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# Changing separating distances between immune receptors as a sensitive mechanism regulating T-cell activation

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It is widely believed that T-cell activation typically requires T-cell receptor (TCR) to form clusters, a necessary prerequisite for activation of proximal signaling proteins. How close the receptor molecules should approach each other to trigger the signaling? Is this just receptor gathering or whether the clusters of the receptors have internal organizational infrastructure that can lead to variations in the proximal signaling?

Because peptide-MHC (pMHC) ligands recognized by TCR are presented on the surface of other cells, positioning of pMHC ligands on the cell membrane may also influence TCR distribution at the interface. MHC proteins have been found to form molecular assemblies with each other and with adhesion molecules on the surface of live target cells.[1] The engagement of adhesion molecules promotes MHC accumulation at the contact surface and facilitates clustering and activation of many TCR augmenting the sensitivity of antigen recognition by T-cells.

Understanding the role of separating distances between MHC and their effect on the sensitivity and quality of T-cell responses requires modeling of MHC clusters and testing their ability to initiate T-cell activation. Even two pMHC proteins that are brought together with a short rigid spacer show the ability to cooperate in activating T-cells.[2] A longer spacer results in loss of pMHC cooperation in the dimer. Packing pMHC proteins with defined biological activities on the surface of nanoparticles allows attaining the close pMHC-pMHC proximity and multivalency in nanoparticles-pMHC conjugates.[3] Such conjugate binds strongly to the surface of T-cells regardless of T-cell specificity in CD8-dependent manner, but induce T-cell response when at least a single agonist pMHC per nanoparticle is present with all others being non-stimulatory. Thus, very few agonist pMHC non-stimulatory ligands displayed in close proximity along with non-stimulatory pMHC can effectively cooperate and potently stimulate T-cells. Nanoparticles bearing only non-stimulatory pMHC bind to the T-cells almost as well as agonist pMHCs nanoparticles, but do not elicit detectable TCR signaling. This is in mark contrast to tetramers containing non-stimulatory pMHC proteins that practically do not interact with T-cells.[4] Most likely, separating distances and the orientation of pMHC subunits within tetramers are different precluding the ability of pMHC proteins to cooperate and to promote CD8-pMHC interactions. We propose that orientation and the separating distances between MHC monomers attached to nanoparticles mimic those in their natural environment. Because separating distances

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between pMHC on the nanoparticles can be varied, nanoparticles-pMHC conjugates represent a tool for examining the effect of proximity between pMHC molecules on triggering of TCR-mediated signaling. Presence of non-stimulatory and agonist pMHC on glass-supported lipid bilayers never revealed cooperative stimulation of T-cells exposed to such bilayers. While pMHC molecules incorporated into the bilayer can freely diffuse, they do not form clusters in which the separating distances between the pMHC molecules would be short enough to allow agonist and non-stimulatory pMHC to cooperate facilitating response against the former.

Although the cooperation between non-stimulatory and agonist pMHC attached to the same nanoparticles is evident, it remains to be determined whether the same pMHC would cooperate when placed on different nanoparticles. It is even more uncertain and interesting whether antagonist pMHC ligands need to be presented on the same or different nanoparticles with an agonist pMHC to exercise their inhibitory activity. It has not been clear thus far whether antagonism require TCR bound to agonist and antagonist pMHC ligand to be a close proximity or can distal communication between activating and inhibitory signaling results in antagonism.

T-cells can still form microclusters containing TCR and activated proximal signaling molecules when they are stimulated with randomly distributed agonist pMHC and adhesion molecules on glass-supported bilayers at low density.[5,6] This suggests that initial productive engagement of a very few TCR on the T-cells can lead to recruitment of additional TCR molecules to the point of initial engagement and TCR-pMHC microcluster formation. In fact, blocking the TCR-pMHC interactions with MHC-specific antibodies precludes the formation of new microclusters, but does not destroy existing microclusters suggesting that TCR and MHC molecules are very tightly packed within microclusters. TCR-coreceptor co-clusters, presumably of a smaller size, are already present on activated T-cells[7] and likely facilitate the formation of a larger molecular assemblies containing signalosome. Additional TCR recruited to the microcluster may not be necessarily bound to agonist pMHC but their close proximity to productively engaged TCR is thought to result in their activation as well. Thus, the signal may spread from few TCR bound to agonist pMHC to other TCR within microcluster.[3] Signaling spread implies that microclusters may have internal infrastructure, which could change during activation process. Although the mechanism of this process is not clear, we propose that a limited amount of activated proximal signaling proteins is sufficient to “activate” a larger number of TCR within individual microclusters. We also propose that the strength and the quality of initial engagement of a limited number of TCR may determine the net result of activation and deactivation of proximal signaling proteins and the formation of either activating of non-activating microclusters. The integration of the signaling occurring in individual microclusters could be translated to propagation of downstream signaling of various strength and quality that can regulate effectiveness and flexibility of T-cell responsiveness.

Thus, shortening the distance between TCR-co-receptor molecules is an important mechanism necessary for the establishing a platform for accumulation and integration of signals from many different TCR. Variations in the separating distances may, therefore, serve to regulate T-cell responses at various stages of T-cell differentiation to diverse TCR ligands.

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