ImmunoTools *special* Award 2017

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DGK zeta limits cytokine-dependent expansion of CD8+ T cells with broad antitumor capacity

The immune system defends the body from foreign invaders. In cancer, tumors disguise as self-body cells and evade immune attack. For this reason, it is important to identify the mechanisms that stop T lymphocytes from recognize and destroy tumors.

The diacylglycerol kinases (DGK) negatively regulate diacylglycerol (DAG)-mediated signaling by catalyzing the conversion of DAG to phosphatidic acid. In T lymphocytes two DGK isoforms: DGKα and DGKζ limit activation of the Ras-ERK pathway by metabolizing the DAG generated after TCR triggering *(Merida, 2015)*. Elevation of the two isoforms is observed when T cells expressing Chimeric Antigen T cell Receptors (CAR T cells) invade solid tumors, suggesting that DGK limit antigen-dependent tumor elimination *(Riese, 2013)*. In agreement, DGKα expression in renal tumor-infiltrating $CD8^+$ T cells correlates with impaired cytotoxic responses *(Prinz, 2012)* and DGKζ deficient mice develop smaller tumors in antigen dependent models *(Riese, 2011)*.

Currently the effectiveness of IL-2 and IL-15 immunotherapy has been proved to boost antitumor immune response. IL-2 or IL-15 cytokines drive expansion and differentiation of cytotoxic $CD8^+$ T cell subsets that express natural killer receptors and eliminate targets via antigen-independent killing. Using DGKα and DGKζ deficient mice, we are investigating antitumor capacity of a CD8 T cell population with high expression of the IL-2/IL-15 common β chain (CD122) that respond to IL-2/ IL-15 independently of antigen recognition. DGKζ but not DGKα deficient mice show an increase in this population and enhance IL-2/IL-15 responses in an antigen-independent manner. We have recently demonstrated using an aggressive BALB/c-derived B cell lymphoma (A20 cells) that DGKζ deficient mice develop smaller subcutaneous tumors and resolve it faster than wild type mice *(Andrada, 2017)*.

Ex vivo incubation of $CD8^+$ T cells with IL-2 or IL-15 in the absence of antigen stimulation promotes differentiation of an innate-like cytotoxic cell population. These

cells called cytokine-induced killers cells have a potent antitumor activity in mouse models and in human clinical assays. We have analyzed the function of DGKζ in the generation of cytokine-induced killers cells. We found that DGKζ deficient cells differentiated with IL-2 presented a more cytotoxic profile than WT cells. In sight of these data, we evaluated the in vivo anti-tumor capacity of these in the A20 model. We found that mice treated with DGKζ deficient cytokine-induced killers cells present a more rapid regression of tumors than mice treated with control cells. Currently we are analyzing the role of DGKζ in the immune response against other low antigenic and more aggressive syngenic tumors models. We are particularly interested in melanoma models in which IL-2 is approved for human treatment. Our preliminary results indicate that DGKζ deficient mice present a better immune response in this model. We want to investigate how pharmacological manipulation of DGKζ combined with cytokine treatment could be employed as cancer immunotherapy.

We are interested in testing ImmunoTools cytokine and chemokines to further investigate how DGKζ controls the response of T cells to other cytokines and its role in the control of T cell tumor recruitment in response to chemokines. We want to demonstrate if DGKζ abilities are related with its function in the control of polarized responses *(Andrada, 2016)*. On the other hand, additional antibodies will allow us to exhaustively characterize the profile of tumor-infiltrating lymphocytes to assess the contribution of DGKζ deficiency to the expression of T cell markers of potential use to delimit different T cells population.

References:

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PE - conjugated anti-human IFN-gamma

recombinant human cytokines: rh IFN-gamma, rh IL-2, rh TGF-beta3

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

FITC - conjugated anti-mouse CD48, Erythroid cells

APC - conjugated anti-mouse CD44

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

recombinant mouse cytokines: rm IFN-gamma, rm IL-15, rm IP-10 / CXCL10

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