ImmunoTools *special* Award 2018

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Diacylglycerol kinase alpha (DGKα) contribution to CD4$^+$ T cell plasticity

T lymphocytes rely on the generation of diacylglycerol (DAG) to commit to correct activation and differentiation. DAG is a lipid second messenger generated in response to T cell receptor (TCR) and costimulatory signals. It binds to proteins that encode Protein Kinase C (PKC) 1 (C1) conserved domains as RasGRP1 and PKCθ, facilitating their localization at the immune synapse and the activation of Ras/ERK (extracellular signal-regulated kinase) and PKCθ-derived cascades. These pathways lead to transcriptional activation of AP-1 (activator protein-1) and of NFκB (nuclear factor kappa-light-chain-enhancer of activated B cells), which in turn foster the expression of certain genes with response elements to these promoters, including the rapidly induced type II C-lectin membrane receptor CD69, as well as CD25, the late activation marker that confers high affinity for IL-2 in activated T cells.

DAG abundance, and thus its signaling in T cells, is tempered by the α and ζ isoforms of the DAG kinase (DGK) enzyme family, which transform DAG into phosphatidic acid (PA) and limit DAG-regulated functions. In tumors, recruitment of immune cells with suppressive capacity and the particular characteristics of the tumor microenvironment promote T cell conversion to a hyporesponsive, anergic state in which the TCR is activated deficiently. Certain DGK isoforms, especially DGKα, are highly expressed in tumor-infiltrating lymphocytes (TIL) and in tumors, suggesting a contribution of this enzyme to the mechanisms that regulate metastasis and immune evasion. DGKα blockade might reinstate T cell attack on tumors and block tumor cell growth directly, making its targeting a promising strategy for coping with cancer.

The intensity of TCR-triggered signals in the periphery modulate the equilibrium between effector and regulatory T cell populations by mechanisms that remain largely unresolved. Commitment to the T regulatory (Treg) cell lineage is regulated by AKT kinase and Forkhead box O (FoxO) family and requires the upregulation of the transcription factor Foxp3. It also has been described that blockade of ERK-derived signaling facilitates commitment to a Treg phenotype in detrimental of the effector T helper (Th) 17 cell population development, suggesting that ERK pathway promotes Th17 cell polarization and suppresses Treg cell differentiation. The development of different T cell subsets is regulated by a combination of the

expression of a master transcriptional regulator and the phosphorylation of a particular STAT protein stimulated by distinct cytokines. IL-12 and IFNγ promote Th1 cells through activation of T-bet/STAT4. TGFβ and IL-2 stimulate Treg cell development through its activation of Foxp3/STAT5. Additionally, TGFβ drives Th17 cell differentiation in the presence of IL-6 through activation of RAR-related orphan receptor (ROR)γt/STAT3.

DGKα limits Ras/ERK activation through negative regulation of RasGRP1. Previous studies in Prof. Dr. Mérida's lab demonstrated that, in $CD8^+$ T cells, DGKα expression is transcriptionally repressed through the PI3K/AKT-mediated phosphorylation of FoxO in response to TCR and IL-2. These studies helped to link FoxO-dependent transcriptional regulation to the intensity of ERK-regulated signals in cytotoxic T cells.

Our project now aims to investigate if the control of DGKα expression in $CD4^+$ T cells is important to regulate the plasticity of different populations in periphery. To this end, we are investigating the kinetics of DGKα expression in *ex vivo* models of Th1, Treg and Th17 differentiation in mouse and human cells. At the same time and using DGKα-deficient mice we pursue to demonstrate that polarization of naïve $CD4^+$ T cells into effector/regulatory T cell populations is altered in the absence of this enzyme. Our final hypothesis aims to demonstrate that DGKα expression in T cells is required to provide the plasticity necessary to differentiate into distinct lineages. For this reason, the diverse offer of reagents from ImmunoTools would be of great help in the development of the different assays that we would like to perform to reach our research interests.

ImmunoTools *special* Award for **Javier Arranz-Nicolás** includes 25 reagents

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recombinant human cytokines: rh IFN-gamma, human TNF-a

human ELISA set (for one 96 plate): human IFN-gamma, human TNF-a

recombinant mouse cytokines: rm IL-1beta, rm IL-6, rm IL-7, rm IL-17A, rm TNF-a

mouse ELISA set (for one 96 plate): mouse IL-17A, mouse TNF-a

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