Abstract

Arguably, the most studied cell types of immune system are T-cells. They are key players of adaptive immunity responsible for targeted action against pathogens or other danger signals. Due to their central importance, any alteration in the regulation of their activity leads often to immunopathology. Thus, the knowledge how to harness their bio-destructive effector functions is of critical importance. Up today, there is only limited consensus on the nature of molecular mechanisms controlling the initiation of T-cell activation. When T-cell receptor (TCR) recognizes its cognate antigen presented on antigen presenting cell (APC), the activation signal is transmitted through the plasma membrane and subsequent phosphorylation of cytoplasmic chains of TCR complex ensues. This is commonly considered as the first biochemical sign of T-cell activation, the process called TCR triggering. How the activation signal gets into the cell and which molecular mechanisms control TCR triggering are two fundamental, yet still unanswered questions. In this study we focused mainly on the latter one. Working within this experimental framework, we investigated three particular problems. The first one concerns the spatiotemporal organization of critical signalling molecules before and after TCR engagement in the context of lipid microdomains that, as we posited, act as an important membrane organizational principal in the regulation of TCR triggering. We mainly focused on Lck kinase which is considered as the main signal-generating element initiating TCR signalling. Using a biochemical approach, we determined membrane distribution of the active form of Lck ($pY394^{Lck}$), the key factor in TCR triggering. In this context we not only showed that in naïve T-cells a limited pool of $pY394^{Lck}$ almost exclusively partition into high molecular weight complexes, but also that after TCR engagement, this pool is significantly increased together with its redistribution to lipid microdomains. Unfortunately, quantitative discrepancies between these and previously reported studies, where $pY394^{Lck}$ levels were found significantly higher and invariant after TCR activation, lead to different conclusions about the role and steady-state levels of active Lck in T-cells. Because $pY394^{Lck}$ drives TCR triggering, the question of the basal level of $pY394^{Lck}$ in naïve T-cell is of central interest. To reconcile these results, in the second line of our research, we provided evidence that most of these discrepancies stemmed from inconsistencies within technical procedures used for sample preparations and that highly saturated levels of $pY394^{Lck}$ were results of uncontrollable spontaneous activation of Lck during cell lysis. Lastly, we previously demonstrated that activation-induced Lck redistribution within plasma membrane was critical for delivery of its function, yet, the mechanism has been unknown. Thus, in the third line of research, we identified, for the very first time, the transient formation of Lck-RACK1-cytoskeleton complexes able to affect Lck redistribution process. The involvement of cytoskeleton in the spatiotemporal organization of signalling molecules provides yet another level of complexity in the regulation of TCR signalling. Taken together, this study provides strong evidence for the contribution of membrane organization to spatiotemporal regulation of Lck activity as well as other signalling components involved in the initiation of TCR signalling.