DISSECTING THE PHOSPHOPROTEOME OF THE DAG-DEPENDENT SIGNALLING PATHWAY IN RESPONSE TO ANTIGEN PRESENTATION

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Lipid second messengers are exquisite signal modulators that finely tune the T cell response to send it to proliferate, get into anergy or apoptosis. Diacylglycerol (DAG) is an important regulator of this response as it mediates the activation of specific targets, such as RasGRP1 and PKCtheta. The key enzymes that control the pool of DAG are the diacylglycerol kinases (DGK). They have been shown to play a critical role as negative regulators of the T cell response although the mechanism of regulation is not fully understood. We decided to center on the impact of DAG-dependent signals on the control of the TCR-driven pathways triggered during a physiological stimulation of T cells using antigen-presenting cells (APC). We are thus setting up a phosphoproteomic platform to monitor specific changes in the phosphoproteome directly emanating from DAG signalling pathways. To this end, specific silencing of DGKs is carried out and the phosphorylation profile upon TCR engagement monitored. We compared different methods of enrichment of phosphoproteins with anti-phosphotyrosine antibodies as well as methods of enrichment of phosphopeptides comparing the TiO_2 and IMAC methodologies. In a first instance, evaluation of phosphoprotein enrichment is performed by western blotting upon stimulation with PMA, CD3/CD28 and then applied to more physiological stimuli (APC pulsed with superantigen). This optimum phosphoprotein enrichment method is then coupled to phosphopeptide enrichment step combining CID/ETD fragmentations for greater identification confidence (Navajas R, et al, *Methods Mol Biol, 2010*). This approach will provide a temporal read out of signalling pathways controlled by TCR and dependent on DAG.