CHAPTER 11

Permissive Geometry Model

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Abstract

Ligand binding to the T-cell antigen receptor (TCR) evokes receptor triggering and subsequent T-lymphocyte activation. Although TCR signal transduction pathways have been extensively studied, a satisfactory mechanism that rationalizes how the information of ligand binding to the receptor is transmitted into the cell remains elusive. Models proposed for TCR triggering can be grouped into two main conceptual categories: receptor clustering by ligand binding and induction of conformational changes within the TCR. None of these models or their variations (see Chapter 6 for details) can satisfactorily account for the diverse experimental observations regarding TCR triggering. Clustering models are not compatible with the presence of preformed oligomeric receptors on the surface of resting cells. Models based on conformational changes induced as a direct effect of ligand binding, are not consistent with the requirement for multivalent ligand to initiate TCR signaling. In this chapter, we discuss the permissive geometry model. This model integrates receptor clustering and conformational change models, together with the existence of preformed oligomeric receptors, providing a mechanism to explain TCR signal initiation.

Introduction

The antigen receptors expressed on lymphocytes belong to the multichain immune recognition receptor (MIRR) family. The B-and T-cell antigen receptors (BCR and TCR) are expressed on B- and T-cells, respectively. BCRs bind to folded native antigens. These ligands can be any chemical substance and therefore can vary enormously in size, shape and geometry. In contrast, TCRs recognize antigenic peptides that are presented on the surface of antigen presenting cells (APCs). Therefore, the basic structure and geometry of the TCR ligand is constant. In both cases, antigen binding leads to MIRR triggering, which in turn activates several cytosolic signaling pathways. To date, the molecular mechanism of how antigen binding evokes MIRR activation is not very well understood and a matter of intense debate. The consensus is that receptor triggering leads to phosphorylation of tyrosine residues in the cytoplasmic portions of the receptor itself. Phosphorylation of the MIRR is the critical event in initiating the signaling cascades.

The TCR comprises the ligand-binding TCR$\alpha\beta$ heterodimer and the signal-transducing dimers CD3$\varepsilon\gamma$, CD3$\varepsilon\delta$ and $\zeta\zeta$ (Chapter 1). The cytoplasmic tyrosines are present within the immunoreceptor tyrosine-based activation motif (ITAM, YxxI/Lx_{6-8}YxxI/L)[1] in the CD3 and ζ subunits.

Several approaches have been undertaken to decipher the changes that the receptor undergoes upon ligand binding. Biochemical studies suggest that MIRRs are only activated by bi- or multivalent ligands, implying that one ligand molecule binds simultaneously to several receptor molecules. Based on these observations, the clustering models for MIRR activation were postulated (Chapter 6). Our

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recent data on induction of conformational changes in the cytoplasmic tails of the receptor upon ligand binding and the fact that resting MIRRs can be found as preformed multimers, motivated us to assert the permissive geometry model of signal initiation by the MIRRs.[2]

On the surface of APCs, the TCR recognizes its ligand, a peptide bound to major histocompatibility complex molecule (MHCp). A given TCR can bind to several distinct, but related, MHCp that differ in the exact sequence of the peptide. In general, these peptides can be agonist, null or antagonist peptides, depending on their affinity for a given TCR and the strength of the signal generated. In the thymus, where only self-peptides are presented, strong activation of immature T-cells leads to apoptosis and, thus, to negative selection of self-reactive lymphocytes. In contrast, MHCp with weak affinity deliver a survival signal for the differentiation into mature lymphocytes. In the periphery, strong signals activate mature T-cells and weak signaling is required for survival.

In the context of an immune response, both self-peptides, derived from endogenous proteins and antigenic peptides are copresented by the APCs. Recent studies have clearly shown that self-peptides can aid in the activation of T-cells by agonist peptides.[3-6] In this Chapter, the mechanism of this phenomenon will be discussed from the perspective of the permissive geometry model.

The Clustering Model of TCR Triggering

Soluble MHCp monomers as well as monovalent Fab-fragments of anti-TCR antibodies can bind to the TCR but fail to stimulate the receptor.[7-10] In contrast, di- and multimeric soluble MHCp as well as complete anti-TCR antibodies can activate T-cells. These findings imply that two or more receptors have to bind simultaneously to one ligand molecule to be activated. In conjunction with the assumption that individual TCRs are distributed equally on the cell surface, these results led to the proposals of the homoclustering and the pseudodimer models of TCR activation[3,11] (Chapter 6).

Briefly, in the homoclustering model (Fig. 1A), monomeric MIRRs are associated with kinases that need cross-wise phosphorylation for full activity. In addition, the kinases cannot phosphorylate the receptors that they are bound to. If a bi- or multivalent ligand simultaneously binds to at least two MIRRs, the kinases are enabled to transphosphorylate each other and the neighboring MIRR, initiating the signaling cascades.

A variant of the homoclustering model is the pseudodimer model. It takes into account the cooperation between antigenic and self-peptides in T-cell stimulation, together with the role of the coreceptor CD4.[3,6] Since MHC molecules of class I and II are most likely multimeric proteins on the surface of the APCs,[12,13] at low antigen concentrations most antigenic peptides (aMHCp) are presented next to a self-peptides (sMHCp). Indeed, heterodimeric aMHCp-sMHCp were shown to activate T-cells.[6] Since sMHCp cannot stably bind to the TCR, the homoclustering model cannot account for this finding. In the pseudodimer model, dimeric MHCp brings two TCRs together in conjunction with CD4. The interaction between dimeric MHCp and two TCRs might therefore be enhanced by simultaneous binding of CD4 to MHCp and the TCR (Chapter 6).

Oligomeric MIRRs

The homoclustering as well as pseudodimer models require that the unstimulated receptor is monomeric. However, several studies have indicated the existence of oligomeric TCRs, i.e., complexes that have several ligand-binding TCRα/β subunits[14,15] (Fig. 1B). Other studies failed to detect them, possibly due to the use of the detergent called digitonin.[16,17] Digitonin disrupts all TCR oligomeric structures[15,18] (Fig. 1B). Likewise, the BCR and the FcεRI might exist as clusters on the surface of unstimulated cells.[19,20]

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 multivalent ligands disturbs the structure of the receptor oligomer.[21,22] The consequent reorientation of the receptor units might lead to conformational changes within the cytoplasmic tails of the receptor, thereby allowing phosphorylation by the associated kinases. Monovalent ligands are not capable of disturbing the structure of the receptor oligomer and thus they are inactive. Therefore,

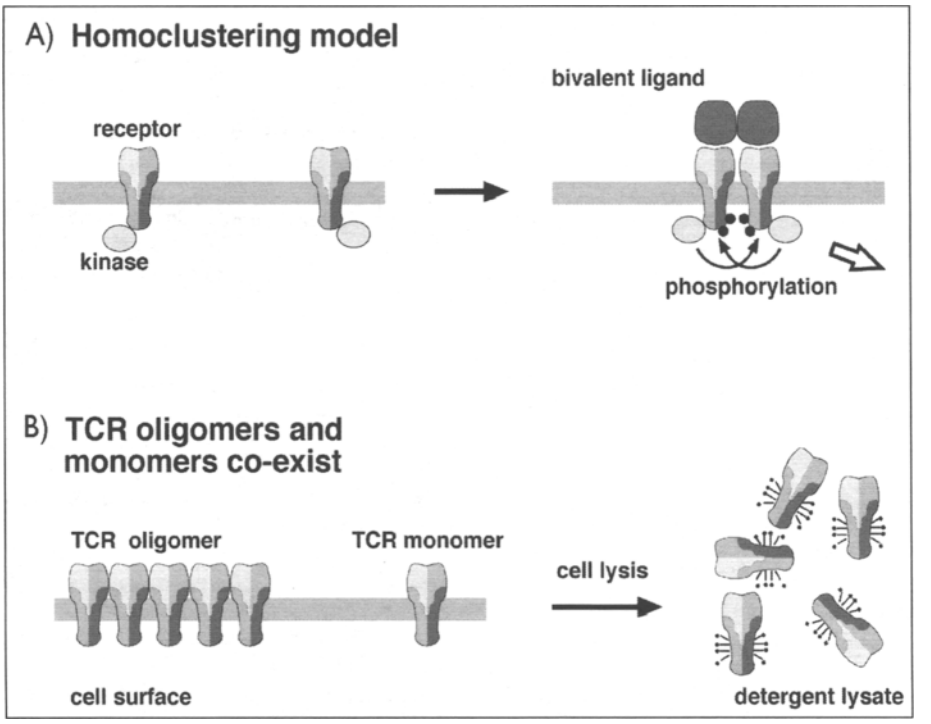

Figure 1. The clustering model is not compatible with preformed TCR oligomers. A) The homoclustering model requires that monomeric TCRs with associated kinases are individually expressed on the cell surface. Stimulation by bi- or multivalent ligand leads to clustering of the receptors, since the ligand simultaneously binds two or more receptors. In these clusters the kinases transphosphorylate each other and the receptors. This initiates the signaling cascades, resulting in activation of the cell. Black dots represent phosphorylated tyrosine residues and the open arrow represents activation of downstream signaling cascades. B) On the T-cell surface, the TCR is expressed as mixture of monomers and oligomers of different sizes. Thus, the requirement for the clustering models is not fulfilled. Note, that cell lysis with commonly used detergents (e.g., digitonin) disrupts the oligomers, hampering their detection by biochemical means.

structural changes of the receptor upon ligand binding might be a useful concept to understand MIRR triggering.

Conformational Changes within the MIRRs

Although the study of structural rearrangements within MIRRs and their consequences in activation are still at a preliminary stage, evidences of conformational changes within the TCR, BCR and FcεRI have been described[23-25] (Chapter 10). Initially, the existence of structural changes in the TCR complex upon ligand binding was proposed to explain early T-cell signaling studies where differences in receptor clustering or antibody affinities were insufficient to explain distinct activation potentials of anti-TCR antibodies.[26] However, crystallographic structures of free and MHCp-bound soluble TCRαβ have revealed induced-fit type changes only in the variable regions at the ligand-binding interface, whereas no alterations were observed at the distal parts of the heterodimer, which are in contact with the CD3 signal-transducing units. Thus, it is difficult to understand how structural changes should be transmitted to the cytoplasmic tails of CD3. Nevertheless, the group of Balbino Alarcón found that a proline-rich region in CD3ε becomes

exposed upon TCR stimulation (Chapter 10).[23] Induction of conformational changes within the TCR complex has mainly been discussed as a direct consequence of ligand binding. But the lack of support from the crystal structures and the absence of a consistent mechanism have generated scepticism. The permissive geometry model integrates the main models of TCR triggering, mainly receptor clustering and conformational changes, together with the presence of preformed oligomeric receptors. It provides a mechanism that accounts for most of the experimental observations regarding TCR activation.

Likewise, the signaling chain homooligomerization (SCHOOL) model (Chapters 12 and 20) introduces the necessity of a defined orientation between two or more MIRRs cross-linked by multivalent ligand. It also explains why initially preformed MIRR oligomers on resting cells do not trigger cell activation.[27]

Permissive Geometry Model

The current clustering models for TCR activation, namely the homoclustering and the pseudodimer models, are not compatible with the presence of preformed oligomeric receptors (Chapter 6). Similarly, the conformational change model, as a direct effect of ligand binding, is not consistent with the requirement for multivalent ligand to initiate cell signaling. Our recent data have evoked a new perspective for the mechanism underlying the TCR activation. We show that conformational changes within the TCR complex can only be induced by multivalent ligands (Fig. 2).[2] The receptor oligomers can either be preformed or achieved by multivalent ligand binding, since homoclustering is necessary but not sufficient to induce conformational changes that initiate signal transduction.

When TCRs are clustered in detergent lysates, the conformational change at CD3 is not induced. This indicates that two TCRs need to be brought not only into close proximity, but also into a defined orientation. Likewise, not all anti-TCR antibodies have the same capability to induce the conformational change, even when they bind to the same number of TCRs. Since these antibodies bind to distinct regions of TCR complex, they should lead to different geometries of the clustered complex. We therefore suggested that the exact geometry determines whether a conformational change takes place or not. A permissive geometry would lead to structural reorganization within the TCR complex (Fig. 2), whereas a different inert geometry would not.[2]

Additionally, we designed an experimental approach that can separate the conformational change at CD3 from receptor clustering. By using ligands that induce conformational changes in the absence of receptor clustering and ligands that can keep two TCRs within close proximity without forcing the conformational change, we showed that both TCR clustering and the conformational change are needed for receptor activation.[2]

The necessity of the conformational change for effective TCR signaling in combination with the permissive geometry requirements, encouraged us to assert the permissive geometry model of signal initiation of the MIRRs (Fig. 2).[2] In the resting state, the cytoplasmic tails of the TCR-CD3 complex might be in a closed conformation and not accessible to kinases and/or adaptor proteins. Monovalent MHCp-binding does not lead to structural changes of TCRα/β outside the direct contact region, thus not rearranging the cytoplasmic tails of CD3 and not leading to TCR activation (Fig. 2, upper panel). Similarly, bi- and multivalent MHCp binding does not change the structure of one TCRα/β (Fig. 2, lower panel). However, since two TCR-CD3 complexes are engaged simultaneously, they have to adjust to the geometry of the preformed MHCp dimer. This results in a reorientation of two TCR$\alpha\beta$ into a permissive geometry. A mechanical force is then exerted on the extracellular and transmembrane regions of the CD3 and ζ subunits, "pushing" them away from their original positions. This rearrangement is transmitted through the membrane and affects the conformation of the cytoplasmic regions of CD3, making them accessible for activation effectors (Fig. 2, lower panel). This model is compatible with preformed receptor oligomers, suggesting that initially they are in a nonpermissive geometry, whereas binding of bi- or multivalent ligands induces the signaling-permissive geometry within the oligomer (Fig. 3).

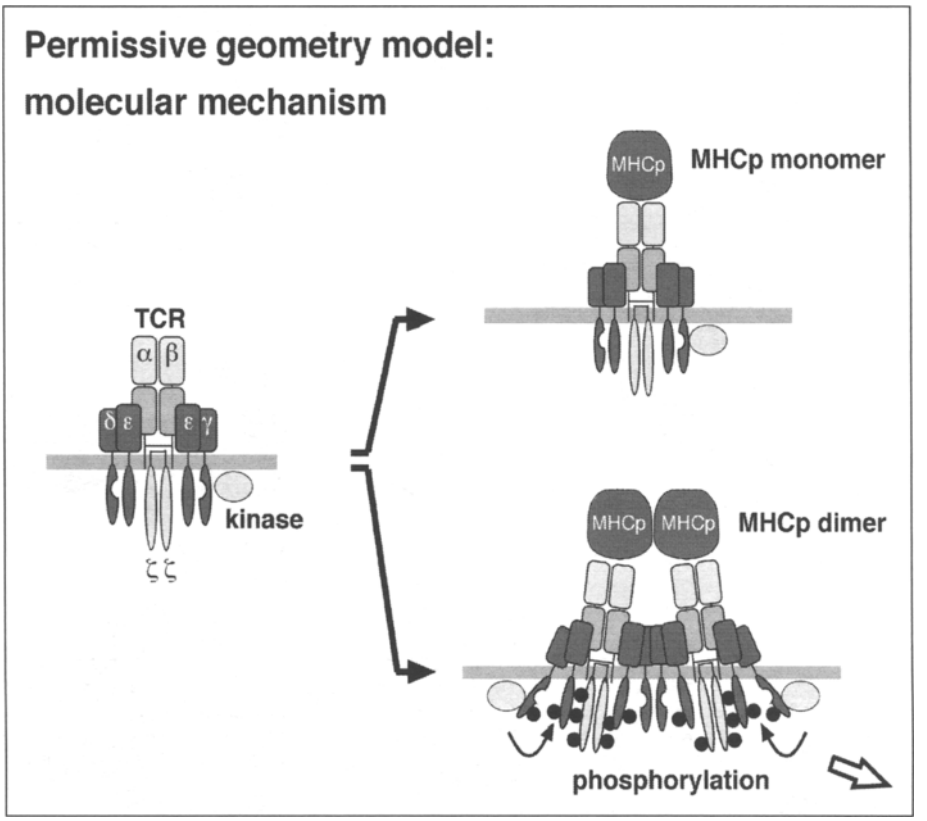

Figure 2. Permissive geometry model. The TCR-CD3 complex is in a closed conformation, preventing phosphorylation by the associated kinases. Monovalent MHCp does not change the structure of TCRα/β. Thus, a conformational change cannot be transmitted to CD3 and the receptor stays in an inactive, nonphosphorylated state (upper panel). In the permissive geometry model, bi- or multivalent MHCp ligands reorientate two TCRα/β without changing the structure of one TCRα/β. The reorientation exerts a mechanical force on CD3, inducing conformational changes in the cytoplasmic tails of CD3 and allowing phosphorylation. Thus, dimeric MHCp activates T-cells due to induction of conformational changes within a TCR oligomer (lower panel). Since monomeric MHCp does not engage several TCRα/β simultaneously, it does not induce conformational changes in CD3.

Similar models have been previously suggested for the TCR[28,29] (dimer conformational change model), the FcεRI[25] and for MIRRs in general (SCHOOL model, Chapters 12 and 20).[27]

Agonist/Self-Peptide-MHC Heterodimers

In the context of the immune response, both antigenic peptides and self-peptides are copresented by the APCs. Recently, it was shown that self-peptides can help agonistic peptides to activate T-cells.[3-6] The role of sMHCp in TCR triggering can be incorporated into the permissive geometry model. The heterodimeric MHCp (aMHCp-sMHCp) would have a sufficient avidity to bind a TCR oligomer stably enough to induce clustering within the permissive geometry and thereby conformational changes at CD3, leading to receptor triggering and cell activation (Fig. 4, middle panel). In contrast, the sMHCp-TCR affinity is not sufficient to cluster two monomeric receptors with a half-life that is long enough for the initiation of signaling (homoclustering model).

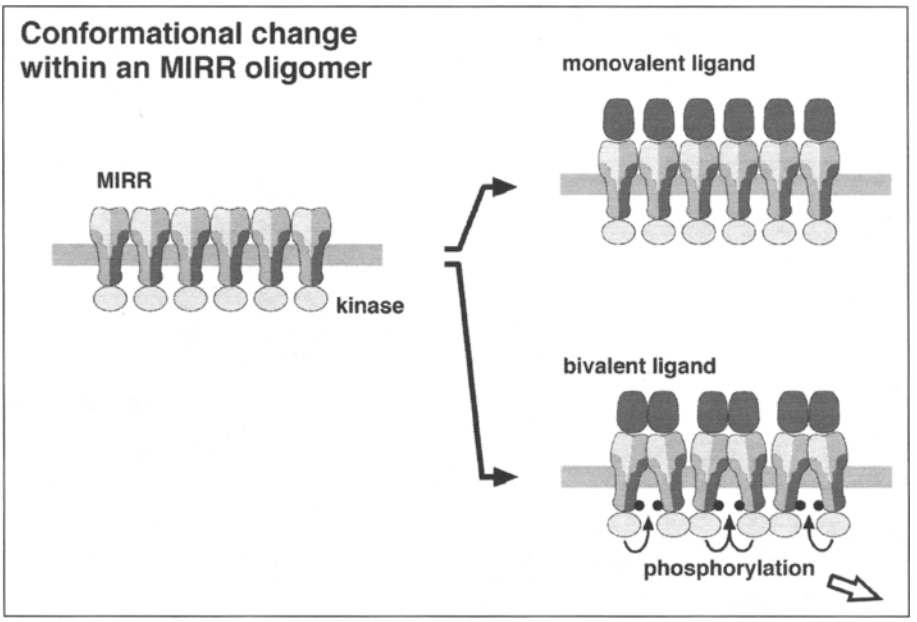

Figure 3. Rearrangement of preformed MIRR oligomers. Resting MIRR oligomers are in a nonpermissive geometry and inactive. MIRR clusters are pre-associated with kinases but due to structural constraints these kinases are not able to transphosphorylate each other and the receptor tails. Monovalent ligands do not disturb the original structure of the oligomer (upper panel) and thus, do not lead to MIRR triggering. By binding simultaneously to at least two receptor units within the cluster, bi- and multivalent ligands change the oligomeric structure into the permissive geometry (lower panel). Consequently, the kinases are able to reach each other and the receptors for their phosphorylation. Thus, this model combines the requirement for a multivalent ligand and the presence of oligomeric receptors.

Mathematical calculations using the permissive geometry model were able to explain how T-cells can respond with extreme sensitivity to low amounts of antigenic peptides without normally being activated by the self-peptides alone.[30] At high antigen doses, sufficient amounts of aMHCp homodimers might be present on the APC surface, allowing homoclustering of two monomeric TCRs and inducing cell activation (Fig. 4, lower panel). This model provides an explanation for the fact that multimeric TCRs become activated at low antigen doses, whereas multi- and monomeric TCRs are activated at high doses.[15] This permissive geometry model does not require the molecular aid of the CD4 or CD8 coreceptors but still allows the coreceptor-mediated enhancement of the signal. This could be due to increased recruitment of kinases of the Src-family or an enhancement of the avidity of TCR/co receptor towards MHCp, prolonging the duration of the TCR-ligand engagement and therefore the strength of the activation signal, as proposed by the kinetic proofreading model[31] (Chapter 8).

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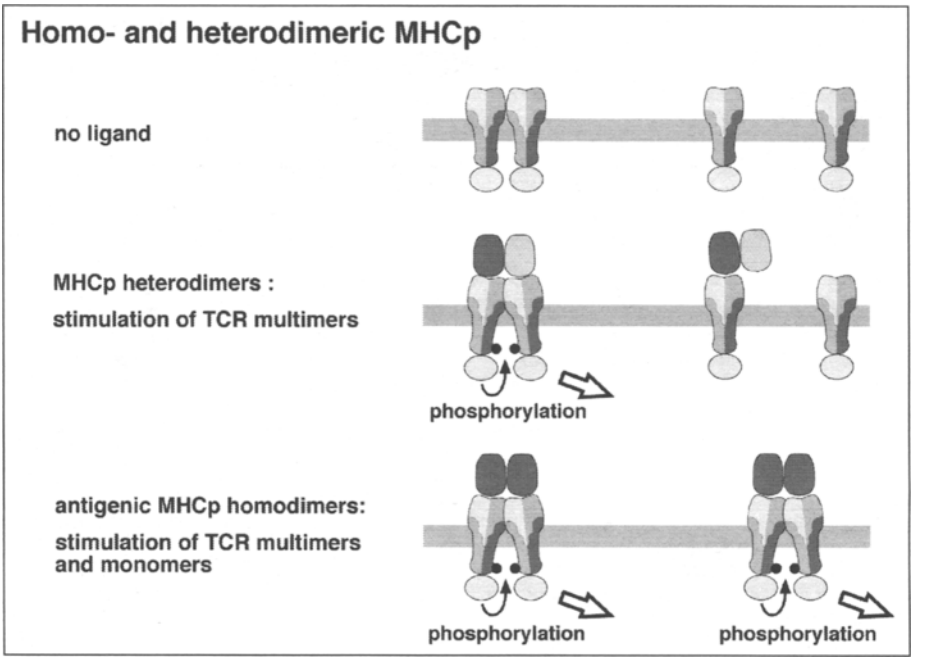

Figure 4. Role of self-peptide-MHC complexes. TCRs are co-expressed as multimers and monomers with associated kinases (upper panel). Heterodimeric aMHCp-sMHCp binds to a TCR oligomer bivalently and with sufficient avidity, inducing rearrangement of the cytoplasmic tails. This results in receptor phosphorylation (middle panel). MHCp heterodimers cannot stably cluster two monomeric TCRs, thus leaving them unphosphorylated. In contrast, homodimers of aMHCp can activate both TCR oligomers and monomers due to the moderate binding affinity of aMHCp molecules (lower panel).

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