

REVIEW ARTICLE

Models of T cell antigen receptor activation: the puzzle still remained

Sumit Deswal[a,b]

[a]Department of Molecular Immunology, Max Planck Institute of Immunobiology and Epigenetics and Faculty of Biology III, University of Freiburg, Stübeweg 51, 79108 Freiburg, Germany
[b]Spemann Graduate School of Biology and Medicine (SGBM), University of Freiburg, Germany

Corresponding address: Max Planck Institute of Immunobiology and Epigenetics, Stübeweg 51, 79108 Freiburg, Germany. E-mail: deswal@immunbio.mpg.de, Tel.: +49-761-5108-314, FAX: +49-761-5108-423

Abstract

T cell antigen receptor (TCR) is a protein-complex expressed on all T cells of the immune system and is responsible for the activation of T cells when infectious agent is presented by an antigen presenting cell (APC) in the form of peptides bound to the major histocompatibility complex (pMHC). Despite numerous studies it is not clear what biochemical changes upon binding of antigen ligand to the extracellular domains of TCR leads to activation of intracellular signaling (a process known as TCR triggering). This review summarizes possible biochemical mechanisms for TCR triggering and discusses their comparative limitations and advantages in explaining various experimental observations.

Keywords: T cell antigen receptor, activation, model

Introduction

T cell antigen receptor (TCR or TCR-CD3) is a protein complex expressed exclusively in T cells and is composed of the variable TCR α and β chains and the constant CD3γ, CD3δ, CD3ε and CD3ζ chains (Figure 1). CD3γ and CD3δ chain are glycoproteins each of which form a heterodimer with non-glycosylated CD3ε chain. Along with the CD3ζ-ζ homodimer, these chains associate with the TCRαβ heterodimer to generate the full TCR-CD3 complex [1]. The TCR-CD3 is responsible for activation of a T cell upon antigen encounter which then help in the activation of B cells by releasing helper cytokines (helper T cells) or kill the target cell directly by the secretion of cytotoxic effector molecules such as granzymes, perforin and granulysin.

Antigenic ligand binding to the TCR leads to the phosphorylation of immunoreceptor

tyrosine-based activation motifs (ITAMs) of the CD3 complex by the Src family protein tyrosine kinase Lck, resulting in the membrane targeting and activation of another kinase, ZAP-70. Once activated, ZAP-70 phosphorylates several substrates, including the transmembrane adaptor protein, linker for activation of T cells (LAT). Phospho-LAT serves as an important point of divergence for signals initiated from the TCR by recruiting several effector molecules to the plasma membrane thus initiating multiple pathways essential for full T cell activation [2].

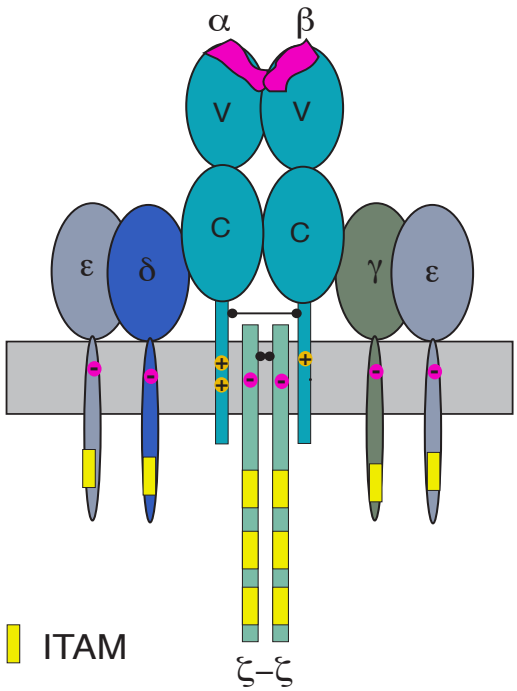

Fig.1: The TCR-CD3 complex. V and C represent variable and constant domains respectively for the TCR α and β. The hypervariable region that binds to the pMHC complex is shown in red color.

Although the downstream signaling mechanisms of T cell activation have been explored to quite detailed extent, the basic mechanism of how ligand binding to extracellular domains of TCRαβ leads to downstream signaling remained unclear. Several models of TCR triggering have been proposed based on the experimental findings, but none of the models so far could explain every aspect of TCR triggering. Broadly, these models can be grouped in three major processes which involve aggregation, conformational change or segregation (Figure 2). This review summarizes the proposed models of TCR triggering and discusses their comparative abilities for explaining various experimental observations (Table 1).

1. Antigen induced clustering

Several forms of clustering have been proposed as the triggering mechanism for the TCR. These include homodimer, heterodimer and the pseudodimer models as discussed below.

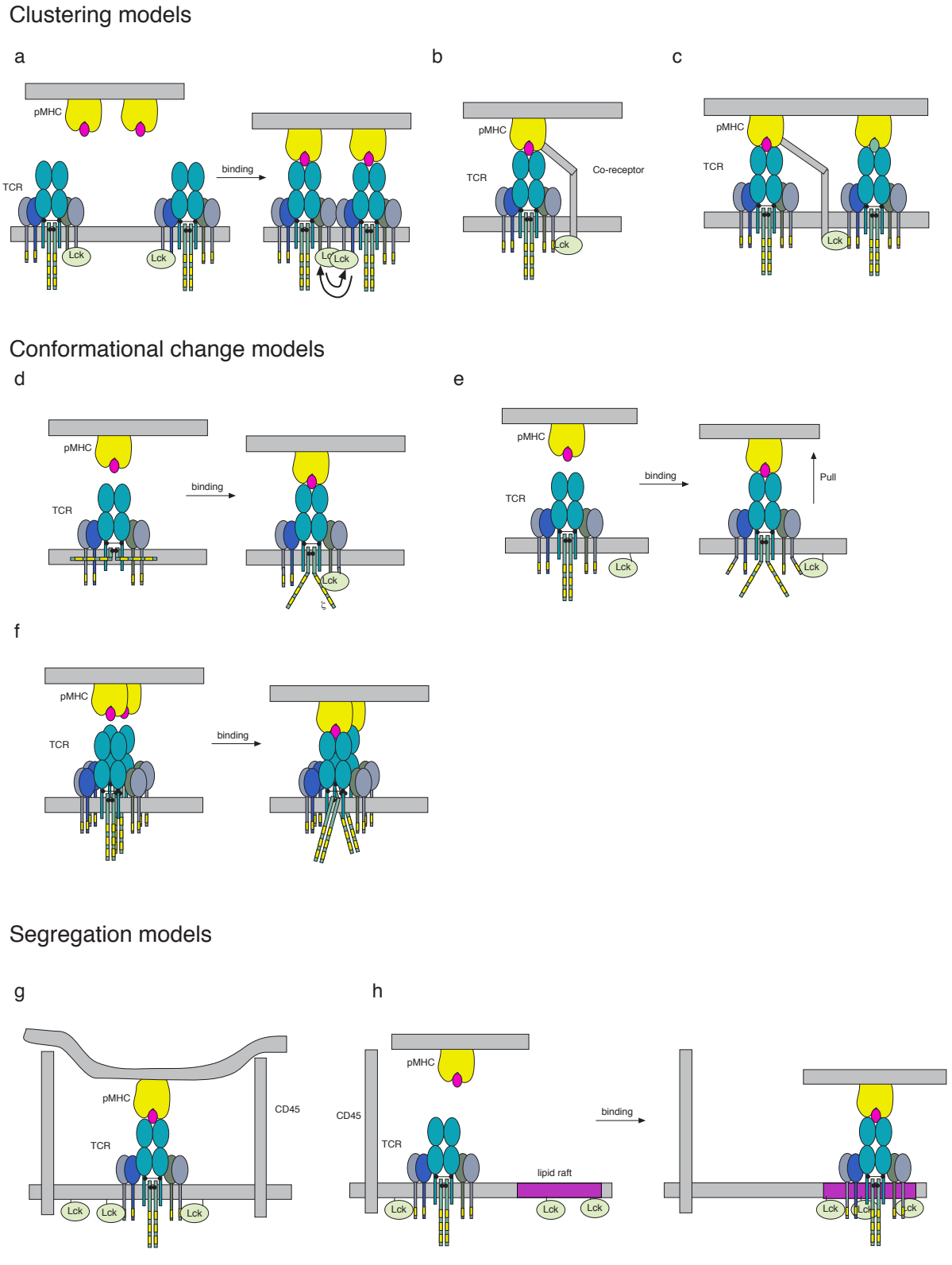

Fig.2: Various models of TCR triggering. a) Homodimer model, b) Heterodimer model, c) Pseudo-dimer model, d) Membrane binding model, e) Kinetic deformation model, f) Permissive geometry model, g) Kinetic segregation, h) Lipid raft mediated segregation model. Full description of each model is given in the main text.

Table 1: Abilities of different models to explain various experimental observations.

Observation	Triggering model							
	Homodimer	**Heterodimer**	**Pseudo-dimer**	**Membrane binding**	**Kinetic deformation**	**Permissive geometry**	**Kinetic segregation**	**Lipid raft**
Pre-clustered TCR	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Coreceptor independent triggering	Yes	No	No	Yes	Yes	Yes	Yes	Yes
Triggering by soluble monomer pMHC	No	Yes	No	Yes	No	No	No	Yes
Triggering by anti-CD3 antibodies	Yes	No	No	Yes	No	Yes	No	No
Activation by pervanadate	No	No	No	No	No	No	Yes	Yes
Inhibition by truncated CD45	No	No	No	No	No	No	Yes	No
Inhibition by elongated pMHC	No	No	No	No	No	No	Yes	No
Triggering in absence of self pMHC	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes

a. Homodimer model

Clustering of TCR–CD3 complexes following TCR engagement could lead to enhanced phosphorylation, for example by increasing the proximity of associated Lck molecules, resulting in the activation of the second receptor in the cluster by *trans*-autophosphorylation (or this enhanced proximity of kinases might lead to their trans-phosphorylation which enhance their activity to phosphorylate the receptor). This model is supported by the observations that artificial ligands such as pMHC tetramer and the anti-TCRαβ and anti-CD3 antibodies that crosslink the TCR lead to its activation whereas Fab fragment of these antibodies or the monomeric pMHC in solution fail to activate the TCR [3]. Also the observations that MHC class I [4, 5] and MHC class II [6] exist in pre-formed clusters, support the homodimer model. However, since several studies have now shown the existence of pre-clustered TCRs on the T cell surface [7, 8], it is difficult to imagine that clustering alone could trigger the

TCR. In fact in a study by Schamel and colleagues [9], both clustering and conformational change were shown to be required for TCR triggering (permissive geometry model, as discussed later).

b. Heterodimer model

In the heterodimer model [10], coreceptor CD8 or CD4 binding to the same pMHC complex as the TCR brings the coreceptor-associated Lck kinase into proximity with TCR–CD3 ITAMs and their phosphorylation. Further it has been proposed that to fully activate the TCR, a pMHC ligand need to interact with a TCR–CD8 pair with a threshold time to induce stable zippering between the membrane-proximal domain of CD8 and the connecting peptide motif in the TCRα [11]. This allows stable association of Lck with the CD3 complex and results in complete phosphorylation of the CD3 ITAMs. A low-affinity pMHC ligand that interacts with a TCR–CD8 pair with less than threshold time induces incomplete zippering and therefore allows only transient Lck association and partial CD3 phosphorylation. However, the observations that T cells can develop and function normally in the mice lacking both CD4 and CD8 suggest that coreceptors are not absolutely required for TCR triggering and T cell activation [12, 13]. Also the fact that anti-CD3 antibodies and their F(ab')2 fragments that do not engage the co-receptors can still activate the TCR [14, 15], indicates that co-receptors are not required for the TCR triggering itself, though they might be important for full activation of a T cell.

c. Pseudo-dimer model

Only a few MHCs carry high affinity agonist peptides for the TCRs, whereas a great majority of MHCs carry endogenous self-peptides. On the basis of the crystallographic studies which showed that the CD4 tail associating with Lck was far from their own TCR/CD3 complex, the pseudodimer model postulates that two TCRs are brought together by binding low-affinity self or high-affinity agonist pMHC ligands and that the CD4 associated with a TCR engaging the agonist pMHC complex can assists in phosphorylation of neighboring TCR (engaged to low affinity self peptide) by the associated kinase Lck [16]. Thus according to this model, a 'pseudodimer' consists of one foreign and one self-antigen engaged receptor linked via CD4 molecule is the primary unit of TCR signal initiation module.

2. Conformational change model

Several studies have proposed conformational

change as a requirement for TCR triggering [17, 18]. However, how the ligand binding to TCRαβ ectodomains leads to a conformational change in the cytoplasmic tails of CD3 subunits is not clear. The situation is complicated by the fact that the crystal structure of the fully assembled TCR-CD3 complex is not known, although individual subunits have been crystallized partially [19-22]. Some of the important models for the mechanism of conformational change in TCR-CD3 are discussed below.

a. Membrane binding model

According to this model, cytoplasmic tails of CD3ε [23, 24] and CD3ζ [25] associate with the lipids present in the plasma membrane of a T cell. Thereby, the ITAMs are buried in the plasma membrane, making them inaccessible for phosphorylation by the kinases. Ligand binding leads to release of these CD3 chains from the plasma membrane, making them accessible to kinase and hence the phosphorylation.

b. Kinetic deformation model

Several line of evidence have proposed that the mechanical effects (such as pulling or shearing) of pMHC binding to the TCR leads to a piston-like displacement of the TCR–CD3 complex [26, 27]. This induces a change in the conformation of the CD3 cytoplasmic domains, allowing ITAM phosphorylation.

c. Permissive geometry model

Based on the experimental evidence for the existence of TCR-CD3s in a pre-clustered form [7] and the fact that pMHC monomers in solution fail to trigger TCR[28, 29] while pMHC dimer or higher oligomer can induce TCR triggering, the permissive geometry model was proposed[30]. According to this model, before ligand binding, the mono and multivalent TCR-CD3s exist in an autoinhibited state where ITAMs are inaccessible for phosphorylation by the kinase. Simultaneous dimeric (or higher order) ligand binding to the two TCR-CD3 complexes leads to a scissor-like movement in the two TCR-CD3 complexes leading to exposure of their cytoplasmic tails, thus making the ITAMs accessible for phosphorylation.

3. Segregation Model

In a resting T cell, phosphorylation of the TCR-CD3 is kept in check by the active phosphotases. This is supported by the fact that pervanadate, a phosphatase inhibitor,

treatment leads to strong phosphorylation of the TCR-CD3 ITAMs and also initiates the downstream signaling [31]. Thus any process that favors the kinase-phosphtase balance towards kinase, can lead to TCR-CD3 phosphorylation and the signaling. Redistribution of the TCR-CD3, kinases and phosphatases in which phosphatase is segregated from the TCR can interfere with the dephosphorylation. There are two mechanisms proposed for such ligand dependent re-distribution, which are discussed below.

a. Kinetic segregation

Kinetic segregation model was proposed based on the topological view of the cell surface molecules at the T cell–APC interface [32, 33]. The tight intercellular contact causes the segregation of the molecules by sizes of their ectodomain resulting in physical separation of TCR from the large inhibitory tyrosine phosphatase CD45, leading to stable phosphorylation of TCR–CD3 ITAMs by Lck. Though sounding good in the context of a T-cell – APC contact, the model fails to explain the activation of T cells by soluble anti-TCRαβ and anti-CD3 antibodies and the pMHC tetramer [14, 34].

b. Lipid raft mediated segregation model

Lipid rafts are the detergent (such as Triton X-100, Brij-series, NP-40 or CHAPS)-resistant membrane microdomains which are enriched in glycosphingolipids, cholesterol and lipid-modified proteins such as the GPI-anchored proteins. Also these microdomains are enriched in double-acylated (myristoylated, palmitoylated) Src-family kinases and the important signaling molecules such as the coreceptors CD4 and CD8 and the adaptor protein LAT. Lipid raft mediated segregation model postulates that pMHC engagement results in partitioning of the TCR–CD3 complex into lipid rafts enriched in Lck and deficient in CD45 [35, 36]. Partial support for this model comes from the observations that palmitoylation-deficient (and raft excluded) mutants of Lck and LAT are functionally defective. In addition, artificial targeting of other cytoplasmic molecules, such as SHP-1 [37], CD45 [38] or PLCγ [39], to membrane rafts has marked functional effects on TCR-induced signaling. A connecting peptide in the TCRα chain and the CD3δ chain were identified as the critical sites essential for effective raft association. Mutations in these components interfere with TCR signaling [40]. The mechanism of directing association of the engaged TCR complex with lipid rafts is not clear but might be based on co-engagement of the raft resident CD4/CD8 coreceptors.

Conclusion

Analysis of various proposed mechanisms reveals that probably a combination of these mechanisms is responsible for TCR triggering. Existence of pre-clustered TCR molecules rules out the homodimerization as a requirement for TCR triggering. It is possible that CD3 ITAMs are inaccessible for phosphorylation by the kinase in the resting state and some sort of conformational change is necessary for exposing the ITAMs, but additional studies are required to find out what mechanism is responsible for such conformational change. Segregation models argue the balance of kinase mediated phosphorylation and phosphatase mediated dephosphorylation shifts in favour of kinase upon ligand binding. Though such segregation might contribute for TCR triggering in the context of a T cell-antigen presenting cell interaction, it seems not the sole mechanism of TCR triggering as the soluble anti-TCR or anti-CD3 antibodies which do not induce segregation can still cause TCR triggering. Further studies on the structure of fully assembled TCR-CD3 complex will be required for a better understanding of the TCR triggering.

References

1. Call ME, Pyrdol J, Wiedmann M, Wucherpfennig KW: **The organizing principle in the formation of the T cell receptor-CD3 complex**. *Cell* 2002, **111**:967-979.

2. Smith-Garvin JE, Koretzky GA, Jordan MS: **T cell activation**. *Annu Rev Immunol* 2009, **27**:591-619.

3. Cochran JR, Aivazian D, Cameron TO, Stern LJ: **Receptor clustering and transmembrane signaling in T cells**. *Trends Biochem Sci* 2001, **26**(5):304-310.

4. Matko J, Bushkin Y, Wei T, Edidin M: **Clustering of class I HLA molecules on the surfaces of activated and transformed human cells**. *J Immunol* 1994, **152**(7):3353-3360.

5. Fooksman DR, Gronvall GK, Tang Q, Edidin M: **Clustering class I MHC modulates sensitivity of T cell recognition**. *Journal of immunology* 2006, **176**(11):6673-6680.

6. Unternaehrer JJ, Chow A, Pypaert M, Inaba K, Mellman I: **The tetraspanin CD9 mediates lateral association of MHC class II molecules on the dendritic cell surface**. *Proceedings of the National Academy of Sciences of the United States of America* 2007, **104**(1):234-239.

7. Schamel WW, Arechaga I, Risueno RM, van Santen HM, Cabezas P, Risco C, Valpuesta JM, Alarcon B: **Coexistence of multivalent and monovalent TCRs explains high sensitivity and wide range of response**. *J Exp Med* 2005, **202**:493-503.

8. Lillemeier BF, Mortelmaier MA, Forstner MB, Huppa JB, Groves JT, Davis MM: **TCR and Lat are expressed on separate protein islands on T cell membranes and concatenate during activation**. *Nat Immunol* 2010, **11**(1):90-96.

9. 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* 2007, **26**(1):43-54.

10. Trautmann A, Randriamampita C: **Initiation of TCR signalling revisited**. *Trends Immunol* 2003, **24**(8):425-428.

11. Palmer E, Naeher D: **Affinity threshold for thymic selection through a T-cell receptor-co-receptor zipper**. *Nat Rev Immunol* 2009, **9**(3):207-213.

12. Locksley RM, Reiner SL, Hatam F, Littman DR, Killeen N: **Helper T cells without CD4: control of leishmaniasis in CD4-deficient mice**. *Science* 1993, **261**(5127):1448-1451.

13. Schilham MW, Fung-Leung WP, Rahemtulla A, Kuendig T, Zhang L, Potter J, Miller RG, Hengartner H, Mak TW: **Alloreactive cytotoxic T cells can develop and function in mice lacking both CD4 and CD8**. *European journal of immunology* 1993, **23**(6):1299-1304.

14. Chang TW, Kung PC, Gingras SP, Goldstein G: **Does OKT3 monoclonal antibody react with an antigen-recognition structure on human T cells?** *Proc Natl Acad Sci U S A* 1981, **78**(3):1805-1808.

15. Kaye J, Janeway CA, Jr.: **The Fab fragment of a directly activating monoclonal antibody that precipitates a disulfide-linked heterodimer from a helper T cell clone blocks activation by either allogeneic Ia or antigen and self-Ia**. *J Exp Med* 1984, **159**(5):1397-1412.

16. Krogsgaard M, Li QJ, Sumen C, Huppa JB, Huse M, Davis MM: **Agonist/endogenous peptide-MHC heterodimers drive T cell activation and sensitivity**. *Nature* 2005, **434**(7030):238-243.

17. Levin SE, Weiss A: **Twisting tails exposed: the evidence for TCR conformational change**. *J Exp Med* 2005, **201**(4):489-492.

18. 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* 2002, **109**(7):901-912.

19. Sun ZY, Kim ST, Kim IC, Fahmy A, Reinherz EL, Wagner G: **Solution structure of the CD3epsilondelta ectodomain and comparison with CD3epsilongamma as a basis for modeling T cell receptor topology and signaling**. *Proc Natl Acad Sci U S A* 2004, **101**(48):16867-16872.

20. Arnett KL, Harrison SC, Wiley DC: **Crystal structure of a human CD3-epsilon/delta dimer in complex with a UCHT1 single-chain antibody fragment**. *Proc Natl Acad Sci U S A* 2004, **101**(46):16268-16273.

21. Sun ZYS, Seok Kim K, Wagner G, Reinherz EL: **Mechanisms contributing to T cell receptor signaling and assembly revealed by the solution structure of an ectodomain fragment of the CD3eg heterodimer**. *Cell* 2001, **105**:913-923.

22. Kjer-Nielsen L, Dunstone MA, Kostenko L, Ely LK, Beddoe T, Mifsud NA, Purcell AW, Brooks AG, McCluskey J, Rossjohn J: **Crystal structure of the human T cell receptor CD3 epsilon gamma heterodimer complexed to the therapeutic mAb OKT3**. *Proc Natl Acad Sci U S A* 2004, **101**(20):7675-7680.

23. Xu C, Gagnon E, Call ME, Schnell JR, Schwieters CD, Carman CV, Chou JJ, Wucherpfennig KW: **Regulation of T cell receptor activation by dynamic membrane binding of the CD3epsilon cytoplasmic tyrosine-based motif**. *Cell* 2008, **135**(4):702-713.

24. Deford-Watts LM, Tassin TC, Becker AM, Medeiros JJ, Albanesi JP, Love PE, Wulfing C, van Oers NS: **The cytoplasmic tail of the T cell receptor CD3 epsilon subunit contains a phospholipid-binding motif that regulates T cell functions**. *J Immunol* 2009, **183**(2):1055-1064.

25. Aivazian D, Stern LJ: **Phosphorylation of T cell receptor zeta is regulated by a lipid dependent folding transition**. *Nat Struct Biol* 2000, **7**(11):1023-1026.

26. Ma Z, Sharp KA, Janmey PA, Finkel TH: **Surface-anchored monomeric agonist pMHCs alone trigger TCR with high sensitivity**. *PLoS Biol* 2008, **6**(2):e43.

27. Ma Z, Janmey PA, Finkel TH: **The receptor deformation model of TCR triggering**. *Faseb J* 2008, **22**(4):1002-1008.

28. Stone JD, Stern LJ: **CD8 T Cells, Like CD4 T Cells, Are Triggered by Multivalent Engagement of TCRs by MHC-Peptide Ligands but Not by Monovalent Engagement**. *J Immunol* 2006, **176**(3):1498-1505.

29. 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* 2000, **12**(3):241-250.

30. Minguet S, Schamel WWA: **A permissive geometry model for TCR-CD3 activation**. *Trends Biochem Sci* 2008, **33**(2):51-57.

31. Secrist JP, Burns LA, Karnitz L, Koretzky GA, Abraham RT: **Stimulatory effects of the protein tyrosine phosphatase inhibitor, pervanadate, on T-cell activation events**. *J Biol Chem* 1993, **268**(8):5886-5893.

32. 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* 2005, **436**(7050):578-582.

33. Davis SJ, van der Merwe PA: **The kinetic-segregation model: TCR triggering and beyond**. *Nat Immunol* 2006, **7**(8):803-809.

34. Boniface JJ, Rabinowitz JD, Wulfing C, Hampl J, Reich Z, Altman JD, Kantor RM, Beeson C, McConnell HM, Davis MM: **Initiation of signal transduction through the T cell receptor requires the multivalent engagement of peptide/MHC ligands [corrected]**. *Immunity* 1998, **9**(4):459-466.

35. Horejsi V: **Lipid rafts and their roles in T-cell activation**. *Microbes Infect* 2005, **7**(2):310-316.

36. Horejsi V: **The roles of membrane microdomains (rafts) in T cell activation**. *Immunol Rev* 2003, **191**:148-164.

37. Su MW, Yu CL, Burakoff SJ, Jin YJ: **Targeting Src homology 2 domain-containing tyrosine phosphatase (SHP-1) into lipid rafts inhibits CD3-induced T cell activation**. *Journal of immunology* 2001, **166**(6):3975-3982.

38. He X, Woodford-Thomas TA, Johnson KG, Shah DD, Thomas ML: **Targeting of CD45 protein tyrosine phosphatase activity to lipid microdomains on the T cell surface inhibits TCR signaling**. *European journal of immunology* 2002, **32**(9):2578-2587.

39. Veri MC, DeBell KE, Seminario MC, DiBaldassarre A, Reischl I, Rawat R, Graham L, Noviello C, Rellahan BL, Miscia S *et al*: **Membrane raft-dependent regulation of phospholipase Cgamma-1 activation in T lymphocytes**. *Molecular and cellular biology* 2001, **21**(20):6939-6950.

40. Werlen G, Hausmann B, Palmer E: **A motif in the alphabeta T-cell receptor controls positive selection by modulating ERK activity**. *Nature* 2000, **406**:422-426.