repiSalud
Repositorio Institucional en Salud

This is the peer reviewed version of the following article:

Wculek, S. K., & Sancho, D. (2019). LKB1 restrains dendritic cell function. *Cell Research, 29*(6), 429-431. doi:10.1038/s41422-019-0169-0

which has been published in final form at: https://doi.org/10.1038/s41422-019-0169-0

LKB1 restrains dendritic cell function

Stefanie K. Wculek and David Sancho*

Immunobiology laboratory, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain.

* Corresponding author:

David Sancho

Centro Nacional de Investigaciones Cardiovasculares (CNIC)

Melchor Fernández Almagro, 3; 28029 - Madrid (Spain)

ORCID: 0000-0003-2890-3984

Tel: (+34) 914531200 Ext. 2010

Email: dsancho@cnic.es

Three independent recent studies support the notion that liver kinase B1 (LKB1), a key nutrient sensor, controls dendritic cell (DC) function. Selective loss of LKB1 in DCs leads to their increased ability to prime effector T cell responses, but the prevailing effect is the expansion of thymus-derived natural regulatory T cells (tTregs) by LKB1-deficient DCs that create a dominant immunosuppressive environment[1–3] (Figure 1).

Tight regulation of T cell priming by DCs is key to maintain tissue homeostasis and orchestrate immunity. As immune sentinels, DCs control the activation of different flavors of immunity including effector CD8+ T cells and CD4+ helper T (Th) cells that comprise IFNγ-producing Th1, IL-4-producing Th2, IL-17-producing Th17 cells and follicular helper T cells (Tfh) that promote B cell differentiation in germinal centers[4,5]. However, DCs also contribute to homeostasis and self-tolerance through the induction of Tregs, which can be Helios^{+} thymus-derived/natural (tTregs) or generated upon antigen exposure in the periphery (Helios^{-} pTregs)[4,6].

DCs integrate environmental cues such as pathogen- or danger-associated molecular patters and cytokines to activate different intracellular signaling pathways. Those adaptions lead to adjustment of antigen uptake, processing and presentation on MHC molecules (signal 1), expression of co-stimulatory molecules (signal 2) and production of specific cytokines (signal 3) by DCs to modulate induction of effector and regulatory T cell responses. Different DC subsets include plasmacytoid (pDCs), conventional type 1 (cDC1s) as well as 2 (cDC2s) DCs and express a varying repertoire of pattern recognition receptors sensing those extracellular cues culminating in diverse functional features[4].

Metabolic alterations in DCs upon sensing the environment have emerged essential for control of DC function in the regulation of adaptive immune responses[5,7]. Resting or tolerogenic DCs preferentially display an active catabolic energy metabolism via the Krebs cycle and oxidative phosphorylation (OXPHOS) that can be fueled by fatty acid oxidation. Immunogenic activation of DCs generally fosters an anabolic metabolism characterized by enhanced glycolysis, and fatty acid synthesis to drive the extension of the Golgi apparatus and endoplasmic reticulum for cytokine production[7]. The early induction of glycolysis regulates many aspects of immunogenic DC activation, including migration,

upregulation of MHC and co-stimulatory molecules and T cell stimulation[5]. The metabolic adaption of DCs upon stimulation is, at least partially, regulated by a balance of AKT/mTOR and AMPK signaling pathways[5,7]. Activation of mTOR can sustain immunogenic DC activation while active AMPK is associated with resting and tolerized DCs. However the precise pathways regulating the metabolic state that drive tolerogenic DC function are poorly understood[5,7].

LKB1 is a serine/threonine kinase that can activate AMPK upon low intracellular ATP to induce catabolic oxidative metabolism. As such, LKB1 is implicated in modulating cell metabolism, survival, differentiation and functional features of hematopoietic stem cells, effector CD4+ and CD8+ T cells as well as Tregs in AMPK-dependent and independent fashions[8–10]. Also, LKB1 is phosphorylated on Ser-428 in lipopolysaccharide (LPS)-stimulated macrophages and restricts their pro-inflammatory functions by inhibition of NF-κB signaling, likely via binding to IκB kinase (IKK) β[11].

To dissect the contribution of LKB1 to DC function, three independent studies have analyzed CD11c-Cre LKB1$^{f/f}$ (CD11cΔLKB1) mice[1–3]. They find an enlarged tTreg pool throughout the body of CD11cΔLKB1 mice, including the thymus, which protects them from experimental allergy or autoimmunity but makes them more susceptible to grafted tumors. LKB1 is phosphorylated in intratumoral DCs[3], while *Escherichia coli*- or LPS-stimulation downregulates LKB1 expression in DCs, which associates with expansion of tTregs[1]. Two studies show that Tregs of CD11cΔLKB1 express higher levels of immune-suppressive molecules and display an enhanced suppressive activity limiting T cell proliferation[2,3]. Further, Wang et al. demonstrate that LKB1-deficient DCs express higher levels of Treg-inducing IL-2, indoleamine 2, 3-dioxygenase (IDO) 1, arginase (Arg) 2 as well as integrin beta (Itgb) 8 than wildtype DCs. These factors contribute to induction of mTOR signaling in tTregs and enhance tTreg proliferation[3].

Notably, LKB1-deficient splenic DCs (subsets) display enhanced MHC and co-stimulatory molecule expression, foremost OX40-ligand (OX40L) and CD86, the latter also being elevated on CD11cΔLKB1 DCs in the thymus[1–3]. Indeed, Pelgrom et al. identify the thymic CD11b+ cDC2s subset, which is associated with regulating tTreg responses[12], but not thymic cDC1s or pDCs, to

be a key player in inducing tTregs upon LKB1 loss. Mechanistically, high CD86 expression, driven by enhanced phospholipase C-β1 (PLC-β1) expression and calcium signaling in thymic CD11cΔLKB1 cDC2s, potentiate tTreg induction[2]. Frequencies of cDC2s are also increased in thymi of CD11cΔLKB1 mice, likely further fostering induction of tTregs[2,3]. Moreover, thymic CD11cΔLKB1 cDC2s express higher levels of CCR7[2], in line with increased presence and CCR7 expression of migratory DCs in lymph nodes and augmented DC-Treg interaction[2,3]. Peripheral CD11cΔLKB1 cDC2s, but not cDC1s, also induce additional tTreg proliferation outside the thymus[2]. Chen et al. report an additional contributing mechanism by showing that LKB1 loss in splenic or lymph node DCs induces non-canonical NF-κB (p65) activation and subsequent upregulation of OX40L, which engages OX40 that is highly expressed on Tregs mediating their expansion in the periphery[1].

Interestingly, the increased T cell-priming ability of LKB1-deficient DCs is not restricted to tTregs. LPS- and ovalbumin-stimulated in vitro generated CD11cΔLKB1 DCs (GM-DCs) more profoundly induce IFNγ- and/or IL-17-producing effector CD8+ and CD4+ T cells after transfer into wildtype mice, which may be related to their enhanced migration to draining lymph nodes and co-stimulatory molecule expression[2]. The effects of those immunogenic roles of LKB1 loss in DCs are likely dampened in vivo by Treg accumulation in CD11cΔLKB1 mice. Nevertheless, Wang et al. observe enhanced generation of Th17 cells by LKB1-deficient CD11cΔLKB1 DCs ex vivo and in vivo, which is partially corroborated by Pelgrom et al[2,3]. Elevated production of IL-6 by CD11cΔLKB1 DCs may cause Th17 induction, which also contributes to the tumor susceptibility of CD11cΔLKB1 mice[3].

Finally, given the involvement of LKB1 in controlling cellular metabolism[8–10] and the influence of metabolic adaptions on DC functions[5,7], the studies from Wang et al. and Pelgrom et al. analyze metabolic parameters and regulation of nutrient-sensing signaling pathways such as AMPK, mTOR and HIF1α in CD11cΔLKB1 DCs, with some contrasting results. Pelgrom et al. report enhanced glucose uptake and unaltered or even reduced mitochondrial fitness, such as mitochondrial mass and membrane potential, in splenic (and thymic) CD11cΔLKB1 cDC1s and cDC2s[2]. Wang et al. corroborate an elevated

extracellular acidification rate (ECAR) by LKB1-deficient splenic DCs, but find a significantly augmented oxygen consumption rate, a readout for OXPHOS. Those partially contradicting findings, which may be due to (FMS-like tyrosine kinase 3 ligand (FLT3L)-mediated DC expansion, limit the potential to correlate a metabolic state or adaption of CD11cΔLKB1 DCs with their observed functional alteration. Nevertheless, the enhanced glycolytic activity of CD11cΔLKB1 DCs together with the observation of an altered cholesterol metabolism and intracellular lipid accumulation[3] may associate with the metabolism of activated DCs[7].

In regard to signaling pathways controlling cell metabolism, the three studies concur in pointing out that LKB1 function in DCs is independent of AMPK, the well-known LKB1 downstream target[8], by analyzing CD11c-Cre AMPKα1$^{f/f}$ (and AMPKα2$^{f/f}$) mice[1–3]. Of note, Pelgrom et al. and Wang et al. find enhanced mTOR signaling in thymic and splenic LKB1-deficient DCs, respectively. Treatment of thymic cDC2s with the mTOR inhibitor rapamycin blocks their potential to induce Treg expansion, but not the enhanced expression of CD86 on DCs[2]. In line, mTOR loss in LKB1-deficient splenic DCs in CD11c-Cre LKB1$^{f/f}$mTOR$^{f/f}$ mice has a small effect on the increased co-stimulatory molecule expression by DCs, but significantly decreases the expansion of Tregs and mTOR activation in Tregs. Those effects appear however independent from HIF1α, which was previously shown to act downstream of mTOR controlling DC metabolism[5,7], as genetic HIF1α depletion in CD11cΔLKB1 does not alter DC phenotype or function[3]. In contrast, Th17 induction potential or IL-6 expression of LKB1-deficient DCs is not influenced by mTOR deletion, suggesting alternative control mechanisms by LKB1[3].

Overall, LKB1 emerges as a fundamental regulator of the core DC function to control T cell responses and maintain their immunological quiescence, at least partially via limiting DC migration, co-stimulatory molecule (CD86 and OX40L) and cytokine (IL-6) expression[1–3]. LKB1 loss in DCs results in their uncontrolled stimulation of T cells, foremost of Tregs by cDC2s in the thymus and periphery as well as peripheral Th17 cells. Prevention of mTOR signaling in DCs, likely in concert with limiting glycolytic metabolism[5,7], appears to account for aspects of LKB1-mediated regulation of T cell immunity by DCs, such as Treg homeostasis.

These studies open a research avenue for the dissection of LKB1 pathway(s) regulating DC function and metabolism, which may offer potential targets to manipulate immunity and tolerance.

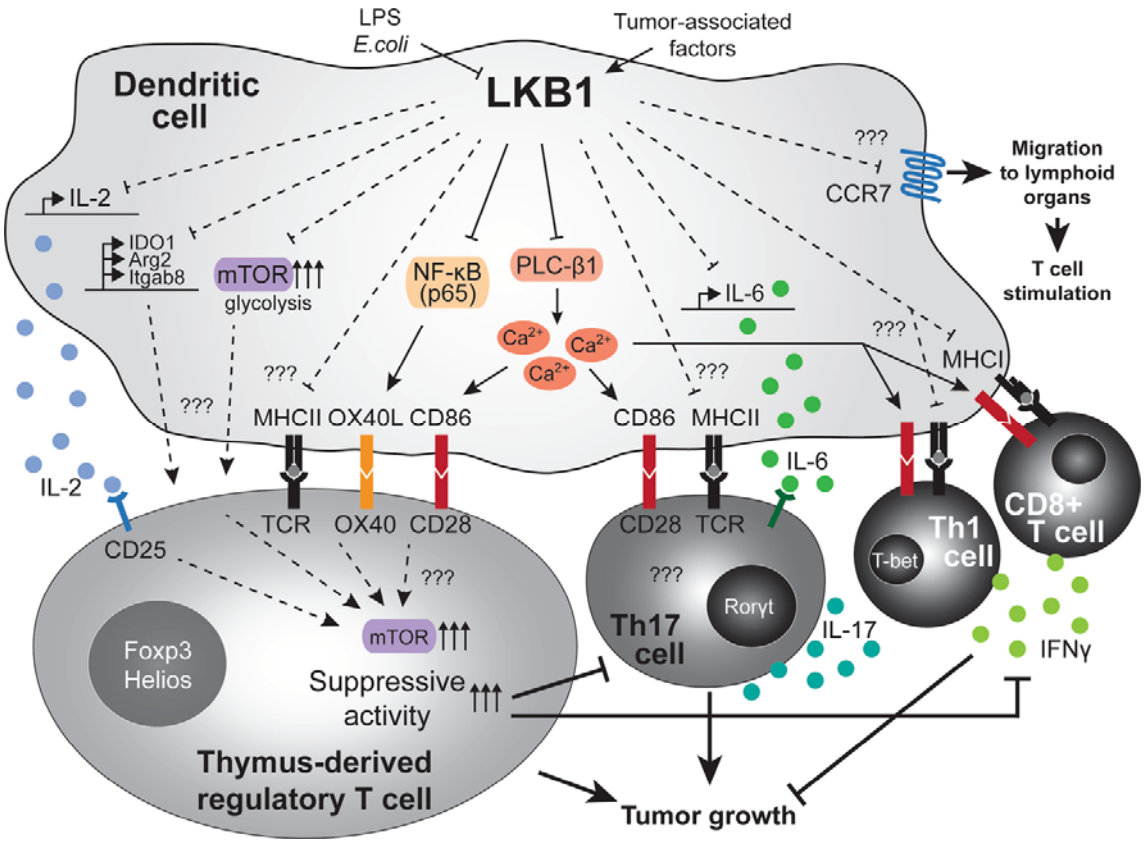
References (max 10)

1. Chen, S. *et al.* Control of Treg cell homeostasis and immune equilibrium by Lkb1 in dendritic cells. *Nat. Commun.* **9**, 5298 (2018).
2. Pelgrom, L. R. *et al.* LKB1 expressed in dendritic cells governs the development and expansion of thymus-derived regulatory T cells. *Cell Res.* (2019).
3. Wang, Y. *et al.* LKB1 orchestrates dendritic cell metabolic quiescence and anti-tumor immunity. *Cell Res.* (2019).
4. Merad, M., Sathe, P., Helft, J., Miller, J. & Mortha, A. The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. *Annu Rev Immunol* **31**, 563–604 (2013).
5. Patente, T. A., Pelgrom, L. R. & Everts, B. Dendritic cells are what they eat: how their metabolism shapes T helper cell polarization. *Curr. Opin. Immunol.* **58**, 16–23 (2019).
6. Pletinckx, K., Döhler, A., Pavlovic, V. & Lutz, M. B. Role of dendritic cell maturity/costimulation for generation, homeostasis, and suppressive activity of regulatory T cells. *Front. Immunol.* **2**, 1–16 (2011).
7. O'Neill, L. A. J. & Pearce, E. J. Immunometabolism governs dendritic cell and macrophage function. *J. Exp. Med.* **213**, 15–23 (2015).
8. Blagih, J., Krawczyk, C. M. & Jones, R. G. LKB1 and AMPK: central regulators of lymphocyte metabolism and function. *Immunol. Rev.* **249**, 59–71 (2012).
9. He, N. *et al.* Metabolic control of regulatory T cell (Treg) survival and function by Lkb1. *Proc. Natl. Acad. Sci. U. S. A.* **114**, 12542–12547 (2017).
10. Yang, K. *et al.* Homeostatic control of metabolic and functional fitness of Treg cells by LKB1 signalling. *Nature* **548**, 602–606 (2017).
11. Zou, M.-H. *et al.* Liver Kinase B1 Suppresses Lipopolysaccharide-induced Nuclear Factor κB (NF-κB) Activation in Macrophages. *J. Biol. Chem.* **290**,

2312–2320 (2014).

12. Proietto, A. I. *et al.* Dendritic cells in the thymus contribute to T-regulatory cell induction. *Proc. Natl. Acad. Sci.* **105**, 19869–19874 (2008).

Fig. 1



LKB1 signaling in dendritic cells limits their T cell-activating potential. LKB1 is phosphorylated in DCs in the tumor microenvironment, while it is depleted by LPS or *E. coli* favoring Treg expansion. LKB1 limits the ability of DCs to induce T cell priming by repressing a variety of activating pathways. These effects lead to LKB1-deficient DCs to promote dysregulated T cell effector activity, with predominant increase in thymus-derived regulatory T cell priming but also increased priming of pro-immunogenic effector Th17, Th1 and $CD8^+$ T cells. Mechanistically, upon loss of LKB1, DCs enhance their expression of MHC molecules, co-stimulatory molecules (e.g., CD86, OX40L), cytokines (e.g., IL-6, IL-2) and migration receptors (e.g., CCR7) — all of which contribute to enhanced T cell priming. The predominant activation of regulatory T cells and Th17 cells upon LKB1 deletion in DCs contributes to tumor growth