Published in final edited form as:

Semin Immunol. 2007 August ; 19(4): 236–244. doi:10.1016/j.smim.2007.04.003.

A role for “self” in T-cell activation

Michelle Krogsgaard[#], Jeremy Juang^Ψ, and Mark M. Davis^{¶,Ψ}

[#] Department of Pathology and NYU Cancer Institute, NYU School of Medicine, New York, New York 10016, USA

[¶] Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305, USA

^Ψ Department of Microbiology and Immunology, Stanford University, Stanford, CA 94305, USA

Abstract

The mechanisms by which $\alpha\beta$ T cells are selected in the thymus and then recognize peptide MHC (pMHC) complexes in the periphery remain an enigmatic. Recent work particularly with respect to quantification of T-cell sensitivity and the role of self-ligands in T-cell activation has provided some important clues to the details of how TCR signaling might be initiated. Here, we highlight recent experimental data that provides insights into the initiation of T-cell activation and also discuss the main controversies and uncertainties in this area.

Keywords

Self-peptide-MHC; T-cell activation; pseudodimer; Thymic selection

Introduction

For an efficient immune response, receptors on T cells must interact with peptide-major-histocompatibility complexes (pMHCs) of antigen presenting cells (APC) to form “immune synapses”, but the role of self-peptides in this process has been unclear. While it is evident that self-peptides are important for positive selection in the thymus and for maintaining T cell numbers in the periphery, it has not until recently been known whether self-peptides contribute to activation of T cells by agonist ligand. Our recent studies have shown that heterodimers of peptide-MHC including an agonist and a self-peptide are the basic units needed to initiate T-cell activation, at least in CD4⁺ T cells¹. This unique ability of T-cells to use self-pMHC to increase the sensitivity to specific agonist pMHC complexes in the periphery suggests a mechanism by which T cells are positively selected in thymus on particular self-pMHC with the purpose of increasing sensitivity to antigen in the periphery¹.

The basic unit of TCR signaling: models of T-cell activation

With regard to the sensitivity of T-cells to peptide-MHC ligand, there have been a variety of estimates for the number of ligands required for optimal T-cell signaling. Early studies estimated that anywhere from 1–400 peptides per antigen-presenting cell were needed to fully activate a T-cell^{2–7}. More recent studies using soluble pMHC dimers suggest that, at least for

Correspondence to: Michelle Krogsgaard.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

CD4⁺ T-cells, a dimer or trimer of agonist pMHC was needed for full T-cell activation^{8, 9}. In contrast to these studies, Irvine et al.¹⁰ and Purbhoo et al.¹¹ demonstrated by single molecule-peptide labeling of fluorescent peptides in the synapses that for both CD4⁺ and CD8⁺ T-cells, even a single agonist pMHC can trigger a transient Ca²⁺ signal recognized on a cell surface. Irvine et al.¹⁰ showed for CD4⁺ T cells that ~10 ligands needed for a sustained calcium response and mature synapse formation whereas Purbhoo et al.¹¹ showed for CD8⁺ T cells that only three peptides are needed for the induction of cytotoxicity. Furthermore, activation of CD4⁺ T-cells is dependent on the co-receptor CD4's engagement only at low densities of antigens¹⁰ whereas the activation of CD8⁺ T-cells and immunological synapse formation is dependent on CD8 co-receptor engagement regardless of antigen density¹¹. So how do we reconcile the different outcomes of these studies using soluble multimers with the cellular data? We think the answer lies in the many endogenous peptides that accumulate at the immunological synapse^{1, 10, 12}. Our work with CD4⁺ T cells suggests a 'pseudodimer' model of activation in which agonist-endogenous pMHC heterodimers, stabilized by the co-receptor CD4, are crucial intermediates for triggering CD4⁺ T lymphocytes. This model is supported by computational and experimental studies which showed that, because helper T-cells were sensitive to as few as ten agonists, a model in which agonist and endogenous ligands act autonomously to trigger the T cell is unlikely¹³. In particular the importance of spatial localization of Lck, a Src family tyrosine kinase, in signaling complexes which, in turn, enable certain endogenous ligands to trigger many TCRs, was also highlighted¹³. Other models have also been suggested for what might be the basic unit of TCR-activation. Two of the most prominent models are the "co-receptor" model and the "dimer of dimers model". The "co-receptor" model states that CD4 binds the same pMHC as the TCR. Failure to stimulate T-cells with soluble pMHC complexes and lack of evidence for why antibody cross-linking of TCR-CD3 molecules is stimulatory has significantly challenged this model. Furthermore, this model provides no evidence for why self-peptides might contribute to T-cell activation when agonist ligand is limiting. The "dimer of dimers" model was first proposed based on work by Brown et al.¹⁴ who observed the presence of a dimer in the three-dimensional structure of HLA-DR1. This model has become quite popular, in particular because of experimental support from Cochran et al.⁸ who showed that a homodimer of agonist pMHC complexes can induce T-cell activation and that pMHC monomers do not activate T-cells. This model is not able to account for the recent findings described above in which that a single agonist pMHC can initiate T-cell activation. Also, subsequent structural data do not show the same dimers of MHC as first seen by Brown et al. not do any TCR-pMHC complexes have 'dimers of dimers' configuration showing that this earlier report is the exception rather than the rule. In addition it is also important to note that even if such dimers existed, it would be very rare events at low peptide concentrations for two agonist pMHC to form dimers (amidst thousands of endogenous pMHC).

Contribution of endogenous peptides in CD4⁺ T-cell activation

The idea that null/endogenous peptides could contribute to T-cell activation was first suggested by Wulfing et al.¹² who labeled null peptides in synthetic lipid bilayers and looked at synapse formation when small amounts of agonist peptide were present. Wulfing et al. also found that some null peptides accumulated into the synapse at high density, whereas other null peptides did not. By labeling pMHC molecules on the APC, they found that there was recruitment of endogenous peptides at the synapse upon recognition of antigen¹². This idea was reinforced and quantified by Irvine et al.¹⁰ showing that 20% of endogenous peptides present on an APC accumulate into the synapse upon recognition of antigen. Stefanova et al.¹⁵ observed that the absence of class II MHC molecules caused a deterioration in the reactivity of CD4⁺ T cells. Recently, we used video fluorescence microscopy to assess the recruitment of endogenous pMHC complexes into the immunological synapse in the presence of minute amounts of agonist ligand¹. We visualized endogenous peptides in the synapse using the approach of Irvine

et al.¹⁰ where MHC are pulsed on APCs with peptides that have long D-amino acid extensions (to prevent proteolysis) outside of the MHC binding portion, terminating in a biotin that can be labeled with streptavidin-Cy3 and then visualized by a sensitive camera. The endogenous peptides tested in these experiments were a panel of I-E^k-specific endogenous peptides that had been previously identified using acidic elution and mass-spectroscopy^{16, 17} from spleen and thymus cells from mice, as well as from an I-E^k specific cell line^{16, 17}. As shown in Fig. 2, not all endogenous peptides are recruited to the cSMAC of the synapse. Rather, a fraction of these which might represent endogenous pMHC with a higher affinity for the TCR. In an effort to understand the mechanisms by which self-peptides convey single molecule sensitivity of T-cells to agonist ligand, and as a critical test of the 'pseudodimer' hypothesis, we made covalently linked heterodimers of agonist and endogenous ligands. For this purpose we used well-defined cross-linking reagents originally described by Cochran et al.⁸ where two thiol-reactive maleimide groups spaced by a flexible backbone can be reacted with pMHC proteins carrying a cysteine residue on the β -chain to form mixed heterodimers of endogenous and agonist pMHCs. As shown in Fig. 3, when we mixed T-cells with these soluble dimers we observed that some specific combinations of agonist pMHC with endogenous pMHC could stimulate T-cells. In contrast monomeric agonist or dimers of endogenous pMHCs were not stimulatory. We verified that the synergistic effect of the endogenous peptides was not due to chemically cross-linking the two ligands by testing the stimulatory effect of mixtures of agonist and endogenous pMHC on artificial membranes and cells. We observed the same synergistic effect of the endogenous peptides as when presented as soluble heterodimers together with agonist. Consistent with theoretical predictions¹³, we demonstrated that stronger agonists were able to synergize with a larger fraction of endogenous peptides than weaker agonist. Results of these experiments strongly support models^{10, 13} in which agonist and endogenous ligands act cooperatively, rather than additively to stimulate T-cell signaling. The role of CD4 in stimulation by heterodimers was also tested in these studies. The original pseudodimer model was partly based on structural data from Reinherz and colleagues¹⁸, showing that CD4 binds to MHC class II at an angle that would not allow a CD4 molecule to bind the same MHC molecule that the TCR is engaging (Fig.4). Supporting this model are also earlier studies showing that a large fraction of TCRs are constitutively associated with CD4 molecules on antigen experienced T cells. To test the contribution of CD4 to pseudodimer formation, we mutated the CD4 binding site on the MHC class II molecules presenting agonist or self-peptide, respectively. We showed activation was significantly impaired if the CD4 binding site on the agonist-pMHC was mutated whereas this mutation on the endogenous-pMHC had no effect¹ (Fig.4). On this basis we developed a modified version of the pseudodimer model as shown in Fig. 1.

The contribution of endogenous peptides in CD8⁺ T cell activation

While there is now rather strong evidence that endogenous peptides and agonist ligands act cooperatively to respond to minute amounts of agonist ligands for CD4⁺ T-cells, the mechanism of CD8⁺ T-cell activation is controversial. Early results implied that endogenous pMHC does not seem to affect the activation of CD8⁺ T-cells¹⁹. Another result showed that soluble monomeric forms of MHC of agonist-pMHC are sufficient to activate CD8⁺ T-cells²⁰. Other work, however, has shown that peptide from soluble monomers can be transferred to endogenous pMHC complexes on a T-cell so the T-cell can act as APCs to activate T-cells, casting doubt on the interpretation that monomers can activate²¹. In one report, it was asserted that endogenous ligands are irrelevant and instead it was suggested that it is a phenomenological model based on competition between positive and negative feed-back loops that leads to multiple steady-states and ultrasensitive responses²². In contrast to these results, three different experimental studies, in addition to the two CD4⁺ studies mentioned above have demonstrated that endogenous peptides or null peptide can contribute to T-cell activation in CD8⁺ T-cells by enhancing the response to small numbers of agonist ligands^{23–25}. The work

by Yachi et al. showed that a CD8⁺ T-cell hybridoma responded equivalently to all non-stimulatory peptides. On that basis they concluded that the TCR is not involved for the CD8 interaction but concentrates CD8 and pMHC complexes in the synapse making the cognate pMHC-TCR-CD3-CD8 interaction more likely and thereby increasing the sensitivity to antigen. This is in contrast to the work by Luescher and colleagues²⁵ and our unpublished data (J.Juang, M. Krogsgaard and M. Davis) which show that different null or endogenous peptides contribute differently to CD8⁺ activation. It is possible that the activation mechanism in CD4⁺ and CD8⁺ T-cells is to some extent different. Several groups have measured the affinity of CD8 binding to MHC class I^{26–29}, whereas binding of CD4 to MHC class II has been much more difficult to demonstrate, suggesting that the affinity of the CD4/MHC class I interaction is at least 25-fold lower³⁰. Furthermore, even though CD8 and CD4 have similar biological roles, they have little structural similarity^{31, 32}. It is therefore possible that CD8 mediates a more stable interaction between the CD8-TCR-CD3 than does CD4 between CD4-TCR-CD3, so a larger fraction of endogenous peptides that contribute to activation by agonist ligand will be seen in CD8 systems than in CD4 systems (J.Juang, M. Krogsgaard and M. Davis, unpublished). Still, there seem to be a consensus in most of the results cited above that activation of T-cells by agonist can be enhanced significantly in the presence of endogenous peptides in both CD4⁺ and CD8⁺ T-cells.

Mechanism of the contribution of endogenous peptides in T-cell activation

What is the mechanism by which endogenous peptides mediate their synergistic effect to activation by agonist ligand? As pictured in Fig. 5 we can imagine two possible ways how this might occur. One is a “structural” option³³. This model is based on the idea that an agonist pMHC induces a conformational change in the TCR which causes “tilting” of the TCR and subsequent phosphorylation of the TCR binding agonist ligand. This result fits well with the suggestion of Janeway and colleagues³⁴ that conformational changes might play an important role in mediating T-cell activation. For a while this hypothesis was disputed because TCR-pMHC structural work showed that in all cases except one there was no change in membrane proximal domains^{35–40}. However, the original study of Garcia et al.³⁵, subsequently confirmed by others^{37–39} demonstrated by comparing ligand-bound and ligand-free receptors that conformational change occurs within the complementarity-determining regions (CDRs) of the TCR, which associate with the pMHC. Complementing these results are studies examining the thermodynamics of TCR-pMHC interactions, in which, in most cases, there has been shown a loss of entropy, indicating a flexible binding site being stabilized by ligand binding^{41–45}. One recent idea that emerged is the piston model^{46–48} or the ‘twist-cap’ model as suggested in Krogsgaard et al.⁴⁵ in which large rearrangements at the binding site could generate a torque on the TCR that changes the relationship with its attendant signaling molecules in a way that facilitates signaling^{46, 49–53}. Supporting translocation of a conformational change through the TCR to the CD3 signaling complex is data from Palmer and colleagues⁵⁰ which reported that both positive and negative selecting ligands induce a conformational change in the CD3 complex of thymocytes. Another option is a ‘catalytic’ model³³. In this model (Fig. 5) we propose that binding of an agonist-pMHC to its cognate TCRs generates an enzyme-like ‘active site’ into which an endogenous pMHC-TCR complex could fit, and where the CD3 chains of the TCR can be quickly phosphorylated. Because the TCR binding to the endogenous pMHC is very weak though it will fall apart very rapidly and allow another TCR-endogenous pMHC to into the site, become phosphorylated and so on, resulting in a faster turnover of triggered TCRs than could be generated by the agonist alone. This model is supported by modeling studies^{13, 54} which suggest that one implication of the pseudodimer model is that serial triggering by endogenous peptide-MHC complexes rather than agonist pMHC complexes could be the critical factor in TCR signaling. The question is if one model excludes the other. Very recent data from Schamel et al.⁵⁵ indicate that this might be not be the case, by providing evidence of a conformational change on CD3 epsilon that seems to be induced by having two

or more TCRs together. Clearly more experimental data are needed to determine the mechanism for the contribution of self-peptide in T-cell activation and future research is needed to determine the structure of the native TCR-CD3 signaling complex in its liganded and unliganded form to answer this question. In addition, new evidence⁵⁶ was provided for a third mechanism for TCR-triggering, known as the kinetic-segregation (K-S) model of TCR triggering^{57, 58} which involves segregation of TCR-CD3 from inhibitory tyrosine phosphatases (CD45) from the synapse which allows close contact between the APC and T-cell membrane. This could increase the phosphorylation of tyrosine kinase substrates such as TCR-CD3 ITAMs within the close-contact region. Triggering occurs when tyrosine kinase substrates remain phosphorylated for long enough to initiate downstream signaling events. It is possible that the initial step in TCR triggering includes the segregation of CD45, followed by pseudodimer formation and phosphorylation of Lck by a conformational change and/or serial triggering.

Linking thymic selection and activation in the periphery

The interaction between self-pMHC and TCR is necessary for negative and positive selection and T cells that react with self-pMHC molecules with weak but sufficient affinity are positively selected and migrate to the periphery. In contrast, T-cells that react too strongly with self-pMHC are eliminated through negative selection⁵⁹. Recent studies by Palmer and colleagues have shown that there is an extremely narrow threshold of affinity for what distinguishes the decision between positive and negative selection in the thymus⁶⁰. They showed that a small increase in ligand affinity leads to marked differences in activation of the MAP kinase pathway and the spatial compartmentalization of RAS and other downstream signaling components suggesting that the purpose of positive selection is to select TCRs with a certain intrinsic affinity to self-ligands. Stefanova et al.¹⁵, provided evidence that endogenous peptides enhance the activation of naïve T cells in the periphery and augments clonal expansion the periphery. Recently, we suggested that T cells are educated to recognize particular self-peptides in the thymus with the purpose of mediating maximal sensitivity to agonist ligand in the periphery¹. As illustrated in Fig. 6, the presence of agonist/high affinity peptides in the thymus would induce death by negative selection and therefore another mechanism must come into play with respect to positive selection in the thymus. Therefore, we have hypothesized^{1, 33} (as illustrated in Fig. 6) that monomers or homodimers of endogenous pMHC stabilized by the CD4 co-receptor could provide a basic initial signaling unit for positive selection. Because thymocytes are significantly more sensitive than peripheral T-cells⁶¹, this could be true in thymic selection despite the fact that endogenous pMHC homodimers are not stimulatory for mature T cells¹. However, it is still unknown if the same self-pMHC complexes contribute to both T-cell development in the thymus and T-cell activation in the periphery. It is possible that thymic development selects for TCRs that can interact with self-peptides with certain kinetic parameters in the periphery. Alternatively, it could be that thymic selection uses particular subsets of self-peptides versus mature T cells to put an extra level of restriction on TCR-recognition of antigen in the periphery. Arguing against this is earlier work of Marrack et al.¹⁶ who found no difference in the most common endogenous peptides bound to an MHC molecule in the thymus versus the periphery.

Conclusion

Here we have discussed some of the recent evidence pertaining to the role of endogenous pMHC in the extraordinary sensitivity of T cells to antigens in the periphery. Although much progress has been made, there are still many unanswered questions and controversies concerning the initiation of T-cell activation and how these events transduce intracellular signaling. This may involve combinations of aggregation, conformation and segregation events. Also, to fully understand the kinetic basis of the contribution of self-ligands in T-cell

sensitivity, it is necessary to determine their biophysical properties. These parameters are difficult to study because their very low affinities ($>300 \mu\text{M}$) cannot be measured by traditional methods (e.g. surface plasmon resonance). We hope that the use of emerging new microscopic techniques, more advanced biophysical/crystallographic techniques, the combination of *in vivo* and *in vitro* experiments combined with mathematical and computer modeling will help us to resolve these important issues in the future.

References

1. Krogsgaard M, Li QJ, Sumen C, Huppa JB, Huse M, Davis MM. Agonist/endogenous peptide-MHC heterodimers drive T cell activation and sensitivity. *Nature* Mar 10;2005 434(7030):238–243. [PubMed: 15724150]
2. Harding CV, Unanue ER. Quantitation of antigen-presenting cell MHC class II/peptide complexes necessary for T-cell stimulation. *Nature* Aug 9;1990 346(6284):574–576. [PubMed: 2115981]
3. Demotz S, Grey HM, Sette A. The minimal number of class II MHC-antigen complexes needed for T cell activation. *Science* Aug 31;1990 249(4972):1028–1030. [PubMed: 2118680]
4. Christinck ER, Luscher MA, Barber BH, Williams DB. Peptide binding to class I MHC on living cells and quantitation of complexes required for CTL lysis. *Nature* Jul 4;1991 352(6330):67–70. [PubMed: 2062379]
5. Sykulev Y, Joo M, Vturina I, Tsomides TJ, Eisen HN. Evidence that a single peptide-MHC complex on a target cell can elicit a cytolytic T cell response. *Immunity* Jun;1996 4(6):565–571. [PubMed: 8673703]
6. Brower RC, England R, Takeshita T, et al. Minimal requirements for peptide mediated activation of CD8+ CTL. *Mol Immunol* Nov;1994 31(16):1285–1293. [PubMed: 7969189]
7. Reay PA, Matsui K, Haase K, Wulfig C, Chien YH, Davis MM. Determination of the relationship between T cell responsiveness and the number of MHC-peptide complexes using specific monoclonal antibodies. *J Immunol* Jun 1;2000 164(11):5626–5634. [PubMed: 10820237]
8. Cochran JR, Cameron TO, Stern LJ. The relationship of MHC-peptide binding and T cell activation probed using chemically defined MHC class II oligomers. *Immunity* Mar;2000 12(3):241–250. [PubMed: 10755611]
9. Boniface JJ, Rabinowitz JD, Wulfig C, et al. Initiation of signal transduction through the T cell receptor requires the multivalent engagement of peptide/MHC ligands [corrected]. *Immunity* Oct;1998 9(4):459–466. [PubMed: 9806632]
10. Irvine DJ, Purbhoo MA, Krogsgaard M, Davis MM. Direct observation of ligand recognition by T cells. *Nature* Oct 24;2002 419(6909):845–849. [PubMed: 12397360]
11. Purbhoo MA, Irvine DJ, Huppa JB, Davis MM. T cell killing does not require the formation of a stable mature immunological synapse. *Nat Immunol* May;2004 5(5):524–530. [PubMed: 15048111]
12. Wulfig C, Sumen C, Sjaastad MD, Wu LC, Dustin ML, Davis MM. Costimulation and endogenous MHC ligands contribute to T cell recognition. *Nat Immunol* Jan;2002 3(1):42–47. [PubMed: 11731799]
13. Li QJ, Dinner AR, Qi S, et al. CD4 enhances T cell sensitivity to antigen by coordinating Lck accumulation at the immunological synapse. *Nat Immunol* Aug;2004 5(8):791–799. [PubMed: 15247914]
14. Brown JH, Jardetzky TS, Gorga JC, et al. Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature* Jul 1;1993 364(6432):33–39. [PubMed: 8316295]
15. Stefanova I, Dorfman JR, Germain RN. Self-recognition promotes the foreign antigen sensitivity of naive T lymphocytes. *Nature* Nov 28;2002 420(6914):429–434. [PubMed: 12459785]
16. Marrack P, Ignatowicz L, Kappler JW, Boymel J, Freed JH. Comparison of peptides bound to spleen and thymus class II. *J Exp Med* Dec 1;1993 178(6):2173–2183. [PubMed: 8245790]
17. Schild H, Gruneberg U, Pougialis G, et al. Natural ligand motifs of H-2E molecules are allele specific and illustrate homology to HLA-DR molecules. *Int Immunol* Dec;1995 7(12):1957–1965. [PubMed: 8746565]

18. Wang JH, Meijers R, Xiong Y, et al. Crystal structure of the human CD4 N-terminal two-domain fragment complexed to a class II MHC molecule. *Proc Natl Acad Sci U S A* Sep 11;2001 98(19):10799–10804. [PubMed: 11535811]
19. Sporri R, Reis e Sousa C. Self peptide/MHC class I complexes have a negligible effect on the response of some CD8+ T cells to foreign antigen. *Eur J Immunol* Nov;2002 32(11):3161–3170. [PubMed: 12555661]
20. Delon J, Gregoire C, Malissen B, et al. CD8 expression allows T cell signaling by monomeric peptide-MHC complexes. *Immunity* Oct;1998 9(4):467–473. [PubMed: 9806633]
21. Ge Q, Stone JD, Thompson MT, et al. Soluble peptide-MHC monomers cause activation of CD8+ T cells through transfer of the peptide to T cell MHC molecules. *Proc Natl Acad Sci U S A* Oct 15;2002 99(21):13729–13734. [PubMed: 12374859]
22. Altan-Bonnet G, Germain RN. Modeling T cell antigen discrimination based on feedback control of digital ERK responses. *PLoS Biol* Nov;2005 3(11):e356. [PubMed: 16231973]
23. Micheletti F, Canella A, Vertuani S, et al. Supra-agonist peptides enhance the reactivation of memory CTL responses. *J Immunol* Oct 15;2000 165(8):4264–4271. [PubMed: 11035060]
24. Yachi PP, Ampudia J, Gascoigne NR, Zal T. Nonstimulatory peptides contribute to antigen-induced CD8-T cell receptor interaction at the immunological synapse. *Nat Immunol* Aug;2005 6(8):785–792. [PubMed: 15980863]
25. Cebecauer M, Guillaume P, Mark S, et al. CD8+ cytotoxic T lymphocyte activation by soluble major histocompatibility complex-peptide dimers. *J Biol Chem* Jun 24;2005 280(25):23820–23828. [PubMed: 15805102]
26. Gao GF, Willcox BE, Wyer JR, et al. Classical and nonclassical class I major histocompatibility complex molecules exhibit subtle conformational differences that affect binding to CD8alphaalpha. *J Biol Chem* May 19;2000 275(20):15232–15238. [PubMed: 10809759]
27. Leishman AJ, Naidenko OV, Attinger A, et al. T cell responses modulated through interaction between CD8alphaalpha and the nonclassical MHC class I molecule, TL. *Science* Nov 30;2001 294(5548):1936–1939. [PubMed: 11729321]
28. Wyer JR, Willcox BE, Gao GF, et al. T cell receptor and coreceptor CD8 alphaalpha bind peptide-MHC independently and with distinct kinetics. *Immunity* Feb;1999 10(2):219–225. [PubMed: 10072074]
29. Kern P, Hussey RE, Spoerl R, Reinherz EL, Chang HC. Expression, purification, and functional analysis of murine ectodomain fragments of CD8alphaalpha and CD8alphabeta dimers. *J Biol Chem* Sep 17;1999 274(38):27237–27243. [PubMed: 10480942]
30. Davis SJ, Ikemizu S, Evans EJ, Fugger L, Bakker TR, van der Merwe PA. The nature of molecular recognition by T cells. *Nat Immunol* Mar;2003 4(3):217–224. [PubMed: 12605231]
31. Zamoyska R. CD4 and CD8: modulators of T-cell receptor recognition of antigen and of immune responses? . *Curr Opin Immunol* Feb;1998 10(1):82–87. [PubMed: 9523116]
32. König R. Interactions between MHC molecules and co-receptors of the TCR. *Curr Opin Immunol* Feb;2002 14(1):75–83. [PubMed: 11790535]
33. Davis MM, Krogsgaard M, Huse M, Huppa J, Lillemeier BF, Li Q. T-cells as Self-referential, sensory 'organ'. *Ann Rev Immunol* 2007;25:681–695.10.1146/annurev.immunol.24.021605.090600 [PubMed: 17291190]
34. Janeway CA Jr. Ligands for the T-cell receptor: hard times for avidity models. *Immunol Today* May; 1995 16(5):223–225. [PubMed: 7779252]
35. Garcia KC, Degano M, Pease LR, et al. Structural basis of plasticity in T cell receptor recognition of a self peptide-MHC antigen. *Science* Feb 20;1998 279(5354):1166–1172. [PubMed: 9469799]
36. Hare BJ, Wyss DF, Osburne MS, Kern PS, Reinherz EL, Wagner G. Structure, specificity and CDR mobility of a class II restricted single-chain T-cell receptor. *Nat Struct Biol* Jun;1999 6(6):574–581. [PubMed: 10360364]
37. Reiser JB, Gregoire C, Darnault C, et al. A T cell receptor CDR3beta loop undergoes conformational changes of unprecedented magnitude upon binding to a peptide/MHC class I complex. *Immunity* Mar;2002 16(3):345–354. [PubMed: 11911820]
38. Reiser JB, Darnault C, Gregoire C, et al. CDR3 loop flexibility contributes to the degeneracy of TCR recognition. *Nat Immunol* Mar;2003 4(3):241–247. [PubMed: 12563259]

39. Kjer-Nielsen L, Clements CS, Purcell AW, et al. A structural basis for the selection of dominant alphabeta T cell receptors in antiviral immunity. *Immunity* Jan;2003 18(1):53–64. [PubMed: 12530975]
40. Krogsgaard M, Davis MM. How T cells 'see' antigen. *Nat Immunol* Mar;2005 6(3):239–245. [PubMed: 15716973]
41. Willcox BE, Gao GF, Wyer JR, et al. TCR binding to peptide-MHC stabilizes a flexible recognition interface. *Immunity* Mar;1999 10(3):357–365. [PubMed: 10204491]
42. Boniface JJ, Reich Z, Lyons DS, Davis MM. Thermodynamics of T cell receptor binding to peptide-MHC: evidence for a general mechanism of molecular scanning. *Proc Natl Acad Sci U S A* Sep 28;1999 96(20):11446–11451. [PubMed: 10500196]
43. Garcia KC, Radu CG, Ho J, Ober RJ, Ward ES. Kinetics and thermodynamics of T cell receptor-autoantigen interactions in murine experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci U S A* Jun 5;2001 98(12):6818–6823. [PubMed: 11391002]
44. Anikeeva N, Lebedeva T, Krogsgaard M, et al. Distinct molecular mechanisms account for the specificity of two different T-cell receptors. *Biochemistry* Apr 29;2003 42(16):4709–4716. [PubMed: 12705834]
45. Krogsgaard M, Prado N, Adams EJ, et al. Evidence that structural rearrangements and/or flexibility during TCR binding can contribute to T cell activation. *Mol Cell* Dec;2003 12(6):1367–1378. [PubMed: 14690592]
46. Sun ZJ, Kim KS, Wagner G, Reinherz EL. Mechanisms contributing to T cell receptor signaling and assembly revealed by the solution structure of an ectodomain fragment of the CD3 epsilon gamma heterodimer. *Cell* Jun 29;2001 105(7):913–923. [PubMed: 11439187]
47. Ottemann KM, Xiao W, Shin YK, Koshland DE Jr. A piston model for transmembrane signaling of the aspartate receptor. *Science* Sep 10;1999 285(5434):1751–1754. [PubMed: 10481014]
48. Davis MM. A new trigger for T cells. *Cell* Aug 9;2002 110(3):285–287. [PubMed: 12176315]
49. Aivazian D, Stern LJ. Phosphorylation of T cell receptor zeta is regulated by a lipid dependent folding transition. *Nat Struct Biol* Nov;2000 7(11):1023–1026. [PubMed: 11062556]
50. Gil D, Schrum AG, Alarcon B, Palmer E. T cell receptor engagement by peptide-MHC ligands induces a conformational change in the CD3 complex of thymocytes. *J Exp Med* Feb 21;2005 201(4):517–522. [PubMed: 15728235]
51. Gil D, Schamel WW, Montoya M, Sanchez-Madrid F, Alarcon B. Recruitment of Nck by CD3 epsilon reveals a ligand-induced conformational change essential for T cell receptor signaling and synapse formation. *Cell* Jun 28;2002 109(7):901–912. [PubMed: 12110186]
52. Schamel WW, Arechaga I, Risueno RM, et al. Coexistence of multivalent and monovalent TCRs explains high sensitivity and wide range of response. *J Exp Med* Aug 15;2005 202(4):493–503. [PubMed: 16087711]
53. Levin SE, Weiss A. Twisting tails exposed: the evidence for TCR conformational change. *J Exp Med* Feb 21;2005 201(4):489–492. [PubMed: 15728230]
54. Qi S, Dinner AR, Krogsgaard M, Davis MM, Chakraborty AK. Molecular models for how endogenous ligands amplify signaling in T cells. 2006Submitted
55. Minguet S, Swamy M, Alarcon B, Luescher IF, Schamel WW. Full activation of the T cell receptor requires both clustering and conformational changes at CD3. *Immunity* Jan;2007 26(1):43–54. [PubMed: 17188005]
56. Choudhuri K, Wiseman D, Brown MH, Gould K, van der Merwe PA. T-cell receptor triggering is critically dependent on the dimensions of its peptide-MHC ligand. *Nature* Jul 28;2005 436(7050):578–582. [PubMed: 16049493]
57. Davis SJ, van der Merwe PA. The structure and ligand interactions of CD2: implications for T-cell function. *Immunol Today* Apr;1996 17(4):177–187. [PubMed: 8871350]
58. Anton van der Merwe P, Davis SJ, Shaw AS, Dustin ML. Cytoskeletal polarization and redistribution of cell-surface molecules during T cell antigen recognition. *Semin Immunol* Feb;2000 12(1):5–21. [PubMed: 10723794]
59. Goldrath AW, Bevan MJ. Selecting and maintaining a diverse T-cell repertoire. *Nature* Nov 18;1999 402(6759):255–262. [PubMed: 10580495]

60. Daniels MA, Teixeira E, Gill J, et al. Thymic selection threshold defined by compartmentalization of Ras/MAPK signalling. *Nature* Dec 7;2006 444(7120):724–729. [PubMed: 17086201]
61. Davey GM, Schober SL, Endrizzi BT, Dutcher AK, Jameson SC, Hogquist KA. Preselection thymocytes are more sensitive to T cell receptor stimulation than mature T cells. *J Exp Med* Nov 16;1998 188(10):1867–1874. [PubMed: 9815264]
62. Holler PD, Kranz DM. Quantitative analysis of the contribution of TCR/pepMHC affinity and CD8 to T cell activation. *Immunity* Feb;2003 18(2):255–264. [PubMed: 12594952]

Fig. 1. Models for the initiation of T-cell activation

Three different models for how T-cell activation is initiated. **A.** The coreceptor model³⁴ in which T-cell activation is initiated by monomeric pMHC together with CD4 and TCR. **B.** The pseudodimer model^{1, 10} in which agonist-endogenous peptide-MHC heterodimers, stabilized by the co-receptor CD4, are crucial intermediates for triggering T lymphocytes. **C.** The dimers of dimers model¹⁴ where the signaling complex consists of two agonist pMHC complex, two TCRs and two CD4 molecules.

Fig. 2. Self-peptide-MHC accumulation at the immunological synapse

A. To detect the accumulation of endogenous pMHC at the T-cell-APC interface APCs are pulsed with biotinylated self-peptides in the presence of minute amounts of unlabeled agonist peptide and the resulting biotin pMHC is labeled with streptavidin-Cy3, which can be detected with 3D fluorescence microscopy. **B.** Representative illustrations of pMHC accumulation at the T-cell-APC interface. The upper panel shows differential interference contrast images overlaid with Ca^{2+} ratio images obtained with fura-2 (340/380 excitations) and the lower panel show the en face view of a 3D reconstruction of Cy3-labeled pMHC. K5 is a strong agonist peptide, ER60, B2M, HSP self-peptides and 99A and 99E “null” peptide (from¹).

Fig. 3. Self-agonist peptide dimers as the basic signaling unit for T-cell signaling in CD4^+ T cells
Experiments from Krogsgaard et al.¹ show that the basic unit for initiation of T-cell signaling is a heterodimer of self and an agonist peptide MHC. **A.** Soluble self pMHC molecules were cross-linked to agonist pMHC (K5) using synthetic crosslinkers as previously described by Cochran et al.⁸. Soluble heterodimers were mixed with T-cell blasts and based on the ratiometric analysis of fura-2, the percentage of cell ($n > 100$) with an elevated Ca^{2+} signal were determined. **B.** CHO-gpi-IE^k APCs were pulsed with 100 μM mixture containing the indicated ratios of endogenous peptide to agonist peptide (MCC). **C.** Lipid bilayers containing B7-1 and ICAM-1 pulsed with 0.08 μM mixture containing the indicated ratio of self I-E^k to I-E^k-MCC.

APCs or bilayers were presented to T-cell blasts loaded with fura-2 and the frequency of T cells with elevated Ca^{2+} signal were determined.

Fig. 4. The contribution of CD4 to the recognition of self-peptide MHC heterodimers

A. Crystal structures from Reinherz and colleagues showing the orientation of the CD4 molecule with respect to the TCR-pMHC complex¹⁸. This would preclude the CD4 molecule from being able to bind the same MHC molecule that the TCR is binding to. B. Mutagenesis strategy for understanding the molecular function of CD4 in recognition of self-agonist-heterodimers¹. A * symbol indicates the site of the mutation abrogating CD4 binding. C. Results of the mutagenesis analysis. Soluble wildtype and mutated heterodimers were mixed with T cell blasts loaded with fura-2 and the frequency of T cells with elevated Ca^{2+} signal was determined.

Fig. 5. Possible mechanism for the contribution of self-ligands to T-cell signaling

At least two possible roles can be envisaged for the self pMHC complexes that act as co-agonists for a particular TCR (from ref. ³²). **A.** The “Structural option”. Self- pMHC may stabilize a conformation induced by the agonist pMHC which allows the phosphorylation of TCR1s CD3 signaling molecules. This could occur in a number of ways, but in the example shown we are proposing a ‘tilting’ mechanism that might allow phosphorylation (**) to proceed. In this mechanism, phosphorylation of the second TCR does not occur because of the very short half-life of the TCR2-endogenous pMHC complex. **B.** The ‘Catalytic’ option, in this case the TCR1-agonist ligand complex acts similarly to the ‘active site’ of an enzyme with TCR2 and (TCR3 etc.) acting as ‘substrates’ and endogenous pMHC co-agonist as ‘co-factors’. Here a succession of TCR-CD3 complexes diffuse into the vicinity of the TCR1-agonist,

become phosphorylated, and then rapidly dissociate. This would explain how even very high affinity (long half-life) TCRs could still signal efficiently⁶².

Fig. 6. The role of self peptides

The specificity and sensitivity of T-cell recognition is vital to the immune response. Self-peptides are presented in both in the thymus and in the periphery on antigen presenting cells. In the thymus self-peptides play an important role for positive and negative selection and we propose that this might be triggering by homodimers (positive selection) or heterodimers (negative selection) of pMHCs that act as co-agonist in the maintaining T cell homeostasis and viability in the periphery when antigen is not present by providing a tonic T-cell stimulation.