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Going Pro to enhance T-cell immunogenicity: easy as π ?

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Abstract

MHC class I molecules bind intracellular oligopeptides and present them on the cell surface for CD8⁺ T-cell activation and recognition. Strong peptide/MHC class I (pMHC) interactions typically induce the best CD8⁺ T-cell responses; however, many immunotherapeutic tumor-specific peptides bind MHC with low affinity. To overcome this, immunologists can carefully alter peptides for enhanced MHC affinity but often at the cost of decreased T-cell recognition. A new report published in this issue of the *European Journal of Immunology* [Eur. J. Immunol. 2013. 43: XXXX-XXXX] shows that the substitution of proline (Pro) at the third residue (p3P) of a common tumor peptide increases pMHC affinity and complex stability while enhancing T-cell receptor recognition. X-ray crystallography indicates that stability is generated through newly introduced CH- π bonding between p3P and a conserved residue (Y159) in the MHC heavy chain. This finding highlights a previously unappreciated role for CH- π bonding in MHC peptide binding, and importantly, arms immunologists with a novel and possibly general approach for increasing pMHC stability without compromising T-cell recognition.

Keywords

altered peptide ligand; MHC class I; peptide; tumor-associated antigen

Major histocompatibility class I (MHC I) molecules are constitutively expressed on the surface of nearly all nucleated cells in jawed vertebrates. MHC I molecules are non-covalently associated trimers consisting of a polymorphic heavy chain, β_2 -microglobulin, and an oligopeptide. The MHC heavy chain folds into two alpha helices and a beta sheet, creating the walls and floor, respectively, of a peptide-binding cleft [1]. Hydrogen bonding between conserved heavy-chain cleft residues and the N- and C-termini of associated peptides typically limit their length to 8-13 residues (though exceptions abound [2]), and strictly dictate peptide directionality. Additionally, six defined pockets (termed A-F) in the cleft confer specificity for peptide side chains oriented towards the groove [3]. Extensive polymorphism between the binding grooves of different MHC allomorphs (more than 7,000 known alleles (and climbing) in human populations with up to 6 allomorphs expressed per person from the HLA A, B, and C loci (<http://hla.alleles.org/nomenclature/stats.html>)), ensures that a wide spectrum of peptides is presented to the immune system, essentially preventing pathogen escape at the population level.

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Conflict of interest

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Despite allelic preferences, common themes guide peptide/MHC (pMHC) binding. Pooled amino acid sequencing of peptides eluted from many different individual class I allomorphs revealed residues over-represented at each position [4, 5], though notably, these allele-specific peptide-binding motifs are also influenced by peptide liberation, transport, and trimming (reviewed in [6]). Most peptide pools exhibit highly dominant specific amino acids (or chemically similar amino acids) at or near their N- and C-termini [4, 5]. These “anchor” residues greatly influence peptide-binding affinity. The more N-terminal anchor is typically located at peptide position 2 or 3 (denoted as p2 and p3) and is accommodated by the B-pocket of the peptide-binding cleft, though it can be located up to p5 (C-pocket, as is the case for the mouse H-2D^b allomorph [4]). The deep F-pocket cradles the C-terminal anchor, typically an aliphatic or aromatic residue for mouse class I allomorphs (some human allomorphs also favor basic C-terminal anchors). Predictably, detailed peptide mapping [7] and high-throughput mass spectrometry [8] identify numerous high-affinity peptides that break these simple rules, increasing both the size of the immunopeptidome and the difficulty of in silico peptide-binding prediction. Class I molecules present tens of thousands of different self-peptides among approximately 10⁵ pMHC complexes on the surface of each cell [9], consistent with their role in tumor immunosurveillance.

How does the cell supply this diverse array of pMHCs? Most MHC class I peptides arise from rapidly degraded polypeptides (DRiPs), ensuring representation among the translome independently of protein stability and minimizing the time to detect viral translation [10]. To enhance immunosurveillance of tumor-associated antigens (TAAs), ribosome subpopulation sampling [11, 12] likely enables surveillance of low abundance bona fide and defective mRNAs [13, 14]. TAA-peptide abundance is critical, since many TAAs derive from non-mutated genes and are thus recognized by low affinity T cells that escape self-tolerance [15].

The affinity of peptides for MHC governs the stability of complexes and hence levels of cell surface expression [16]. Consequently, the immunogenicity of pMHCs can potentially be improved by increasing peptide affinity [17]. Though peptide-binding algorithms have greatly enhanced rational peptide design, they are far from perfect. Further, despite their orientation away from the TCR, anchor residue substitutions can change pMHC conformation to negatively impact TCR recognition. What is needed then is a bit of magic: a general method for increasing peptide affinity while minimizing changes in TCR specificity.

In this issue of the *European Journal of Immunology*, while seeking to improve the CD8⁺ T-cell response to the melanocyte differentiation Ag Gp100, Uchtenhagen et al. [18] appear to achieve the impossible, or at least the improbable. Gp100 expression is greatly enhanced in melanoma, making it an attractive therapeutic vaccine target. Human gp100₂₅₋₃₃ peptide (KVPRNQDWL (KVP)) presented by the mouse class I D^b allomorph elicits self-reactive mouse CD8⁺ T cells, while the orthologous mouse peptide (EGSRNQDWL (EGS)) does not [19, 20]. Both peptides possess canonical p5N and p9L anchor residues for D^b (which has a motif of XXXXNXXX[IML], where X represents any amino acid) [4]. Despite identical anchors, EGS binds D^b with 100-fold lower affinity than KVP [15], evincing the contribution of non-anchor residues to D^b binding [21, 22]. Systematic crosswise substitution of p1-3 between KVP and EVS revealed greatly enhanced peptide binding [15] and pMHC stability when simply replacing p3 of EGS with Pro (EGPRNQDWL (EGP)) [23]. Immunization with EGP elicited higher numbers of EGS-specific CD8⁺ T cells than EGS itself, and critically, protected against tumor challenge while the homologous peptide did not [23].

Uchtenhagen et al. [18] scrutinized the structural basis for enhanced EGP peptide affinity with surprising and potentially generally applicable findings. X-ray crystallography of D^b complexed with Gp100 peptides KVP, EGS, or EGP revealed a conserved peptide

conformation and similar peptide-D^b hydrogen bonding in each complex [23]. Thus, the EGP's increased affinity was not due to large structural alterations in the complex. Notably, in EGP, the pyrrolidine ring of p3P and the hydroxyphenyl group of D^b Y159 formed CH- π interactions, which affords substantial intermolecular binding energy [24, 25] (and see <http://www.tim.hi-ho.ne.jp/dionisio/page/whatis.html>).

To examine the contribution of CH- π interactions (which occur with aromatic residues) to EGP/D^b stability, Uchtenhagen et al. substituted Y159 with either another aromatic (F) or aliphatic residues with a short (A) or long (L) side chain. Intriguingly, the enhanced pMHC stability of EGP versus EGS was abrogated with D^b-Y159A or D^b-Y159L. An intermediate effect was observed with Y159F, consistent with reduced energetic stabilization of Phe-Pro CH- π interactions compared with that of Tyr-Pro. To confirm CH- π interactions between p3P and Y159F, the authors crystallized EGP/D^b-Y159F and found nearly identical peptide and 159 side-chain orientation as in wild-type D^b.

The most important aspect of the study is the effect of the CH- π interaction on TCR recognition of the modified peptide. EGP/D^b complexes bind *better* to the cognate TCRs than complexes with wild-type peptide, providing a double advantage of the Pro substitution. To gain insight into this effect, Uchtenhagen et al. used high-powered computers to simulate the simultaneous movements of individual atoms of the structure. Such "molecular dynamics" analysis suggests that increased TCR affinity results from increased rigidity of the peptide within the D^b cleft.

As with all good science, discoveries beget questions. Most pragmatically, can the increased pMHC affinity, pMHC stabilization, and TCR recognition afforded by the p3P substitution be generally extended to other peptide/MHC combinations for enhanced vaccine efficacy? Previous work by Achour and colleagues suggests that p3P altered peptides bind to D^b or K^b with increased affinity [23]. Since Y159 is highly conserved among human HLA genes and alleles, this likely applies to human pMHC complexes, particularly for those unusual allomorphs that do not bind with strong P2 anchors (such as B*0801). Can other aromatic residues within the peptide-binding cleft be exploited for CH- π interactions, and if so, will tertiary structure be preserved to maintain TCR recognition? Is increased peptide rigidity generally positive for TCR recognition? Does increased binding uniformly extend to endogenous peptides when they are loaded on to class I in the ER by the peptide-loading complex? Although binding of exogenous peptides to class I is generally considered to precisely mimic the binding of endogenous peptides, peptides can bind to class II in multiple conformations, depending on how they are loaded, with major biological consequences [26].

The work of Uchtenhagen et al. [18] beautifully illustrates the importance of continued research on problems thought to be "solved." It is essential for young scientists in particular to appreciate that nature's secrets are boundless, and that the critical information for practical applications often springs from surprising sources that are best accessed by curiosity-driven research.

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Abbreviations

MHC   major histocompatibility complex

p1-p9	peptide positions 1-9
pMHC	peptide/MHC complex
TAA	tumor-associated antigen
TCR	T cell receptor

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D^b BindingT-cell
Activation

strong

good

weak

much worse

strong

better

Figure 1.

“One π to Rule Them All”. Residues at peptide positions 5 and 9 interact with deep pockets in the Db binding groove, and have a large effect on peptide binding affinity. Uchtenhagen et al. show that position 3 can also make a large contribution to binding. Pro not only increases Db binding affinity of the 9-mer peptide, but also increases T-cell activation. This effect is due to pi-binding of the proline to Tyr at position 159 of Db. 159 residue is highly conserved among class I molecules, raising the possibility that substituting Pro at position 3 will be a more general strategy for increasing peptide immunogenicity.