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Effect of N-myristoylation on ontogenesis and function of T lymphocytes

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N-myristoylation refers to an irreversible protein modification through the covalent attachment of myristate, a C14 fatty acid, to the N-terminal glycine of proteins, promoting protein-protein and protein-membrane interactions. Myristoyl-CoA:protein N-myristoyltransferase (NMT) is the enzyme catalyzing this modification. N-myristoylated proteins are involved in a variety of cellular processes including proliferation, differentiation and apoptosis, and have been shown to be essential for mouse embryogenesis. Increased expression and activity of NMT has been demonstrated in several malignant tumors and its presence is essential for the viability of numerous human pathogens. For selected proteins, the impact of myristoylation on their function has been investigated in vitro. However, it is still largely unknown how myristoylation influences cellular processes in vivo.
Firstly, to be able to investigate N-myristoylation in vitro a non-radioactive, easy and robust ELISA-like method was developed. It is based on the myristoylation reaction between a FLAG-tagged peptide and an azido analog of myristoyl-Coenzyme A. After the reaction, the acylated peptide is coupled to phosphine-biotin via the Staudinger ligation, captured by plate-bound anti-FLAG antibodies and detected by streptavidin-peroxidase. Using this assay, it could be shown that thymus has a prominent NMT activity. Further studies were therefore focused on the role of myristoylation in T cell development. To this end, mice with floxed Nmt1, Nmt2 and both genes in combination were crossed with mice expressing Cre under the control of the proximal Lck promoter which is activated early during T cell development. Absence of Nmt activity led to two major impairments during T cell development: a partial block at the double negative 3 stage and a block during the transition from double positive to CD4+ and CD8+ single positive T cells, a process strongly dependent on αβ TCR signaling. γδ T cells, which lacked myristoylation, were not affected. Analysis of the TCR signaling ex vivo in NMT-deficient thymocytes revealed that N-myristoylation is crucial for both early TCR signaling events like the activation of CD3ζ and Zap70, and distal TCR signaling, like the activation of Erk and the expression of CD69. Similarly, upon stimulation with phorbol 12-myristate 13-acetate (PMA), late TCR signaling and cytokine secretion were impaired in the double knockout mice.
When investigating the myristoylation of Lck, a kinase essential for the initiation of the TCR signaling, it was found not to be myristoylated and to be mainly localized in the cytoplasm in the double knockout mice. As a consequence, non-myristoylated Lck cannot reach the plasma membrane and phosphorylate CD3ζ chains for the initiation and propagation of the TCR signaling.

In summary, it could be demonstrated that myristoylation is important for T cell differentiation and the transmission of pre-TCR and TCR signals upon TCR-dependent and independent stimulation.