here we show that the binding of the extracellular domain of the TCR to pMHCI does not transmit structural changes to the pMHCI-CD8 binding site that would alter the subsequent pMHCI/CD8 interaction.

Keywords: TCR; CD8-co-receptor; T cell; Antigen recognition; K_D; Surface plasmon resonance

1. Introduction

Cytotoxic T lymphocyte (CTL) antigen recognition and CTL activation are mediated by T-cell receptor (TCR) engagement of peptide-major histocompatibility complex class I (pMHCI). However, the activities of the CD8 co-receptor (expressed mainly on MHC class I-restricted CTLs) (Cantor and Boyse, 1975) can increase T-cell antigen sensitivity, and aid activation (Janeway, 1992). The CD8 co-receptor binds to the same pMHCI complex as the TCR, but at a spatially distinct site from the TCR/pMHCI antigen-specific interaction (Gao et al., 1997; Janeway, 1992), enabling the possibility for tripartite TCR/pMHCI/CD8 interactions to take place. This system of two receptors engaging one ligand to generate intracellular signal transduction is unique to T cells. Cooperative binding of the TCR and CD8 during pMHCI engagement may be an important mechanism by which T cell activation is achieved, and has therefore been the subject of many studies (Garcia et al., 1996; Wooldridge et al., 2005; Wyer et al., 1999).

Experiments using soluble pMHCI have determined that CD8 stabilizes the TCR/pMHCI interaction at the cell surface (Luescher et al., 1995; Wooldridge et al., 2005) by about two-fold and there has been considerable interest in the possibility that the binding of the extracellular domain of CD8 to pMHCI modifies the TCR binding site (Garcia et al., 1996; Wyer et al., 1999). The first study to examine whether the extracellular domains of the TCR and CD8 cooperate in binding pMHCI concluded that CD8 enhances formation of TCR/pMHCI complexes

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(Garcia et al., 1996). However, the surface plasmon resonance (SPR) data presented in this study exhibited characteristics consistent with the presence of protein aggregation (Wooldridge et al., 2005) and our own subsequent studies utilizing the same techniques have reached the opposite conclusion (Wyer et al., 1999). More recently, fluorescent resonance energy transfer (FRET) based examinations of the TCR/pMHCI/CD8 antigen recognition complex have shown that the TCR binds before CD8 (Yachi et al., 2006). This order of antigen engagement is likely to be important in ensuring that the specific interaction between the TCR and pMHCI dominates CTL recognition. Thus the question of whether TCR binding alters the binding site for CD8 is more relevant to T cell biology than the question that has been addressed previously of whether CD8 binding affects TCR engagement.

Here, we used a range of ligands known to exhibit the full spectrum of CD8-dependencies, from total CD8 dependence to near CD8 independence in order to elicit T cell activation, in order to study whether the engagement of pMHCI by the TCR affects subsequent CD8 binding. By utilizing engineered high affinity TCRs that bind to pMHCI with the same overall conformation as the wildtype TCRs, but with half-lives of ∼1 h as opposed to a few seconds for the wildtype TCR/pMHCI interactions, we were able to use SPR to measure CD8 binding before and during TCR/pMHCI engagement in real time.

2. Materials and methods

2.1. Generation of expression plasmids

The HLA-A*0201 telomerase$_{540–548}$ restricted wildtype TCR (Tel TCR), the HLA-A*0201 telomerase$_{540–548}$ high affinity TCR (c13 TCR) and the HLA A*0201 NY-ESO-1$_{157–165}$ restricted high affinity TCR (c49c50 TCR) α and β chains, the CD8 α chain and the HLA-A*0201 (A2), A*02402 (A24) (Cole et al., 2006) and B*0801 (B8) α chain and β2m sequences were generated by PCR mutagenesis (Stratagene) and PCR cloning. All sequences were confirmed by automated DNA sequencing (Lark Technologies). The high affinity c13 TCR and c49c50 TCR were produced using a phage display library based on the HLA-A*0201 telomerase$_{540–548}$ (ILAKFLHWL) antigen (A2 Tel) and the HLA A*0201-restricted NY-ESO-1$_{157–165}$ (SLLMWITQC) antigen (A2 NY-ESO-1) respectively, the method of which has been previously reported (Li et al., 2005). The Tel TCR, the c13 TCR and the c49c50 TCR were constructed using a disulphide linked construct to produce the soluble domains (variable and constant) for both the α (residues 1–207) and β chains (residues 1–247) (Boulter et al., 2003; Garboczi et al., 1996). The A2, A24 (Cole et al., 2006) and B8 (Kjer-Nielsen et al., 2002) soluble heavy chain (residues 1–248) (α1, α2 and α3 domains), tagged with a biotinylation sequence, and β2m (residues 1–100) were also cloned and used to make the pMHCI complexes. For CD8, the α chain domain (residues 1–120) was cloned to make the CD8 αα homodimer. The TCR α and β chains, CD8, the A2 α chain and β2m sequences were inserted into separate pGMT7 expression plasmids under the control of the T7 promoter (Garboczi et al., 1996).

2.2. Protein expression, refolding and purification

BL21 Rosetta DE3 *E. coli* cells were used to produce the TCR α and β chains, the CD8 α chain and the A2, A24 and B8 heavy and β2m chains in the form of inclusion bodies (IBs) using 0.5 mM IPTG to induce expression as described previously (Garboczi et al., 1996). For a 1 L refold, 30 mg of TCR α chain IBs were incubated at 37 °C for 15 min with 10 mM DTT and added to 1 L of cold refold buffer (50 mM TRIS pH 8.1, 2 mM EDTA, 2.5 M urea, 6 mM cysteamine hydrochloride and 4 mM cystamine). After 10–15 min, 30 mg of TCR β chain, incubated for 10–15 min at 37 °C with 10 mM DTT, was added. For a 1 L pMHCI refold, 30 mg of α chain was mixed with 30 mg of β2m, 4 mg of synthetic peptide and 10 mM DTT at 37 °C for 15 min, which were then added to 1 L of cold refold buffer (50 mM TRIS pH 8.1, 2 mM EDTA, 400 mM L-arginine, 6 mM cysteamine hydrochloride and 4 mM cystamine). For the pMHCI/CD8 binding analysis before and during TCR/pMHCI engagement, three different A2 complexes were refolded with three different 9 mer peptides derived from the screening of a mutant peptide library based on the A2 Tel antigen. These included; A2 Tel, A2-ILGKFLHWL (A2 3G) and A2-ILAKYHWL (A2 5Y). The A2 NY-ESO-1 protein was also refolded for this analysis. Furthermore, we screened CD8 binding against a number of other pMHCI complexes in order to compare CD8 binding over a broad range of different ligands. These were as follows; A*0201-LLFGYPVYV (A2 Tax), A*2402-PYLFWLAAI (A24 EBV), A*0201-YLEPGPVTV (A2 GP100), A*0201-ELAGIGILTV (A2 Mel), A*0201-GILGFVFTL (A2 Flu) and B*0801-FLRGRAYGL (B8 EBNA). Refolding of TCR and pMHCI was performed at 4 °C for >1 h. Dialysis was carried out against 10 mM TRIS, pH 8.1 until the conductivity of the refolds was under 2000 μS. The refolds were then filtered through a 0.45 μM filter, ready for purification steps. The refolded TCR and pMHCI proteins were purified initially by ion exchange using a Poros 50HQ^{TM} column.

Refolding of CD8 was carried out as previously described (Cole et al., 2007b; Gao et al., 1998) with some modifications. For a 1 L refold, 60 mg of CD8 α chain IBs were incubated in 6 mL of 6 M guanidine buffer at 37 °C for 15 min with 10 mM DTT. The denatured IBs were then added to 1 L of cold refold buffer (100 mM TRIS-base, 76 mM TRIS-acid, 1 mM EDTA, 600 mM L-arginine, 6 mM cysteamine hydrochloride and 4 mM cystamine). The CD8 refold was then mixed at 4 °C for >1 h. Dialysis was carried out against 10 mM MES pH 6 until the conductivity of the refolds was under 2000 μS. The refolds were then filtered through a 0.45 μM filter, ready for purification steps. The CD8 protein was initially purified by cation exchange using 10 mM MES pH 6 as binding buffer and 10 mM MES pH6, 1 M NaCl as elution buffer, using a Poros50 HS^{TM} column.

All of the soluble proteins (TCR, pMHCI and CD8) were then gel filtered into BIAcore buffer (10 mM HEPES pH 7.4, 150 mM, NaCl, 3 mM EDTA and 0.005% (v/v) Surfactant P20), using a Superdex 200HR^{TM} column. This step was implemented on the day of analysis, and concentration of the proteins was kept to a minimum in order to minimize protein aggregation. Proteins quality was analyzed by Coomassie-stained SDS-PAGE.