DUAL INHIBITION OF DGKα AND DGKζ INCREASES T CELL AND NK CELL ACTIVITY


Background: Diacylglycerol kinases (DGKs) play a role in regulating the responsiveness of immune cells to activating stimuli, making them an emerging immunotherapeutic target. DGKs phosphorylate the signaling lipid diacylglycerol (DAG) to produce phosphatidic acid, thus depleting the pool of DAG that can serve as a second messenger within a cell. In T cells, DAG is rapidly generated following T cell receptor (TCR) activation, forming a gradient around the immunological synapse. DAG accumulation leads to the activation of DAG-binding proteins that are critical for T cell activation and effector function, including the Ras/ERK activating guanine nucleotide-exchange factor, RasGRP1. Blocking DGK activity can delay the metabolism of DAG, leading to enhanced downstream signaling, thus increasing the strength of T cell responses to TCR stimulation and potentially protecting against T cell exhaustion. Notably, DGKs have also been shown to regulate DAG signaling in NK cells, extending the prospective anti-tumor capacity of these targets beyond T cells.

Methods: Transcript analysis and flow cytometry-based profiling were used to assess the expression of different DGK isoforms in human peripheral blood mononuclear cells (PBMCs) and isolated primary CD8 T and NK cells. Efficacy of DGK inhibition in T and NK cells was examined using CRISPR-based deletion strategies or small molecules selectively targeting DGKα and/or DGKζ isoforms in various in vitro functional assays.

Results: Analysis of PBMCs by flow cytometry confirmed expression of both DGKα and DGKζ in CD8 T cells, in alignment with transcript data from purified CD8 T cells. T cell receptor stimulation using CD3/CD28 binding antibody complexes showed increased ERK phosphorylation, cytokine production, proliferation, and CD69 expression in T cells treated with DGKα, DGKζ, or dual DGKα/ζ-inhibitors. Additionally, CD8 T cell cytokine production increased with DGK inhibition when PBMCs were treated with HLA class I-restricted viral peptides. Similarly, NK cells expressed both DGKα and DGKζ isoforms and showed increased cytokine production upon DGK inhibitor treatment in the presence of stimuli. In both T and NK cell systems, the greatest increases in activity occurred when DGKα and DGKζ were simultaneously inhibited. High throughput screening of DGK inhibitors using the Jurkat T cell line further indicated that TCR stimulation-mediated effects on IL-2 production were more robust using dual DGKα/ζ inhibitors.

Conclusions: Inhibition of DGKα and DGKζ increased the activation potential of T cells and NK cells, with simultaneous inhibition of DGKα and DGKζ showing the strongest effects, based on in vitro analysis.

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