CHAPTER 6

Clustering Models

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Abstract

Ligand binding to the multichain immune recognition receptors (MIRRs) leads to receptor triggering and subsequent lymphocyte activation. MIRR signal transduction pathways have been extensively studied, but it is still not clear how binding of the ligand to the receptor is initially communicated across the plasma membrane to the cells interior. Models proposed for MIRR triggering can be grouped into three categories. Firstly, ligand binding invokes receptor clustering, resulting in the approximation of kinases to the MIRR and receptor phosphorylation. Secondly, ligand binding induces a conformational change of the receptor. Thirdly, upon ligand-binding, receptors and kinases are segregated from phosphatases, leading to a net phosphorylation of the receptor. In this review, we focus on the homoclustering induced by multivalent ligands, the heteroclustering induced by simultaneous binding of the ligand to the MIRR and a coreceptor and the pseudodimer model.

Introduction

Multichain immune recognition receptor (MIRR) family members are transmembrane multiprotein complexes that are activated by binding to their appropriate antigens (or ligands). This binding event transmits information across the plasma membrane to the cytoplasm in a process termed signal transduction. The first measurable biochemical change that takes place upon antigen recognition is phosphorylation of tyrosine residues in the cytoplasmic portions of the receptor itself. These tyrosines are part of the immunoreceptor tyrosine-based activation motif (ITAM).[1] Depending on the receptor type, ITAM tyrosines are phosphorylated by kinases of the Src-family[2] and Syk/ZAP70-family.[3] Phosphorylation of the MIRR is the critical event in initiating the signaling cascades, since phosphotyrosines serve as binding sites for proteins with src homology 2 (SH2) domains. Consequently, these proteins are recruited to the receptor and activate signaling pathways.

Ligand binding to the MIRRs induces molecular changes in the receptor structure or in the distribution of the receptor within the membrane, leading to receptor activation. Although crucial for lymphocyte activation, these events still remain an enigma. One experimental possibility to decipher the molecular mechanisms underlying receptor activation is to study the structural requirements of the ligand. Ligands of interest are altered ligands that still bind to the receptor, but are unable to activate the receptor. It was demonstrated that the T-cell antigen receptor (TCR) and the high-affinity IgE receptor (FcεRI) are only activated by bi- or multivalent ligands.[4-7] This has led to the clustering models of signal initiation by the MIRRs. In case of the B-cell antigen receptor (BCR) however, it is still controversial whether monovalent antigen can induce BCR triggering.[8]

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In this chapter, emphasis will be taken on the TCR, since it is the best studied MIRR. The ligand for the TCR is a peptide bound to a major histocompatibility complex molecule (MHCp) on the surface of antigen-presenting cells (APCs). One single TCR can bind to several distinct, but related, MHCp that differ in the exact sequence of the peptide. MHC presenting antigenic peptides (aMHCp) bind with moderate affinity and lead to activation of the mature T-cell. MHC molecules also present self-peptides (sMHCp) derived from endogenous proteins. sMHCp bind with weak affinity to the TCR and cannot activate the cells by themselves. TCR engagement to sMHCp, however, leads to positive selection of immature T-cells in the thymus and T-cell survival in the periphery. In the context of the immune response, both types of peptides are copresented by the APC. In this case, self-peptides aid in the recognition of agonistic peptides by the T-cell.[9-12] These features of the TCR allow the development of sophisticated MIRR triggering models (pseudodimer and permissive geometry models), which have not been extended to the BCR or FcεRI.

Homoclustering

Early stimulation experiments have shown that bivalent anti-TCR-CD3 antibodies can activate T-cells, whereas monovalent Fab fragments of these antibodies fail to do so, although they do bind to the TCR-CD3 complex.[13,14] The same holds true for the FcεRI.[6,7] Later on, soluble MHCp monomers or multimers were prepared and, as expected, only the multimeric forms could trigger the TCR.[4,5] These experiments indicate that the ligands for the TCR and FcεRI have to be multivalent, in order to be functional. This implies that two or more receptors 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 homoclustering model of MIRR activation[15] (Fig. 1). This model has also been named cross-linking, aggregation or multimerization model. Support for this model was provided by imaging approaches that have shown that MIRRs cluster on the cell surface upon multivalent ligand binding and that TCR microclusters are formed upon interaction with an APC.[16] Indeed,

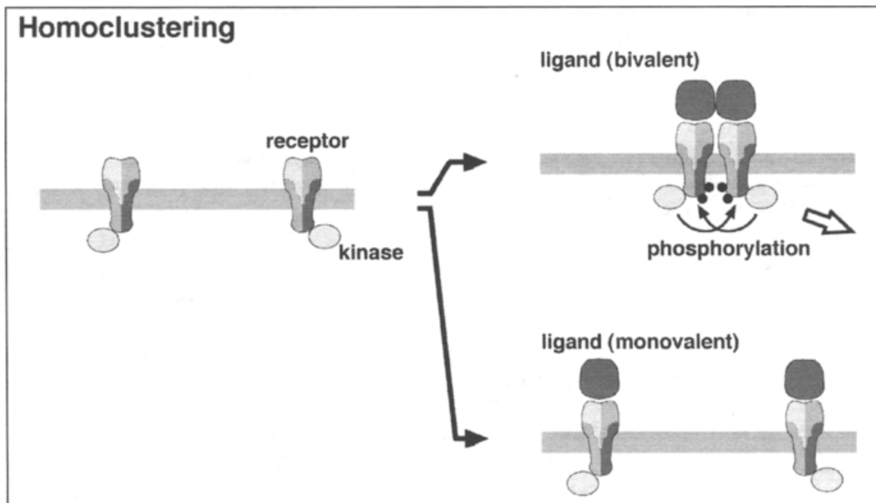

Figure 1. Homoclustering model.Monovalent receptors with associated kinases are individually expressed on the cell surface and randomly distributed. Stimulation by bi- or multivalent ligand leads to clustering of the receptors, since the ligand simultaneously binds two or more receptors. In these clusters, the kinases can transphosphorylate each other and the receptors. This represents the first step in the activation of the cell. Monovalent ligands do not cluster the receptors and thus cannot induce phosphorylation and activation. Black dots represent phosphorylated tyrosine residues. The open arrow shows activation of downstream signaling cascades.

these TCR microclusters have been shown to be the structures that initiate and sustain TCR signaling (see Chapter by Dustin).

Within the homoclustering model, each monomeric receptor is supposed to be associated with a kinase, that needs cross-wise phosphorylation for its full activity. In addition, it is possible that due to sterical reasons the kinase cannot phosphorylate the receptor that it is bound to. As random encounters of two receptors by diffusion are infrequent, the amount of phosphorylated receptor stay below the activation threshold of the cell. If a bi- or multivalent ligand simultaneously binds to at least two MIRRs and brings them into close proximity, the kinases are enabled to transphosphorylate each other and the neighboring MIRR. Thus, the cell becomes activated. A variant of the homoclustering model is the signaling chain homooligomerization (SCHOOL) model,[17] which is subject of chapters by Sigalov.

Concerning B-cell activation, studies in the 80th have shown that the higher the valency of an antigen is, the better is the B-cell response (the immunon model).[18] In addition, monovalent anti-BCR antibody Fab fragments do not trigger the BCR, whereas bivalent antibodies do.[8] Therefore, the homoclustering model also seems to apply for the BCR. This assumption was recently challenged by the finding that a monovalent antigen could activate the BCR.[8] Therefore, the triggering mechanism for the BCR is still a mystery.

The homoclustering model is widely accepted, but has several caveats. First, recent publications have questioned that the receptors are equally distributed on the cell surface.[19-22] Second, there exist antireceptor antibodies that do dimerize the receptor but do not elicit a full activation response.[6,23,24] Therefore, an alternative model has been put forward, known as the permissive geometry model[25] (Chapter by Minguet and Schamel).

The physiological ligand for the TCR is not a soluble but membrane-bound MHCp molecule. The MHC molecules of class I and II are most likely multimeric on the surface of the APCs.[26,27] Therefore, the requirement of the homoclustering model for multivalent ligand seems to hold true. In addition to a possible aMHCp, an APC also presents sMHCp. The affinity (or half-life) of an sMHCp-TCR interaction is too low (or short) to yield an effective sMHCp-TCR complex. This is the reason why sMHCp do not activate T-cells. Interestingly, an APC can activate the T-cell if it presents only around 10 aMHCp molecules that are intermingled within 10,000-100,000 sMHCp.[9] In these situations, the probability of two antigenic peptides being present on two adjacent MHC molecules is negligible. Thus, one has to postulate that TCRs can be activated by MHCp heterodimers in which one MHC molecule is loaded with the antigenic peptide and the second with a self-peptide.[9-11] Indeed, recent work has shown that it is possible to activate T-cells with those MHCp heterodimers.[12] The homoclustering model cannot explain the activating effect of the MHC heterodimers (aMHCp-sMHCp).

Heteroclustering

Variable portions of the MHCp complex, representing the peptide sequences and the adjacent regions of the MHC molecules, bind to the variable immunoglobulin domains of the TCRα/β.[28,29] In addition, constant regions of the MHC class I and class II molecules bind to the CD8 and CD4 coreceptors, respectively.[30] The simultaneous binding of MHCp to the TCR and the coreceptor leads to a heteroclustering of TCR with CD4 or CD8. Interestingly, the cytoplasmic tails of CD4 and CD8 have been shown to interact constitutively with the Src-family kinase Lck[31] that when recruited to the TCR, could phosphorylate the receptor (heteroclustering model, Fig. 2).

Formation of TCR-CD4 heterodimers by chimeric antibodies leads to TCR triggering,[32] enforcing the heteroclustering model. In favour of this model are also findings that demonstrate a requirement for the coreceptor in T-cell activation.[33] However, in other experimental systems the coreceptor is not needed.[34] The reason for this discrepancy is not clear but might be related to the affinity and amount of ligand used. The findings that a substantial proportion of TCRs is constitutively associated with coreceptors[35] and that monomeric MHCp does not activate T-cells,[4,5] argue against the heteroclustering model. Also, this model cannot explain why T-cells are triggered by anti-TCR-CD3 antibodies.[13,14] However, it seems likely that co-engagement of

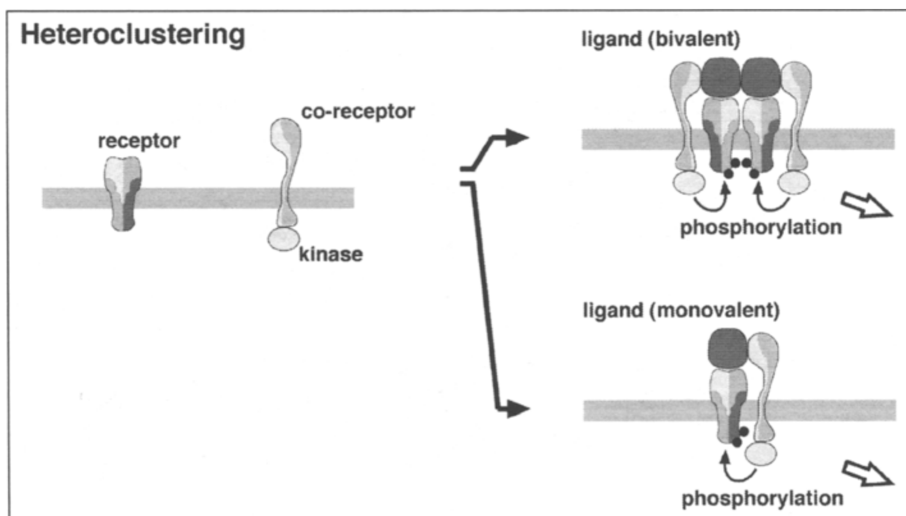

Figure 2. Heteroclustering model. TCRs are not constitutively associated to kinases, but the coreceptors CD4 and CD8 are. Multimeric and monomeric MHCp ligands simultaneously engage the TCR and the coreceptor. Hence, the kinase is brought into the vicinity of the TCR allowing phosphorylation with subsequent activation of the cell. Notably, the heteroclustering model does not apply to the BCR or the FcεRI.

the TCR-CD3 with CD4 or CD8 lowers the threshold for T-cell activation by augmenting the avidity of MHCp binding to the T-cell and/or by recruiting additional kinases (and possibly other signaling proteins) to the receptor.

The antigens for the BCR and the FcεRI can be multimeric substances of nearly any structure, shape and geometry. Therefore, signaling cannot be initiated by clustering of these MIRRs with a second receptor on the cell surface. Nevertheless, there exist coreceptors for these MIRRs that can either suppress or augment the signal emitted by the BCR or the FcεRI. Antigen that is already bound to an antibody can promote heteroclustering with FcγRs which bind to the constant regions of the antibody. FcγRs associate with the SH2 domain-containing protein tyrosine phosphatase SHP-1 and therefore weaken the signal emitted by the BCR or the FcεRI. In contrast, antigen that is bound by complement can simultaneously bind to the antigen receptor and the complement receptor CD19/CD21, resulting in an augmentation of the signal.

Pseudodimer Model

With the beginning of the new millennium it became clear that the sMHCp complexes play a role not only in positively selecting T-cells in the thymus but also in T-cell activation.[9-11] They are accumulated in the center of the immune synapse and enhance recognition of aMHCp. Final proof of their stimulating activity came from the finding that aMHCp-sMHCp heterodimers can trigger the TCR.[12] This cooperativity between antigenic and self-peptides in T-cell stimulation together with the data concerning the role of the coreceptor CD4 lead to the development of the pseudodimer model (Fig. 3).[9] In this model, one TCR binds to aMHCp, whereas the second TCR binds to sMHCp. Importantly, the low-affinity sMHCp-TCR interaction is strengthened by the binding of MHC to CD4. At the same, time this CD4 molecule interacts with the first TCR. Indeed, crystallography studies suggested that in a trimeric CD4-pMHC-TCR complex the cytoplasmic tail CD4 cannot come close to the TCR.[30] Thus, the Src-family kinase that is associated to the CD4 cytoplasmic tail can phosphorylate only the second TCR. The strength of this model is that it takes into account the effects of the self-peptides. However, the model cannot

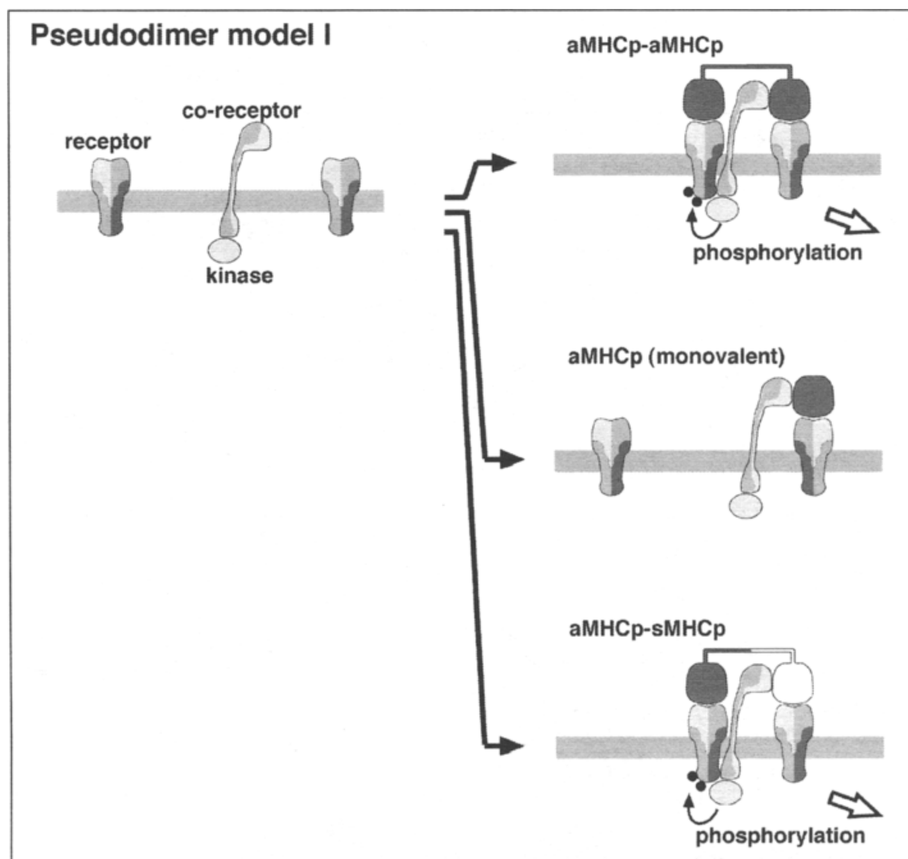

Figure 3. Pseudodimer model I. The TCR is monovalent and not bound to the kinase. In contrast, the coreceptor CD4 is associated to a Src-family kinase. Upon bi- or multivalent MHCp-binding TCRs become clustered and CD4 that binds to both the first TCR and the second MHCp, is recruited, allowing TCR phosphorylation (upper panel). Monovalent MHCp would be inactive (middle panel). At low antigenic peptide concentrations, aMHCp-sMHCp heterodimers are presented by the APC. This allows formation of a pseudodimer:one TCR binds aMHCp and the second TCR binds sMHCp (lower panel). The weak sMHC-TCR interaction is enforced by CD4 that binds to sMHCp and the first TCR. Abbreviations: antigenic peptide-MHC complex, aMHCp; self-peptide-MHC complex, sMHCp.

explain the coreceptor-independent stimulation of T-cells. In a new version of the pseudodimer model, CD4 interacts with aMHCp and the TCR bound to sMHCp (Fig. 4).[12]

Homo- and Heteroclustering and Lipid Rafts

Ligand-induced clustering of the MIRRs could also play a role in changing the lipid environment of the receptor within the plasma membrane, thereby initiating signal transduction. Central to this concept are the so-called lipid rafts (see Chapter by Dopfer et al). They represent microdomains that are enriched in sphingolipids and cholesterol and devoid of phospholipids.[36] Membrane proteins are thought to be located either inside (kinases of the Src-family) or outside (e.g., CD45) the lipid rafts. Early studies have suggested that most of the resting TCR, BCR and FcεRI receptor complexes are located outside lipid rafts. Upon ligand binding, some of them move into the rafts and become phosphorylated (Fig. 5A).[37-40] When lipid rafts are disrupted by

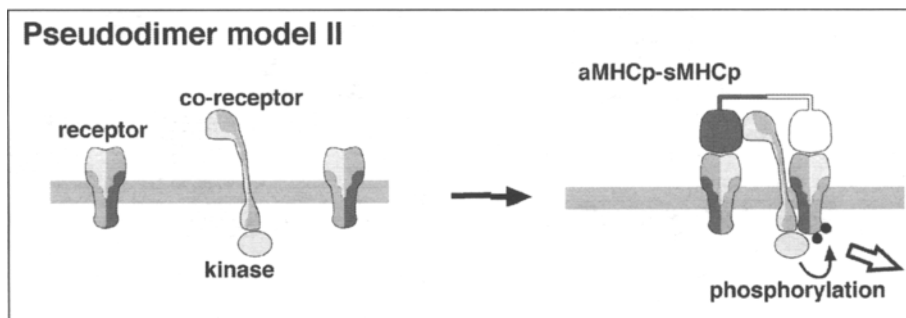

Figure 4. Pseudodimer model II. In the latest version of the pseudodimer model, the coreceptor CD4 binds to aMHCp but not to sMHCp. At the same time, CD4 binds to the second TCR that also associates to the sMHCp. Consequently, kinases bound to the cytoplasmic tail of CD4 phosphorylate the second TCR.

extracting cholesterol from the membrane, stimulation of the MIRRs does not lead to an increase in tyrosine phosphorylation, suggesting that activation of the treated cells is blocked.[41] Likewise, mutants of Src-kinase family members unable to localize to lipid rafts do not initiate signaling upon receptor triggering.[42]

The fundamental question of this model is the molecular change that ligand binding exerts on the receptor, making it move to the lipid rafts. Since signal processes are not required, as shown by inhibiting Src-kinases or disrupting the actin cytoskeleton.[39,43,44] This molecular change might be one of the crucial steps of signal initiation. For the FcεRI, an important role of its transmembrane region has been shown, indicating that interactions with membrane lipids might be critical.[45] Therefore, it can be postulated that monomeric receptors have a low affinity for lipid rafts. Homoclustering of the receptors by multivalent ligands increases their avidity for rafts so that the receptor oligomers move to lipid rafts.[46]

In contrast, other research groups have found that the receptors are already present in lipid rafts prior to stimulation.[43,47] In this case, it is postulated that receptor and kinase are in different separated rafts and that homoclustering of the receptors leads to raft coalescence that also includes kinase-possessing rafts (Fig. 5B).[48] Thus, receptors and Src-kinases come into contact only upon multivalent ligand binding.

Alternatively, CD4 and CD8 might be present in the same lipid rafts as the Src-kinases[49] and therefore heteroclustering with the TCR would bring the MIRR in contact with the kinase (Fig. 6). As discussed above, monomeric ligand cannot activate the MIRRs making the heteroclustering model rather unlikely.

Proving or disproving the "lipid raft localization" models is hampered by the current technical difficulties in studying lipid rafts[50] that might be more dynamic than previously appreciated.

The PreTCR and PreBCR

The pre-antigen receptors (preBCR and preTCR) are expressed on immature lymphocytes and play a role in their development to mature cells. Most likely, these receptors signal in the absence of ligand. Studies of this constitutive signal have shown that both receptors localize to lipid rafts without the need for ligand binding.[51,52] In addition, they might be present in preformed oligomeric structures.[53,54] These findings provide further support for the clustering models of MIRR triggering.

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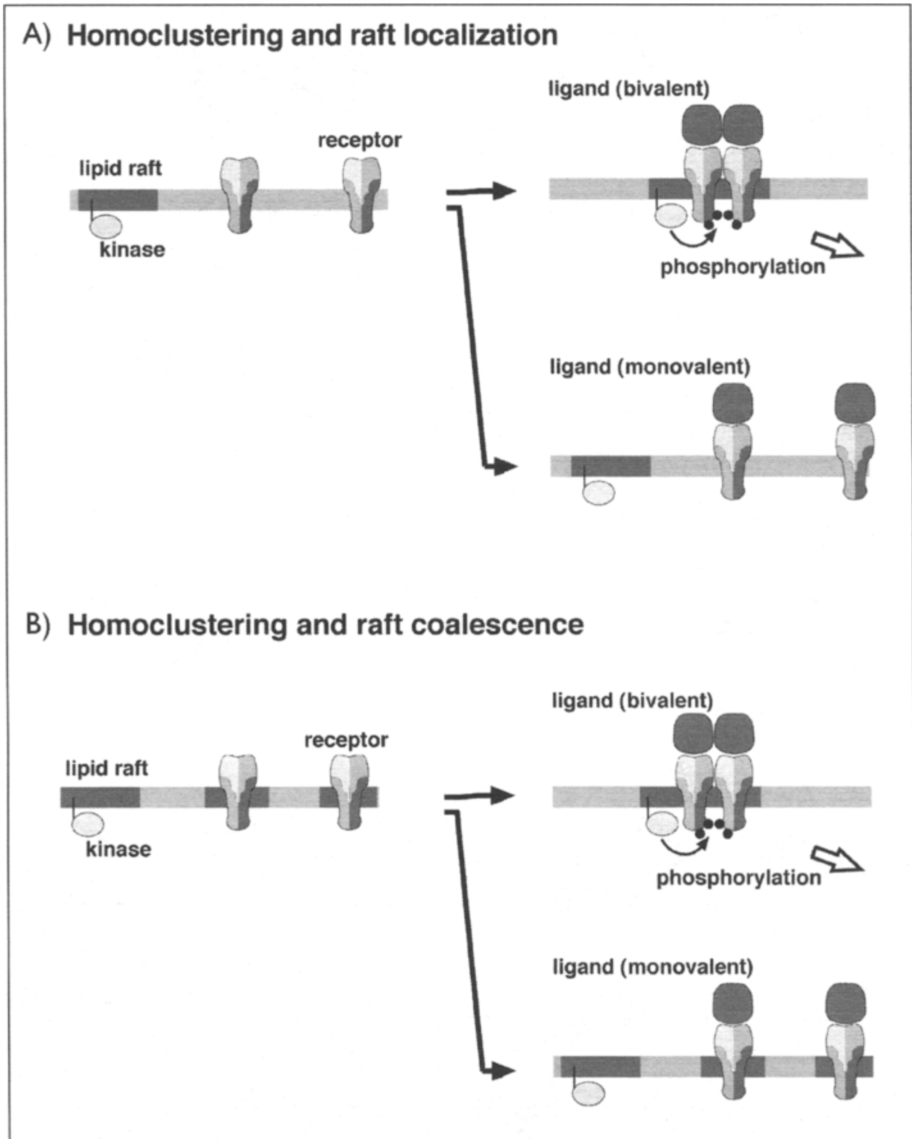

Figure 5. Homoclustering and lipid rafts. A) In the absence of ligand binding, monomeric MIRRs are located outside the cholesterol-rich microdomains, the so-called lipid rafts, due to low affinity of the interaction between MIRR and raft. Because of their fatty acid modification, the Src-family kinases are located inside the rafts. Homoclustering of the receptor by bi- or multivalent ligand increases the avidity of the receptor-raft interaction. Therefore, clustered MIRRs move to the rafts where they become phosphorylated. Monovalent ligands do not induce MIRR clustering and do not lead to colocalization of the receptor with the kinases. B) Alternatively, receptors and kinases are constitutively raft-associated but partitioned into distinct rafts. Homoclustering of the MIRR-associated rafts by bi- or multivalent ligand binding induces raft coalescence including nonreceptor rafts. Thus, the kinases come into proximity to the MIRRs. Again, monomeric ligands are inactive.

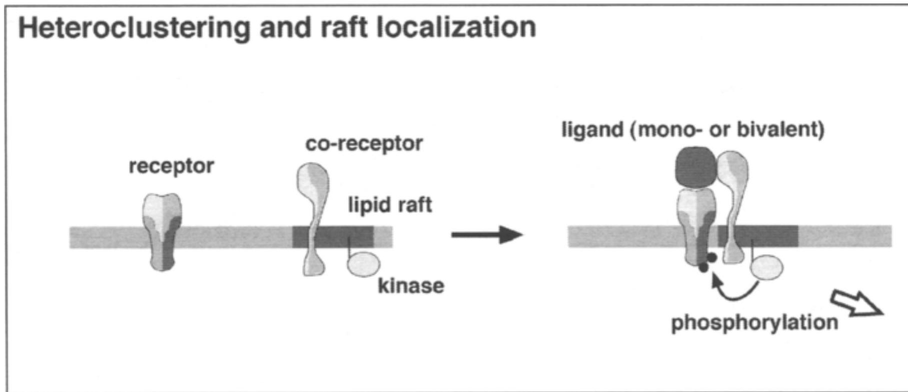

Figure 6. Heteroclustering and lipid rafts. Similar to that suggested for the heteroclustering model (Fig. 2), the ligand (MHCp) binds to the TCR and the coreceptor and simultaneously brings the TCR into the vicinity of the coreceptor CD4 or CD8 . In the "heteroclustering model for raft localization", the coreceptors are localized to the same rafts as the Src-kinases but do not directly bind to the kinases. If TCRs and kinases become coclustered by mono- or multivalent MHCp (not shown), the receptor becomes phosphorylated, activating signaling pathways.

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