

Chapter 2

Activation of the TCR Complex by Peptide-MHC and Superantigens

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Abstract Drug hypersensitivity reactions are immune mediated, with T lymphocytes being stimulated by the drugs via their T-cell antigen receptor (TCR). In the nonpathogenic state, the TCR is activated by foreign peptides presented by major histocompatibility complex molecules (pMHC). Foreign pMHC binds with sufficient affinity to TCR $\alpha\beta$ and thereby elicits phosphorylation of the cytoplasmic tails of the TCR $\alpha\beta$ -associated CD3 subunits. The process is called TCR triggering. In this review, we discuss the current models of TCR triggering and which drug properties are crucial for TCR stimulation. The underlying molecular mechanisms mostly include pMHC-induced exposure of the CD3 cytoplasmic tails or alterations of the kinase-phosphatase equilibrium in the vicinity of CD3. In this review, we also discuss triggering of the TCR by small chemical compounds in context of these general mechanisms.

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2.1 Introduction

Drug hypersensitivity reactions are a health problem worldwide, but they are difficult to predict and to cure. These reactions are immune mediated, i.e., the drug stimulates the immune system in an undesired way. The major responsive cells are T lymphocytes, being either directly or indirectly stimulated by the drug via their T-cell antigen receptor (TCR).

The TCR is a multiprotein transmembrane complex comprising the TCR $\alpha\beta$ (or TCR $\gamma\delta$), CD3 $\epsilon\delta$, CD3 $\epsilon\gamma$, and CD3 $\zeta\zeta$ dimers (Alarcon et al. 2003; Kuhns and Davis 2012) (Fig. 2.1a) and facultatively the TRIM₂ dimer (Swamy et al. 2010). TCR $\alpha\beta$ possesses variable immunoglobulin domains that bind the ligand, an antigenic peptide bound to major histocompatibility complex (pMHC) molecules. TCR $\alpha\beta$ forms contacts to the peptide as well as to the MHC (Garboczi et al. 1996; Garcia et al. 1996) (Fig. 2.1b). The CD3 chains contain tyrosine residues in their cytoplasmic tails, that are phosphorylated upon successful ligand binding to TCR $\alpha\beta$ and that transmit the signal inside the cell. These tyrosines are part of the immunoreceptor tyrosine-based activation motif (ITAM) (Reth 1989) and are phosphorylated by the kinase Lck belonging to the Src-family (Iwashima et al. 1994). Phosphorylation is the critical event in initiating downstream signaling cascades, since phosphotyrosines serve as binding sites for proteins with src homology 2 (SH2) domains. Consequently, these proteins (e.g., the kinase ZAP-70) are recruited to the receptor and activate signaling pathways, such as activation of phospholipase C γ (PLC γ , Fig. 2.1b) and consequent calcium influx into the cytosol, resulting in the activation of the T cell. In addition, the CD3 ϵ chain has a proline-rich sequence in its cytoplasmic tail that can bind to the signaling molecule Nck (Gil et al. 2002). For the TCR (as well as for most receptors), it is still not well understood which biochemical changes are induced by ligand binding to the receptor, that are transmitted via the transmembrane regions to the cytoplasmic tails, thereby leading to the phosphorylation of the tails. However, a number of ideas and reasonable models have been put forward for the TCR, which we will discuss here.

For each model we discuss which properties a drug needs to possess, in order to stimulate the TCR. An overview on how drugs modify pMHC, in order to activate the TCR, is found in our second review entitled “Activation of the TCR complex by small chemical compounds.”

2.2 Selection of the TCR Specificity in the Thymus

During the development of T cells in the thymus, the genes encoding for the TCR β and TCR α chains are randomly rearranged (mutated), so that TCRs with any random specificity are generated. This includes TCRs that strongly bind to MHC loaded with self-peptides derived from endogenous proteins. Thus, T cells

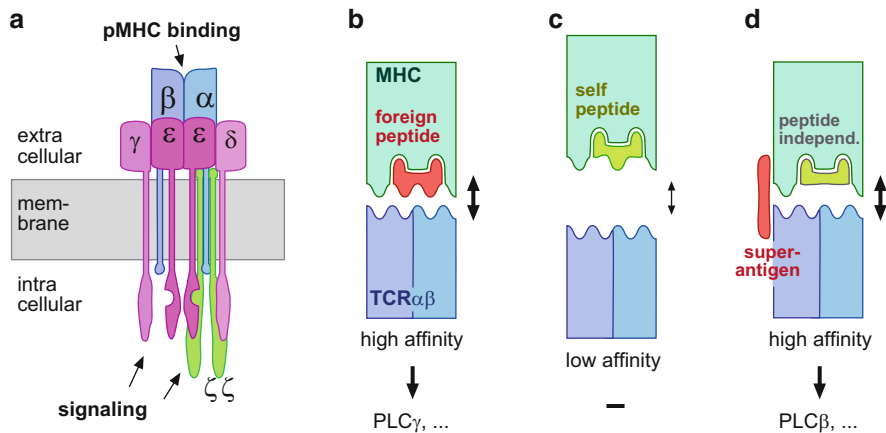


Fig. 2.1 The T-cell antigen receptor (TCR). (a) In its minimal form, the TCR is composed of the pMHC-binding TCR $\alpha\beta$ and the signal-transducing CD3 $\epsilon\delta$, CD3 $\epsilon\gamma$, and CD3 $\zeta\eta$ dimers. (b) Foreign peptides bound to MHC have a high affinity for an appropriate TCR. Both the peptide and the MHC molecule have contacts with TCR $\alpha\beta$, triggering intracellular signaling events, such as the activation of PLC γ and other proteins, leading to T-cell activation. (c) Due to negative selection in the thymus, self-peptide-MHC only has a weak affinity for TCRs in mature T cells. Self-pMHC does not perfectly fit to TCR $\alpha\beta$, thus not triggering their TCR. (d) Superantigens simultaneously bind to MHC in a peptide-independent manner and to the constant regions of TCR $\alpha\beta$. Thus, pMHC is bridged to the TCR largely independent of pMHC-TCR $\alpha\beta$ contacts. Superantigen stimulation leads to the activation of PLC β and other proteins, resulting in T-cell activation

expressing these TCRs are stimulated in the thymus by self-pMHC, resulting in their death. This process is called negative selection. In sharp contrast, T cells possessing TCRs that weakly bind to self-pMHC survive this process and are positively selected (Starr et al. 2003). Further maturation and subsequent tissue allocation leads to the directed distribution of matured T cells in the body. This process ensures that TCRs do not bind strongly to any self-peptides loaded onto MHC molecules in the periphery, preventing autoimmune reactions (Fig. 2.1c). If a TCR binds strongly to pMHC in the periphery, it most likely is a foreign peptide, such as derived from viruses or bacteria, being loaded onto MHC (Fig. 2.1b). Hence, a T cell whose TCR is strongly triggered in the periphery will become activated and will initiate an immune response.

Thus, drugs that alter self-pMHC, so that a potent ligand for the TCR is generated, will stimulate T cells, and this leads to the hypersensitivity reactions observed for a number of different drugs or metal ions.

2.3 TCR Triggering Models

The TCR can be activated (i.e., the tyrosine residues of the CD3 chains can be phosphorylated) using a number of different ligands, such as membrane-bound pMHC, soluble pMHC multimers, anti-TCR $\alpha\beta$ antibodies, and bacterial superantigens (Fig. 2.1d) (all binding to TCR $\alpha\beta$) or anti-CD3 antibodies. Consequently, a number of mechanistic models have been put forward to explain how the TCR is triggered, i.e., how ligand binding at TCR $\alpha\beta$ causes CD3 phosphorylation.

2.3.1 *The Homoclustering Model of TCR Triggering*

Early experiments showed that bivalent anti-TCR $\alpha\beta$ or anti-CD3 antibodies can activate T cells, whereas monovalent Fab fragments of these antibodies fail to do so (Chang et al. 1981; Kaye and Janeway 1984). Likewise, the TCR is only activated by bi- or multivalent soluble, recombinant pMHC (Boniface et al. 1998; Cochran et al. 2000). These experiments indicated that the soluble ligands for the TCR have to be multivalent, in order to be functional. This implies that two or more TCRs have to bind simultaneously to one ligand molecule in order to be activated. In conjunction with the hypothesis that individual receptor molecules are distributed equally on the cell surface, these findings led to the cross-linking (homoclustering) model of TCR activation (Ashwell and Klausner 1990) (Fig. 2.2a). However, it was shown that the TCR can be pre-clustered in the resting, non-ligand-bound state (Alarcon et al. 2006; Lillemeier et al. 2010; Molnar et al. 2010, 2012; Schamel et al. 2005), which is standing in contrast to this model. Nevertheless, these TCR nanoclusters can be co-expressed with TCR monomers (Fig. 2.2b).

According to the homoclustering model, small chemical compounds would need to bind to two adjacent self-peptide-MHC molecules, in order to allow both of them to simultaneously bind to two TCRs.

2.3.2 *Conformational Change Models of TCR Triggering*

How can the requirement for a multivalent ligand and the presence of preformed oligomeric receptors be integrated into a unique model? One intriguing possibility is that binding of bivalent (or multivalent) ligands leads to a reorientation of one TCR in respect to the second TCR. The consequent alteration of the quaternary structure of the receptors might lead to conformational changes within the cytoplasmic tails of CD3, therefore exposing the tyrosines to allow phosphorylation (Minguet and Schamel 2008; Minguet et al. 2007). Otherwise, the CD3 cytoplasmic tails are in a closed conformation and not accessible to kinases. This model is called the permissive geometry model (Minguet and Schamel 2008) (Fig. 2.3). According

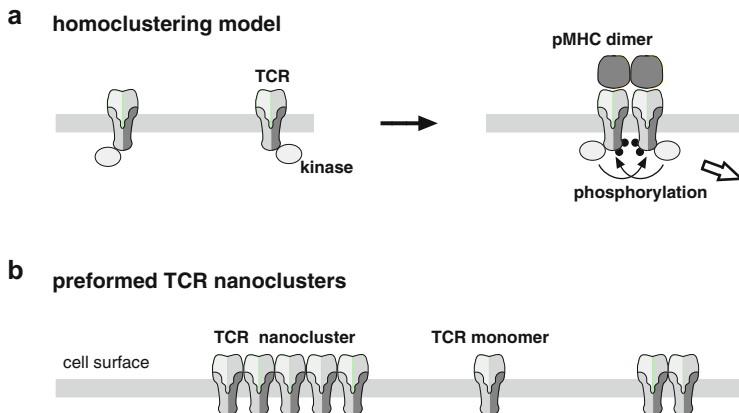


Fig. 2.2 The homoclustering model and TCR nanoclusters. **(a)** Monovalent TCRs are individually expressed on the cell surface. Stimulation by bivalent (or multivalent) pMHC leads to clustering of the TCRs. Consequently associated kinases can phosphorylate each other and the CD3 subunits. Monovalent pMHC do not cluster the TCRs and thus do not induce CD3 phosphorylation. *Black dots* represent phosphorylated tyrosine residues. The *open arrow* shows activation of downstream signaling cascades. **(b)** On the T-cell surface, the TCR is expressed as a mixture of monomers and nanoclusters of different sizes. Thus, the requirement for the homoclustering model is not fulfilled

to this model, monovalent ligands are not capable of reorienting one TCR in respect to another one and are thus inactive. The main inspiration for the conformational change model was the seminal finding that a proline-rich sequence in the cytoplasmic tail of CD3 ϵ becomes exposed upon multivalent TCR stimulation (Gil et al. 2002). The permissive geometry model is supported by the finding that an artificially selected pMHC, binding in a different geometry to TCR $\alpha\beta$, cannot trigger the TCR (Adams et al. 2011).

A “lipid-based model” proposes that the tyrosines of the cytoplasmic tails of CD3 ϵ and ζ are embedded in the inner leaflet of the plasma membrane and are thus not accessible for phosphorylation. Indeed, peptides corresponding to these tails bind to and partially integrate into artificial membranes (Aivazian and Stern 2000; DeFord-Watts et al. 2011; Xu et al. 2008). pMHC binding to the TCR would modulate the lipid environment of the TCR, so that the tyrosines become exposed and available for phosphorylation (Shi et al. 2013).

If drugs and other small compounds activate TCRs according to the permissive geometry model, they would have to approximate two pMHC molecules to two TCRs, in a way that structural changes in the TCR/CD3s are induced. Considering the “lipid-based model,” the small chemical compounds have to mimic or modify pMHC in a way that binding to the TCR frees the cytoplasmic tails from being shielded by the membrane.

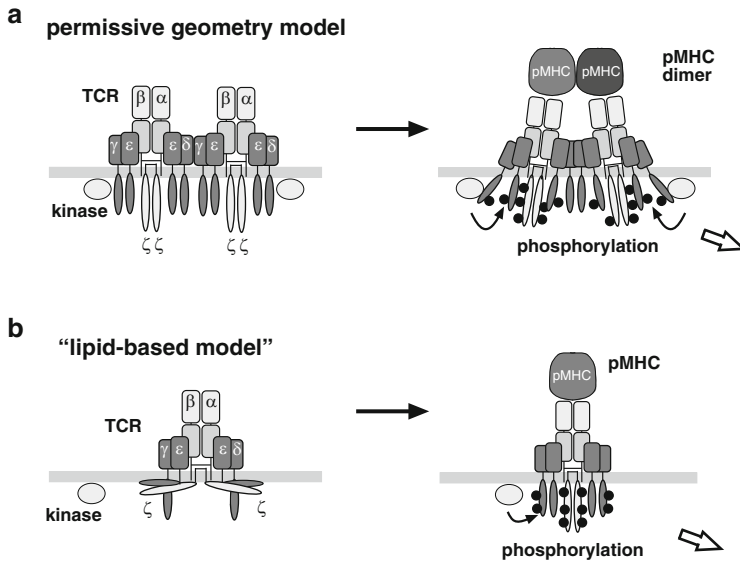


Fig. 2.3 Conformational change models. **(a)** The permissive geometry model. In the resting cell the cytoplasmic tails of CD3 are in a closed conformation, preventing phosphorylation. Bivalent (or multivalent) pMHC reorientates two TCR $\alpha\beta$ towards each other without changing the structure of the individual TCR $\alpha\beta$. This reorientation induces conformational changes in the cytoplasmic tails of CD3, exposing the tyrosines, which now become available for phosphorylation. Since monomeric MHCp does not engage several TCR $\alpha\beta$ simultaneously, it does not induce these conformational changes in CD3 (not shown). **(b)** In the “lipid-based model,” the tyrosines of the cytoplasmic tails of CD3 ϵ and ζ are embedded within the membrane, thus not being accessible for the kinases. pMHC binding to the TCR removes the cytoplasmic tails from the membrane, e.g., by modulating the lipids, thus making the tyrosines available for phosphorylation

2.3.3 The Pseudodimer Model of TCR Triggering

In addition to a possible foreign peptide-MHC, an antigen-presenting cell (APC) and other cells can also present self-peptide-MHC. In contrast to foreign pMHC, the affinity of a self-pMHC-TCR interaction is too low to stimulate a TCR. Mostly, foreign peptides are presented next to self-peptides, although there is a possibility for enriching foreign peptides in certain membrane areas (Lu et al. 2012). Importantly, self-peptides aid in the recognition of foreign peptides by the T cell (Irvine et al. 2002; Stefanova et al. 2002; Wülfing et al. 2002). Indeed, it was shown that soluble pMHC dimers with one agonistic (foreign, high affinity) and one self-peptide (low affinity) could stimulate TCRs (Krogsgaard et al. 2005). This underlying model is called pseudodimer model (Fig. 2.4a) (Irvine et al. 2002) and is a variant of the homoclustering model. Furthermore, the permissive geometry model could also allow simultaneous binding of one foreign and one self-peptide loaded onto MHC, in order to induce the structural changes at CD3 (Minguet and Schamel 2008).

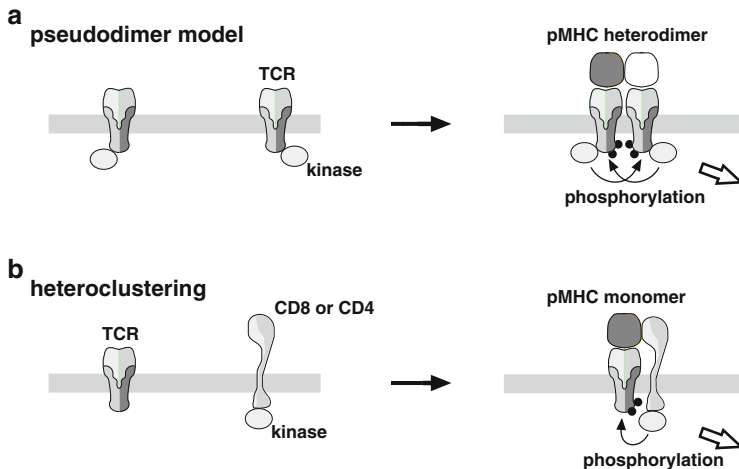


Fig. 2.4 The pseudodimer and heteroclustering models. (a) In the pseudodimer model TCRs are individually expressed. Upon bivalent (or multivalent) MHCp binding, TCRs become clustered and kinases can phosphorylate CD3 (see also the homoclustering model). On the APC foreign (high affinity), peptides are presented on MHC next to self (low affinity) peptides. Thus, heterodimeric pMHCs, with one foreign and one self-peptide, activate the TCR. In the original model, binding of CD4 to the heterodimeric pMHC aids in binding to the TCR (see also the heteroclustering model). (b) In the heteroclustering model, TCRs are not constitutively associated to kinases, but the co-receptors CD8 and CD4 are. Monomeric (and multimeric) pMHC simultaneously engages the TCR and CD8/CD4. Hence, the kinase is brought into the vicinity of the TCR allowing phosphorylation of CD3 with subsequent activation of the T cell

According to this model, it would be sufficient that small chemical compounds only bind to one pMHC of a preformed pMHC cluster, thus inducing high affinity binding of this pMHC with one TCR. The other TCR would be bound by a self-pMHC.

2.3.4 The Heteroclustering Model of TCR Triggering

As well as binding to the TCR, pMHC also binds with its constant regions to the CD8 and CD4 co-receptors. The simultaneous binding of pMHC to the TCR and CD8/CD4 could lead to a heteroclustering of the TCR with CD8/CD4. The CD8 and CD4 cytoplasmic tails interact constitutively with Lck (Kim et al. 2003) that, when recruited to the TCR, could phosphorylate CD3 (heteroclustering model, Fig. 2.4b). The findings that monomeric pMHC does not activate TCRs (Boniface et al. 1998; Cochran et al. 2000) and that anti-TCR $\alpha\beta$ or anti-CD3 antibodies stimulate TCRs clearly argue against the heteroclustering model.

In contrast to mature T cells, all Lck is CD4- or CD8-bound in thymocytes (Van Laethem et al. 2007). Thus, in thymocytes the co-receptors have to be recruited, in

order to bring Lck in the vicinity of the TCR. Most likely, this occurs simultaneously to another triggering event, such as exposure of the CD3 tyrosines.

If one aims to explain the activity of small chemical compounds using the heteroclustering model, then the compound would need to induce high affinity binding of one self-pMHC to one TCR and at the same time allow binding of the pMHC to either CD8 or CD4.

2.3.5 The Kinetic Segregation Model of TCR Triggering

The physiological ligand for the TCR is a membrane-bound pMHC molecule. However, due to technical reasons, most experiments to elucidate the mechanism of TCR triggering are done with soluble pMHC (see above). We think that the obtained results still hold true for membrane-bound pMHC, since key findings also hold true for stimulations with membrane-bound pMHC. For example, stimulation of T cells by APCs led to the exposure of the proline-rich sequence in CD3 ϵ (Risueno et al. 2005), thus inducing a similar conformational change to stimulation with soluble pMHC multimers (Gil et al. 2002); and MHC class I and class II molecules form clusters on the surface of the APCs (Krishna et al. 1992; Schafer et al. 1995); therefore, the requirement for multivalent pMHC binding to TCRs seems to hold true. Anyhow, considering membrane-bound pMHC allows formulating additional models of TCR triggering (Choudhuri and van der Merwe 2007).

CD3 phosphorylation by kinases is counteracted by phosphatases (Mustelin et al. 2004). The main phosphatase in the plasma membrane of lymphocytes is CD45 (Trowbridge and Thomas 1994). Therefore, antigen binding could result in removal of the phosphatase from the vicinity of the TCR (Davis and van der Merwe 2006), thus initiating signal transduction. The TCR and pMHC are small molecules (Garboczi et al. 1996; Garcia et al. 1996), whereas CD45 has a bulky ectodomain (Davis and van der Merwe 2006). If the TCR and pMHC interact, then CD45 has to be “squeezed out” from these so-called close-contact zones (Davis and van der Merwe 2006) or microclusters (Yokosuka et al. 2005) (Fig. 2.5a). Indeed, confocal microscopy has shown that CD45 is excluded from TCR microclusters, where signaling is initiated at the immune synapse (Varma et al. 2006) (please note that formation of the synapse is a consequence of TCR triggering and not the cause). This model is called the kinetic segregation model since TCRs and kinases are temporally segregated from phosphatases, allowing CD3 phosphorylation. The model is supported by the finding that elongation of MHCp or shortening of CD45 results in inhibition of TCR triggering (Choudhuri et al. 2005; James and Vale 2012).

According to the kinetic segregation model, small chemical compounds presented by APCs need to be small enough so that the T cell and APC membranes come into close contact where the large phosphatases are excluded.

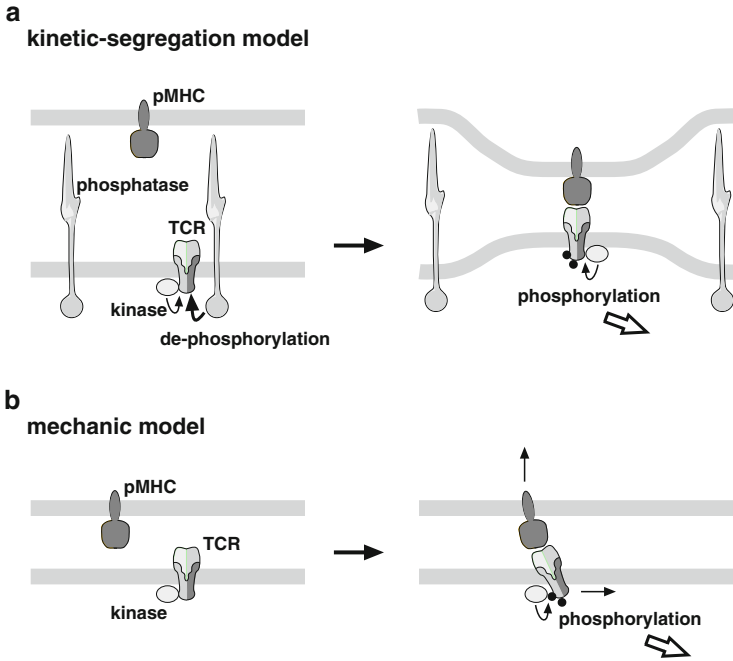


Fig. 2.5 The kinetic segregation and mechanical models. **(a)** TCRs, kinases, and transmembrane phosphatases intermingle on the membrane, resulting in constitutive phosphorylation and dephosphorylation of CD3. When the TCR interacts with membrane-bound pMHC, the two opposing membranes come into close contact. The small intermembrane distance at the TCR does not provide enough space for the large phosphatases. Therefore, phosphatases are segregated from the TCRs, resulting in accumulation of phosphorylated CD3. TCRs that do not bind to pMHC rapidly move out of these close-contact zones. In contrast, pMHC-bound TCRs stay for a sufficient amount of time inside the phosphatase-free areas. **(b)** Mechanical forces are applied to the TCR that change its structure allowing kinases to phosphorylate CD3. In the different mechanical models, it is either the APC that moves away from the T cell or it is the TCR being laterally moved by the actin cytoskeleton

2.3.6 Mechanical Models of TCR Triggering

Another model includes mechanical forces that are generated when pMHC binds to the TCR (Kim et al. 2009; Ma and Finkel 2010; Wang and Reinherz 2012). These forces distort the TCR, inducing conformational changes that are transmitted to the cytoplasmic domains (Fig. 2.5a). The forces are generated by a movement of either the TCR, e.g., mediated by the actin cytoskeleton, or the pMHC, e.g., by movements of the APC.

In contrast to the permissive geometry and other models, these membrane-bound pMHC-based models would be in line with monomeric pMHC stimulating the TCR. However, they fail to explain T-cell activation via soluble anti-TCR $\alpha\beta$ or anti-CD3 antibodies (Chang et al. 1981; Kaye and Janeway 1984) or soluble bi- or

multivalent pMHC (Boniface et al. 1998; Cochran et al. 2000; Krogsgaard et al. 2005; Minguet et al. 2007).

According to the mechanical models, it would be sufficient if drugs modify self-pMHC in a manner that they would bind to TCRs with high affinity, without any further constraints.

2.3.7 TCR Triggering by Superantigens

Superantigens, also called enterotoxins, are secreted by *Staphylococcus aureus* and *Streptococcus pyogenes* and can trigger a massive immune response that can lead to lethal toxic shock. This is caused by a peptide-independent activation of the TCR (Fraser and Proft 2008; Petersson et al. 2004). Enterotoxins bind both to the MHC class II and the outer face of TCR V β domains, triggering the TCR (Fig. 2.1d). The activated T cells divide and produce cytokines. As superantigens activate all T cells expressing certain V β domains (up to 40 % of all T cells), they can cause the toxic shock. Since superantigens bridge the TCR and MHC, all of the abovementioned models potentially hold true. However, superantigens approximate the TCR and MHC in a different geometry compared to the TCR-peptide-MHC interaction (Saline et al. 2010; Wang et al. 2007) and simultaneously to the TCR and MHC also bind to the co-receptor CD28 (Arad et al. 2011).

These differences might contribute to the fact that superantigens partially stimulate different signaling pathways than pMHC. Superantigens induce the canonical “pMHC-TCR” signaling pathways, which includes phosphorylation of CD3 by Lck, recruitment of ZAP70, and activation of PLC γ (Fig. 2.1b). In addition, a second Lck-independent pathway is triggered, in which the small heterotrimeric G protein G α q11 is activated, leading to the stimulation of PLC β (Bueno et al. 2007, 2006) (Fig. 2.1d). Both, PLC γ and PLC β , cleave inositol lipids resulting in the increase in cytoplasmic calcium concentrations—one of the important second messengers in T-cell activation.

Considering superantigen-mediated TCR triggering, small chemical compounds could induce self-pMHC binding to TCR $\alpha\beta$ in any orientation and may be even independent on any peptide bound to the MHC molecule.

2.4 TCR $\alpha\beta$ Binds to pMHC in a Conserved Orientation

pMHC always binds in a diagonal orientation to TCR $\alpha\beta$, with variations in the binding angle of only 35° (Rudolph et al. 2006). The N-terminus of the peptide binds to TCR α and the C-terminus to TCR β . Whether this orientation is germ line-encoded and dictated by semi-conserved TCR $\alpha\beta$ -MHC interactions (Garcia 2012; Scott-Browne et al. 2009) or whether this orientation is necessary to activate the TCR (Minguet and Schamel 2008) is unknown.

Our data suggested that the diagonal orientation with which $\text{TCR}\alpha\beta$ binds to pMHC is crucial for the structural change and the activation of the TCR (permissive geometry model) (Minguet et al. 2007). Since superantigens lead to an approximation of MHC with the TCR in different orientations, they might partially activate the TCR in a different manner. If true, then drug-induced TCR activation might be accomplished by pMHC binding to $\text{TCR}\alpha\beta$ in different geometries.

2.5 The Kinetics of the pMHC- $\text{TCR}\alpha\beta$ Interactions Determine the Stimulation Outcome

A high affinity pMHC- $\text{TCR}\alpha\beta$ interaction leads to T-cell activation, as observed with foreign, agonistic pMHC. In contrast, a low affinity pMHC- $\text{TCR}\alpha\beta$ interaction does not induce T-cell activation, as seen with self-peptide-MHC molecules, preventing autoimmune reactions (see above). How can the TCR interpret different affinities? One solution is the kinetic proofreading scheme (Dushek et al. 2011; McKeithan 1995). High affinity pMHC would be bound for sufficient time to a TCR, to allow forming an activatory signalosome at the TCR. Low affinity pMHC would only bind for short time, so that the signalosome cannot form and an activatory signal is not generated. Thus, the half-life of the pMHC- $\text{TCR}\alpha\beta$ interaction is crucial in discriminating between high and low affinity ligands.

Recently, it has been argued that also the on-rate of the pMHC- $\text{TCR}\alpha\beta$ interaction might play a role (Aleksic et al. 2010). Thus, small chemical compounds might need to alter self-pMHC, so that the modified pMHC binds for sufficient amount of time to the TCR. This would then translate into a high affinity interaction. Likewise, a fast on-rate might be beneficial.

2.6 TCR Triggering by Non-MHC Molecules

In mature T cells, a lot of the kinase Lck molecules are not bound to CD4 or CD8 and thus are free to interact and phosphorylate the TCR. In contrast, all Lck molecules are bound to CD4 and CD8 in developing T cells, called thymocytes (Van Laethem et al. 2007). Thus, stimulation of the TCR for positive selection in thymocytes requires the co-engagement of pMHC with the TCR and CD8/CD4, in order to allow Lck to approximate the TCR. Still, the TCR triggering models discussed above could hold true, such as that the cytoplasmic tyrosines of CD3 have to be exposed (Aivazian and Stern 2000; Minguet and Schamel 2008). In a recent experiment, MHC as well as CD4 and CD8 were deleted in the mouse, so that free Lck molecules are present in the thymocytes (Tikhonova et al. 2012). This then allowed T cells to develop that do not recognize pMHC, but for instance

CD155. This experiment clearly shows that TCRs can be triggered by other molecules than MHC.

Stimulation of TCRs by non-MHC molecules is also in line with the activating capacity of anti-TCR $\alpha\beta$ and anti-CD3 antibodies (Chang et al. 1981; Kaye and Janeway 1984) or activation of a chimeric TCR by artificial ligands (Minguet et al. 2007).

These findings open the interesting possibility that drugs could also alter other proteins than pMHC in order to generate high affinity ligands for TCR $\alpha\beta$ or even for CD3.

2.7 Conclusion

Although triggering of the TCR is one of the most important events in adaptive immunity, there is still no consensus on the underlying molecular mechanism. This is not due to lack of interest, but due to the fact that the TCR is one of the most complicated transmembrane receptors known to date. In fact, a large number of models have been put forward that mostly are not mutually exclusive. Thus, a combination of the proposed mechanisms might be close to what happens in reality.

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References

- Adams JJ, Narayanan S, Liu B, Birnbaum ME, Kruse AC, Bowerman NA, Chen W, Levin AM, Connolly JM, Zhu C, Kranz DM, Garcia KC (2011) T cell receptor signaling is limited by docking geometry to peptide-major histocompatibility complex. *Immunity* 35:681–93
- Aivazian D, Stern LJ (2000) Phosphorylation of T cell receptor zeta is regulated by a lipid dependent folding transition. *Nat Struct Biol* 7:1023–6
- Alarcon B, Gil D, Delgado P, Schamel WW (2003) Initiation of TCR signaling: regulation within CD3 dimers. *Immunol Rev* 191:38–46
- Alarcon B, Swamy M, van Santen HM, Schamel WWA (2006) T-cell antigen-receptor stoichiometry: pre-clustering for sensitivity. *EMBO Rep* 7:490–5
- Aleksic M, Dushek O, Zhang H, Shenderov E, Chen JL, Cerundolo V, Coombs D, van der Merwe PA (2010) Dependence of T cell antigen recognition on T cell receptor-peptide MHC confinement time. *Immunity* 32:163–74
- Arad G, Levy R, Nasie I, Hillman D, Rotfogel Z, Barash U, Supper E, Shpilka T, Minis A, Kaempfer R (2011) Binding of superantigen toxins into the CD28 homodimer interface is essential for induction of cytokine genes that mediate lethal shock. *PLoS Biol* 9:e1001149
- Ashwell JD, Klausner RD (1990) Genetic and mutational analysis of the T-cell antigen receptor. *Annu Rev Immunol* 8:139–67

- Boniface JJ, Rabinowitz JD, Wülfing C, Hampl J, Reich Z, Altman JD, Kantor RM, Beeson C, McConnell HM, Davis MM (1998) Initiation of signal transduction through the T cell receptor requires the peptide multivalent engagement of MHC ligands. *Immunity* 9:459–66
- Bueno C, Lemke CD, Criado G, Baroja ML, Ferguson SS, Nur-Ur Rahman AK, Tsoukas CD, McCormick JK, Madrenas J (2006) Bacterial superantigens bypass Lck-dependent T cell receptor signaling by activating a G α 11-dependent, PLC- β -mediated pathway. *Immunity* 25:67–78
- Bueno C, Criado G, McCormick JK, Madrenas J (2007) T cell signalling induced by bacterial superantigens. *Chem Immunol Allergy* 93:161–80
- Chang TW, Kung PC, Gingras SP, Goldstein G (1981) Does OKT3 monoclonal antibody react with an antigen-recognition structure on human T cells? *Proc Natl Acad Sci U S A* 78:1805–8
- Choudhuri K, van der Merwe PA (2007) Molecular mechanisms involved in T cell receptor triggering. *Semin Immunol* 19:255–61
- Choudhuri K, Wiseman D, Brown MH, Gould K, van der Merwe PA (2005) T-cell receptor triggering is critically dependent on the dimensions of its peptide-MHC ligand. *Nature* 436:578–82
- Cochran JR, Cameron TO, Stern LJ (2000) The relationship of MHC-peptide binding and T cell activation probed using chemically defined MHC class II oligomers. *Immunity* 12:241–50
- Davis SJ, van der Merwe PA (2006) The kinetic-segregation model: TCR triggering and beyond. *Nat Immunol* 7:803–9
- DeFord-Watts LM, Dougall DS, Belkaya S, Johnson BA, Eitson JL, Roybal KT, Barylko B, Albanesi JP, Wulfing C, Van Oers NS (2011) The CD3 zeta subunit contains a phosphoinositide-binding motif that is required for the stable accumulation of TCR-CD3 complex at the immunological synapse. *J Immunol* 186:6839–47
- Dushek O, Aleksic M, Wheeler RJ, Zhang H, Cordoba SP, Peng YC, Chen JL, Cerundolo V, Dong T, Coombs D, van der Merwe PA (2011) Antigen potency and maximal efficacy reveal a mechanism of efficient T cell activation. *Sci Signal* 4:ra39
- Fraser JD, Proft T (2008) The bacterial superantigen and superantigen-like proteins. *Immunol Rev* 225:226–43
- Garboczi DN, Ghosh P, Utz U, Fan QR, Biddison WE, Wiley DC (1996) Structure of the complex between human T-cell receptor, viral peptide and HLA-A2. *Nature* 384:134–41
- Garcia KC (2012) Reconciling views on T cell receptor germline bias for MHC. *Trends Immunol* 33:429–36
- Garcia KC, Degano M, Stanfield RL, Brunmark A, Jackson MR, Peterson PA, Teyton L, Wilson IA (1996) An alphabeta T cell receptor structure at 2.5 Å and its orientation in the TCR-MHC complex. *Science* 274:209–19
- Gil D, Schamel WW, Montoya M, Sanchez-Madrid F, Alarcon B (2002) Recruitment of Nck by CD3 epsilon reveals a ligand-induced conformational change essential for T cell receptor signaling and synapse formation. *Cell* 109:901–12
- Irvine DJ, Purbhoo MA, Krogsgaard M, Davis MM (2002) Direct observation of ligand recognition by T cells. *Nature* 419:845–9
- Iwashima M, Irving BA, van Oers NS, Chan AC, Weiss A (1994) Sequential interactions of the TCR with two distinct cytoplasmic tyrosine kinases. *Science* 263:1136–9
- James JR, Vale RD (2012) Biophysical mechanism of T-cell receptor triggering in a reconstituted system. *Nature* 487:64–9
- Kaye J, Janeway CA Jr (1984) 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* 159:1397–412
- Kim PW, Sun ZY, Blacklow SC, Wagner G, Eck MJ (2003) A zinc clasp structure tethers Lck to T cell coreceptors CD4 and CD8. *Science* 301:1725–8
- Kim ST, Takeuchi K, Sun ZY, Touma M, Castro CE, Fahmy A, Lang MJ, Wagner G, Reinherz EL (2009) The alphabeta T cell receptor is an anisotropic mechanosensor. *J Biol Chem* 284:31028–37

- Krishna S, Benaroch P, Pillai S (1992) Tetrameric cell-surface MHC class I molecules. *Nature* 357:164–7
- Krogsgaard M, Li QJ, Sumen C, Huppa JB, Huse M, Davis MM (2005) Agonist/endogenous peptide-MHC heterodimers drive T cell activation and sensitivity. *Nature* 434:238–43
- Kuhns MS, Davis MM (2012) TCR signaling emerges from the sum of many parts. *Front Immunol* 3:159
- Lillemeier BF, Mortelmaier MA, Forstner MB, Huppa JB, Groves JT, Davis MM (2010) TCR and Lat are expressed on separate protein islands on T cell membranes and concatenate during activation. *Nat Immunol* 11:90–6
- Lu X, Gibbs JS, Hickman HD, David A, Dolan BP, Jin Y, Kranz DM, Bennink JR, Yewdell JW, Varma R (2012) Endogenous viral antigen processing generates peptide-specific MHC class I cell-surface clusters. *Proc Natl Acad Sci U S A* 109:15407–12
- Ma Z, Finkel TH (2010) T cell receptor triggering by force. *Trends Immunol* 31:1–6
- McKeithan TW (1995) Kinetic proofreading in T-cell receptor signal transduction. *Proc Natl Acad Sci U S A* 92:5042–6
- Minguet S, Schamel WWA (2008) A permissive geometry model for TCR-CD3 activation. *Trends Biochem Sci* 33:51–7
- Minguet S, Swamy M, Alarcon B, Luescher IF, Schamel WW (2007) Full activation of the T cell receptor requires both clustering and conformational changes at CD3. *Immunity* 26:43–54
- Molnar E, Deswal S, Schamel WW (2010) Pre-clustered TCR complexes. *FEBS Lett* 584:4832–7
- Molnar E, Swamy M, Holzer M, Beck-Garcia K, Worch R, Thiele C, Guigas G, Boye K, Luescher IF, Schwille P, Schubert R, Schamel WW (2012) Cholesterol and sphingomyelin drive ligand-independent T-cell antigen receptor nanoclustering. *J Biol Chem* 287:42664–74
- Mustelin T, Alonso A, Bottini N, Huynh H, Rahmouni S, Nika K, Louis-dit-Sully C, Tautz L, Togo SH, Bruckner S, Mena-Duran AV, al-Khouri AM (2004) Protein tyrosine phosphatases in T cell physiology. *Mol Immunol* 41:687–700
- Petersson K, Forsberg G, Walse B (2004) Interplay between superantigens and immunoreceptors. *Scand J Immunol* 59:345–55
- Reth M (1989) Antigen receptor tail clue. *Nature* 338:383
- Risueno RM, Gil D, Fernandez E, Sanchez-Madrid F, Alarcon B (2005) Ligand-induced conformational change in the T-cell receptor associated with productive immune synapses. *Blood* 106:601–8
- Rudolph MG, Stanfield RL, Wilson IA (2006) How TCRs bind MHCs, peptides, and coreceptors. *Annu Rev Immunol* 24:419–66
- Saline M, Rödström KE, Fischer G, Orekhov VY, Karlsson BG, Lindkvist-Petersson K (2010) The structure of superantigen complexed with TCR and MHC reveals novel insights into superantigenic T cell activation. *Nat Commun* 1:119
- Schafer PH, Pierce SK, Jardetzky TS (1995) The structure of MHC class II: a role for dimer of dimers. *Semin Immunol* 7:389–98
- Schamel WW, Arechaga I, Risueno RM, van Santen HM, Cabezas P, Risco C, Valpuesta JM, Alarcon B (2005) Coexistence of multivalent and monovalent TCRs explains high sensitivity and wide range of response. *J Exp Med* 202:493–503
- Scott-Browne JP, White J, Kappler JW, Gapin L, Marrack P (2009) Germline-encoded amino acids in the alphabeta T-cell receptor control thymic selection. *Nature* 458:1043–6
- Shi X, Bi Y, Yang W, Xingdong G, Jiang Y, Wan C, Lunyi L, Bai Y, Guo J, Wang Y, Chen X, Wu B, Sun H, Liu W, Wang J, Xu C (2013) Ca²⁺ regulates T-cell receptor activation by modulating the charge property of lipids. *Nature* 493:115–25
- Starr TK, Jameson SC, Hogquist KA (2003) Positive and negative selection of T cells. *Annu Rev Immunol* 21:139–76
- Stefanova I, Dorfman JR, Germain RN (2002) Self-recognition promotes the foreign antigen sensitivity of naive T lymphocytes. *Nature* 420:429–34

- Swamy M, Siegers GM, Fiala GJ, Molnar E, Dopfer EP, Fisch P, Schraven B, Schamel WW (2010) Stoichiometry and intracellular fate of TRIM-containing TCR complexes. *Cell Commun Signal* 8:5
- Tikhonova AN, Van Laethem F, Hanada K, Lu J, Pobeziński LA, Hong C, Guinter TI, Jeurling SK, Bernhardt G, Park JH, Yang JC, Sun PD, Singer A (2012) alphabeta T cell receptors that do not undergo major histocompatibility complex-specific thymic selection possess antibody-like recognition specificities. *Immunity* 36:79–91
- Trowbridge IS, Thomas ML (1994) CD45: an emerging role as a protein tyrosine phosphatase required for lymphocyte activation and development. *Annu Rev Immunol* 12:85–116
- Van Laethem F, Sarafova SD, Park JH, Tai X, Pobeziński L, Guinter TI, Adoro S, Adams A, Sharrow SO, Feigenbaum L, Singer A (2007) Deletion of CD4 and CD8 coreceptors permits generation of alphabetaT cells that recognize antigens independently of the MHC. *Immunity* 27:735–50
- Varma R, Campi G, Yokosuka T, Saito T, Dustin ML (2006) T cell receptor-proximal signals are sustained in peripheral microclusters and terminated in the central supramolecular activation cluster. *Immunity* 25:117–27
- Wang JH, Reinherz EL (2012) The structural basis of alphabeta T-lineage immune recognition: TCR docking topologies, mechanotransduction, and co-receptor function. *Immunol Rev* 250:102–19
- Wang L, Zhao Y, Li Z, Guo Y, Jones LL, Kranz DM, Mourad W, Li H (2007) Crystal structure of a complete ternary complex of TCR, superantigen and peptide-MHC. *Nat Struct Mol Biol* 14:169–71
- Wülfing C, Sumen C, Sjaastad MD, Wu LC, Dustin ML, Davis MM (2002) Costimulation and endogenous MHC ligands contribute to T cell recognition. *Nat Immunol* 3:42–7
- Xu C, Gagnon E, Call ME, Schnell JR, Schwieters CD, Carman CV, Chou JJ, Wucherpfennig KW (2008) Regulation of T cell receptor activation by dynamic membrane binding of the CD3epsilon cytoplasmic tyrosine-based motif. *Cell* 135:702–13
- Yokosuka T, Sakata-Sogawa K, Kobayashi W, Hiroshima M, Hashimoto-Tane A, Tokunaga M, Dustin ML, Saito T (2005) Newly generated T cell receptor microclusters initiate and sustain T cell activation by recruitment of Zap70 and SLP-76. *Nat Immunol* 6:1253–62

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