Investigating the parameters influencing the rate of phosphorylation of CD3 subunits by Lck.

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T cells are multifunctional adaptive lymphocytes that are crucial cells in the immune system. They are involved in a vast amount of processes ranging from the killing of virally infected and cancerous cells to regulation of immune responses and prevention of autoimmunity. The T cell Receptor (TCR) is the most important feature of the T cell as it can specifically recognise peptides bound to major histocompatibility complexes (pMHC) and distinguishes between 'self' and 'non-self' and also between dangerous and harmless molecules. For this reason the TCR is pivotal to our understanding of how these principal cells function.

The extracellular pMHC binding subunits of the TCR do not contain sufficient intracellular portions to facilitate downstream signalling independently, therefore they must associate with CD3 complexes on the inner plasma membrane in order to initiate intracellular signalling. The CD3 complex consists of six domains, a single δ and γ chain and two ε and ζ chains. Their unstructured cytoplasmic tails contain a total of 10 immunoreceptor tyrosine-based activation motifs (ITAM). Upon TCR binding to pMHC these ITAM motifs on CD3 become phosphorylated by Lck, creating binding sites for adaptor proteins such as ZAP70. This binding initiates sequential signaling, propagating downwards from the membrane and ultimately leading to T cell activation.

Using techniques such as bilayer interferometry and surface plasmon resonance we are defining the parameters that influence the rate of phosphorylation and subsequent activation of the CD3 subunits by the kinase Lck. There is evidence of an ionic interaction between the basic residue-rich sequence in the CD3ε chain and acidic residues in the unique domain of Lck (1). This binding may tether Lck to the CD3 complex and result in an increased rate of phosphorylation of local ITAM motifs (2). We have found there is no interaction between Lck and the CD3ζ chain however there is a weak interaction with CD3ε, although both were efficiently phosphorylated. The weak interaction observed using the full-length protein could indicate that native Lck adopts a conformation that precludes binding. Therefore we analysed the mechanism of action of a series of drugs developed by InterK that augment the kinase activity of Lck. The addition of these drugs resulted in a high level of Lck binding to the CD3ζ subunit, suggesting the drugs may alter the conformation of Lck to allow binding.

The results so far indicate the interaction between Lck and the CD3 subunits is dependent on the conformational states of Lck, which could be manipulated by the Lck augmenting drugs. In the future we aim to define this interaction and investigate the effect of this interaction on local phosphorylation rates and downstream signalling and T cell activation via ZAP70. Understanding the mechanism of action between the TCR and Lck is pivotal to our understanding of T cells and for the development of cancer immunotherapies, primarily for the optimization of adoptive cell transfer systems.

Bibliography

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