2. DIACYLGLYCEROL KINASE ALPHA

2.1. The immunological side

Diacylglycerol Kinase alpha (Dgkα) belongs to the type I family of Dgks and displays, starting from the N-terminal toward the C-terminal part of the protein, two EF-hand domains, which make this isoform responsive to calcium, two atypical C1 domains [8] and a catalytic domain [2].

The role of Dgkα has been widely studied in T cells both *in vitro* and *in vivo*.

In the mouse T cell line CTLL-2, IL-2 activates Dgkα in a Src-like kinases- and PI 3-K- dependent manner [9]. In fact, the increase in cellular levels of PI 3-K products is sufficient to induce Dgkα activation, allowing Dgkα relocation to the intact lymphocyte plasma membrane, while cell treatment with PP2, a Src-family inhibitor, prevents both Dgkα and Akt activation induced by IL-2. In IL-2 triggered signaling, Dgkα activation is necessary for IL-2-induced G1-to-S transition [10,11].

Furthermore in T cells, appropriate TCR-dependent signal intensity and duration is essential for correct progression of the T-cell activation program. PLC-mediated DAG generation is a key signal initiated after TCR triggering, and DAG level modulation alters T-cell activation parameters. The PKCθ isoform, which is rapidly relocated to the plasma membrane during T-cell activation, is essential for TCR signaling. In response to increased DAG levels, and modulated by tyrosine phosphorylation, PKCθ translocates to the immune synapse, where it activates c-Jun N-terminal kinase and initiates the nuclear factor-kB cascade. RasGRP are guanine exchange factors (GEF) for Ras family proteins; they contain one C1 domain and provide a direct link between DAG generation and Ras/Raf/Mitogen-activated protein kinase (MAPK) activation. TCR triggering induces rapid, DAG-dependent RasGRP1 relocalization to internal membranes [12]. In T lymphocytes, following TCR engagement, Dgkα is recruited to the plasma membrane in a rapid, transient and tyrosine kinase-dependent manner and regulates the clearance of PLC-generated DAG [13]. Thus, by metabolizing DAG to PA, Dgkα functions as negative regulator of both PKCθ and Ras signaling, by reversing respectively PKCθ and RasGRP membrane translocation [14,15].
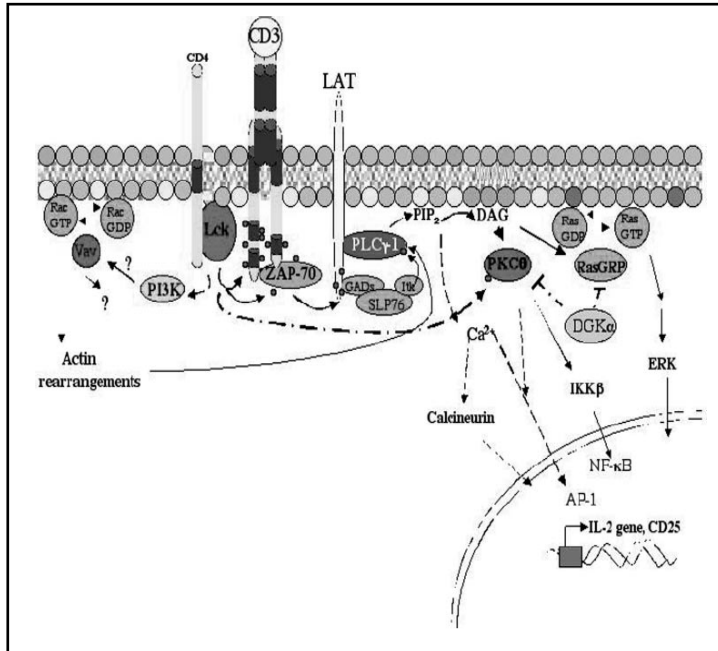
Dgkα as negative regulator of PKCθ and RasGRP downstream of TCR signaling.
From Diaz-Flores *et al.*, J. Biol. Chem. 2003 (Ref. 14).

During the development of the fetus, most autoreactive T cells are eliminated through a non completely effective negative selection called central tolerance. As a result, self-reactive T cells are still detectable in adult organisms and T cell tolerance must be maintained by additional mechanisms, including "ignorance" of the antigen, action of regulatory T cells and T cell clonal anergy, which is a state of antigen unresponsiveness induced by TCR stimulation in absence of costimulatory signals. Anergic T cells have multiple defects in TCR signaling pathways, do not produce IL-2 nor proliferate after reencounter with the antigen and fail to appropriately activate PLC-γ1 and Ras/MAPK pathway after TCR stimulation [16]. Indeed, active Ras restores IL-2 production and MAPK signaling in anergic T cells [17].

Accordingly with the model in which Dgkα functions as a negative regulator of RasGRP, Dgkα is found to be upregulated in anergic T cells. Moreover, Dgkα overexpression suppresses IL-2 production in T lymphocytes, while Dgkζ overexpression resulted in minimal effect on IL-2 production, thus underscoring the specificity of Dgkα-mediated signaling in the induction of T cell anergy [17]. These data are absolutely coherent with the phenotype of the Dgkα -/- mice. In fact, when stimulated in anergy-producing conditions, T cells lacking Dgkα proliferate and produce IL-2 [16]. Beside Dgkα, T cells express the Dgk isoforms ζ and δ, but their function appears to be non-redundant, as they have structurally distinct regulatory domain and differ in their subcellular localization [16].

Anaplastic large-cell lymphomas (ALCLs), a subgroup of non-Hodgkin lymphomas predominantly of T- or null-type, are frequently characterized by the presence of constitutively active forms of the product of the anaplastic lymphoma kinase (*ALK*) gene, which encodes for a large, glycosylated 200-kDa membrane-spanning tyrosine kinase receptor, whose expression is physiologically restricted to components of the nervous system. The oncogenic forms of ALK are the result of somatic chromosome translocations that fuse the ALK cytoplasmic domain to the 5′ region from different partner genes. The most frequent oncogenic version of ALK is represented by nucleophosmin (NPM)/ALK, an 80-kDa hybrid protein created by the t(2;5)(p23;q35) rearrangement. Several signaling molecules have been identified that associate and/or are activated by ALK, including growth factor receptor–bound protein 2 (Grb2), Src homology and collagen (Shc), insulin receptor substrate-1 (IRS-1), phospholipase C-γ (PLC-γ), Src kinases, and phosphatidylinositol 3-kinase. The contribution of Src has been recently evaluated in NPM/ALK-positive cell lines and demonstrated through the effects of Src down-regulation and pharmacologic inhibition on cellular proliferative rate. Additional relevant effectors of NPM/ALK-mediated lymphomagenesis are represented by signal transducer and activator of transcription 3 (Stat3) and Stat5. Bacchiocchi *et al.*, in collaboration with my laboratory, demonstrated that Dgkα is constitutively activated, in a Src-dependent manner, downstream of NPM/ALK and is involved in ALK-mediated mitogenic properties [18].

2.2. On the other side of Dgkα: endothelia and epithelia

Though abundant in T cells, Dgkα is expresses also in endothelial and epithelial cells, fibroblasts and oligodendrocytes. In my laboratory, the involvement of Dgkα in tyrosine kinase receptors signaling has been reported for the first time [19].

Hepatocyte Growth Factor (HGF), through binding to its tyrosine kinase receptor Met, induces a range of biological responses, including scattering of epithelial cells, proliferation, motility and branching morphogenesis of both epithelial and endothelial cells, and invasiveness of carcinoma cells. HGF is required for early development of liver, placenta and limb muscles, is involved in kidney and liver regeneration and induces angiogenesis *in vivo* in infracted myocardium. Signal transduction of HGF occurs through receptor recruitment and activation of several

intracellular signaling transducers. Gab1 and Gab2 membrane and receptor recruitment, and Ras and PI 3-K activation are all required for HGF-induced cell migration, tubulogenesis and proliferation. HGF-induced Rac activation is required for cell motility, while activation of both Src and Rho is required for tyrosine phosphorylation of focal adhesion proteins and formation of stress fibres (see references in 19). Cutrupi *et al.* demonstrated that HGF activates Dgkα in both epithelial and endothelial cells and that Dgkα activation is required for HGF-induced cell motility of PAE endothelial cells. Moreover, HGF induces the association of Dgkα with Src, which in turn phosphorylates and mediates HGF-induced activation of Dgkα [19].

Consistently, Baldanzi *et al.* demonstrated that VEGF induces the activation of Dgkα, again in Src-dependent manner [20]. VEGF-A stimulates angiogenesis by activating several signaling pathways in a spatially and timely coordinated manner, leading to endothelial cells migration, proliferation and organization in tubular structures. Among the numerous pathways activated, the generation of phosphatidylinositol(4,5)bis-phosphate ($PI(4,5)P_2$)-derived second messengers plays a crucial role in VEGF-A angiogenic signaling. Indeed, VEGF-A-induced angiogenic signaling requires both phosphorylation and hydrolysis of $PI(4,5)P_2$, mediated, respectively, by PI 3-K and PLC-γ to generate $PI(3,4,5)P_3$ and diacylglycerol. While $PI(3,4,5)P_3$ is required for activation of Akt, which mediates VEGF-A-induced survival signaling, DAG is required for activation of PKC-α, which mediates VEGF-A-induced proliferation and chemotaxis (see references in 20). In endothelial cells systems, Dgkα activation was demonstrated to be required for VEGF-dependent chemotaxis, proliferation and *in vitro* angiogenesis, highlighting that Dgkα generates a signal essential for both proliferative and migratory responses to VEGF [20].

Taken together, the data produced in both epithelial and endothelial cells suggest that the role of Dgkα downstream of tyrosine kinase receptors accomplishes several biological functions and is highly biologically significant.