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Regulatory T Cells under the Mercy of Mitochondria

Mrinmoy Das,[1] Fawaz Alzaid,[2] and Jagadeesh Bayry[2,*]
[1]Division of Immunology, Boston Children's Hospital, Harvard Medical School, Boston, MA 02115, USA
[2]Institut National de la Santé et de la Recherche Médicale, Centre de Recherche des Cordeliers, Sorbonne Université, Université Paris Descartes, Sorbonne Paris Cité, Paris F-75006, France
*Correspondence: jagadeesh.bayry@crc.jussieu.fr
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Mitochondria, the powerhouse of the cell, known for producing energy through oxidative phosphorylation and the Krebs cycle, continue gaining notoriety for roles beyond bioenergetics. Recently in *Nature*, Weinberg et al. (2019) reported that mitochondrial complex III is indispensable for suppressive function of regulatory T cells, thus highlighting the importance of mitochondria in immune tolerance.

An increasing number of reports in the field of immunometabolism have mapped the metabolic needs of immune cells associated with their differentiation and function. Notably, type-1 immune responses depend largely on cytoplasmic glycolysis and type-2 immune responses depend on mitochondrial metabolism. These two energy systems are perfectly suited to the bioenergetic requirements of the job at hand. In as much, glycolytic metabolism allows rapid, yet unsustainable production of energy from glucose to support the immediate type-1 response to bacterial or viral stimuli. The process appears to be regulated by the PI3K and Akt pathway and the transcription factors Myc and estrogen-related receptor-α (ERRα) (Wang et al., 2011). Mitochondria, on the other hand, can metabolize lipids, amino acids, or by-products of glycolysis to generate ATP in a slower but sustainable manner. Mitochondrial metabolism occurs in two distinct phases, first through engaging the Krebs cycle in the mitochondrial matrix, products of which are substrates for the second phase, the electron transport chain (ETC). The ETC is a series of four complexes anchored to the inner mitochondrial membrane that carry out oxidative phosphorylation through a series of redox reactions. Metabolic alterations are common features of various pathologies including cancer, autoimmune, and inflammatory disorders (Huang and Perl, 2018). As an additional layer of complexity on how metabolic processes regulate the immune responses, Weinberg et al. (2019) now report that mitochondrial complex III plays a critical role in controlling the suppressive functions of regulatory T cells (Tregs).

$CD4^+CD25^+FOXP3^+$ Tregs are renowned for their immunosuppressive roles. Tregs assure appropriate resolution of inflammation and ensure self-tolerance by dampening the autoimmune responses (Ohkura et al., 2013). Tregs are known to have a "metabolic edge" for their survival, through their bias toward mitochondrial respiration. Predominant mitochondrial metabolism effectively supports their known immunosuppressor functions but also allows their survival in lactate-rich environments (Angelin et al., 2017). FoxP3, the lineage-specific transcription factor implicated in Treg function, has also been shown to induce the transcriptional programs responsible for Treg metabolic adaptation.

A number of recent reports have shown that the Treg numbers and their plasticity are regulated by metabolic processes. Through innovative approaches, Weinberg et al. (2019) report that mitochondrial complex III of ETC is critical for the suppressive function of Tregs (Figure 1). But the number, proliferation, and survival of Tregs as well as expression of FoxP3 are not controlled by mitochondrial complex III. Mitochondrial complex III has 11 subunits including Rieske iron sulfur protein (RISP) and ubiquinone-binding protein (QPC). RISP is required for superoxide generation during the ubiquinone cycle while QPC functions downstream of superoxide generation. Treg-specific deletion of *uqcrsf1* (encodes the RISP) (RISP-KO mice) or *Uqcrq* (encodes QPC) (QPC-KO mice) in mice led to a fatal inflammatory disorder. Both RISP-KO and QPC-KO mice exhibited multiple symptoms suggestive of a profound immune response, including thymic atrophy, enlargement of lymph nodes and spleen, increased lymphocytic cell infiltration in multiple organs, and increase in activated $CD4^+$ and $CD8^+$ T cells in lymph nodes and spleen. The mice also die by week 4 of age, similar to those observed with Scurfy mice. But unlike Scurfy mice that lack Tregs, RISP-KO and QPC-KO mice had normal Treg number and expressed FoxP3 despite drastically shifting metabolic flux away from mitochondria and toward cytoplasmic glycolysis. Of note, a recent study reports that FoxP3 reprograms T cell metabolism by suppressing Myc and glycolysis, enhancing oxidative phosphorylation that attributes to Treg survival in lactate-rich niches (Angelin et al., 2017). This suggests an intimate cooperation between FoxP3-mitochondrial respiratory complexes to ensure proper functioning of Tregs. However, when the mitochondrial respiratory chain is disrupted as reported by Weinberg et al., Tregs show increased glycolytic flux. How FoxP3-mediated suppression of Myc is relieved in such conditions and what are the mechanisms of lactate processing (a known by-product of anaerobic glycolysis) in Tregs are subjects for future investigation.

The centerpiece of this publication is brought about through innovative use of the natural phenomenon of X-inactivation. Through generating female mice floxed for RISP and heterozygote for a $FoxP3^{YFP\text{-}Cre}$, random inactivation of the X chromosome leads to a mix of RISP-deficient (YFP^+) and -competent (YFP^-) Tregs in each mouse. Comparison of transcriptomic signatures of RISP-deficient and RISP-competent cells from chimeric mice and from RISP-KO mice allowed the authors to discern cell-autonomous transcriptomic shifts caused by loss of a functional mitochondrial complex III,

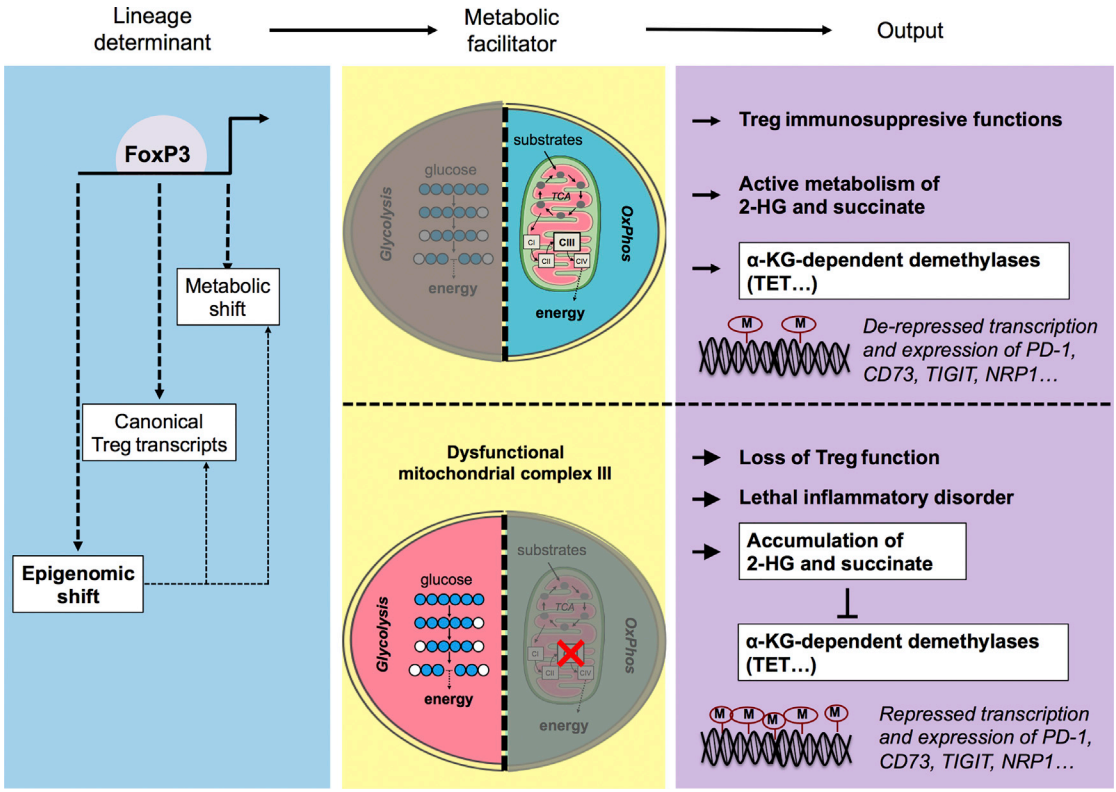

Figure 1. Mitochondrial Complex III Activity Sustains Regulatory T Cell Function through Maintaining Metabolite Homeostasis and DNA Methylation Status

Immunosuppressive regulatory T cells (Tregs) commit to their lineage upon expression of the FoxP3 transcription factor. FoxP3's transcriptional activity induces pathways associated with canonical Treg functions as well as pathways adapting cellular metabolism to meet Treg requirements. Tregs have a metabolic profile biased toward mitochondrial metabolism (like other type-2 immune effector cells) that allows efficient lactate clearance and conversion to energy through the tricarboxylic acid cycle (TCA cycle or Krebs cycle) and oxidative phosphorylation (OxPhos). Importantly, engaging mitochondrial respiration and the role of the mitochondrial complex III (CIII) allows the maintenance of a homeostatic balance of succinate and L-(s)-2-hydroxyglutarate (2-HG) through their further metabolism. Loss of complex III functionality leads to accumulation of these two metabolites and the downstream repression of α-ketoglutarate (α-KG)-dependent demethylases (e.g., TET). Such metabolite imbalance through complex III inactivation results in DNA hypermethylation, repressing transcripts required for Treg functionality (like PD-1, CD73, TIGIT, and NRP1). The role of mitochondrial metabolism, and specifically complex III, thus extends beyond fueling cells to maintenance of homeostatic levels of epigenetically active metabolites. TET, ten-eleven translocation (TET) family of DNA demethylases; PD-1, programmed cell death 1; TIGIT, T cell immunoreceptor with Ig and ITIM domains; NRP1, Neuropilin 1.

independently of the host's inflammatory status.

The profound inflammation in RISP-KO and QPC-KO mice indicated impaired suppressive function of Tregs. Indeed, the authors found that Tregs from RISP-KO had abrogated suppressive capacity. Transcriptomic profile of RISP-deficient Tregs revealed that genes encoding for inhibitory molecules such as PD-1, CD73, NRP1, and TIGIT as well as their surface expression were reduced compared to wild-type Tregs. Also, the loss of complex III in Tregs resulted in DNA hypermethylation and altered gene expression without affecting the methylation status of Foxp3. The loss of complex III also increased the expression of Treg CTLA-4 but did not affect the Treg-cytokines such as TGF-β, IL-10, and IL-35 (S.E. Weinberg and N. Chandel, personal communication) that are implicated in immunosuppression. Foxp3 expression and its hypomethylation are known to be a key regulator of development and suppressor function of Tregs (Fontenot et al., 2003; Ohkura et al., 2013) while CTLA-4 has been reported to be a major molecule implicated in the Treg-mediated suppressive functions. The current report by Weinberg et al. adds an additional layer of regulation in the form of mitochondrial complex III, which governs Treg functions.

Further fueling the arguments linking cellular metabolic flux as epigenetic modifiers, an analysis of the cellular metabolites demonstrates an increased abundance of succinate and L-(S)-2-hydroxyglutarate (2-HG), known antagonists of the α-ketoglutarate (α-KG)-dependent histone demethylases (Lu and Thompson, 2012). α-KG is a known by-product of the Krebs cycle, metabolically functioning upstream of the ETC. An insufficiency of complex III activity in Tregs leads to accumulation of the metabolite antagonists to demethylases like the ten-eleven translocation (TET) family of DNA demethylases, thus explaining hypermethylation observed in RISP deficiency. Whether altered metabolism also leads to changes in the expression of other transcriptional regulators like microRNA remains to be investigated.

It is important to validate the current findings in humans for translational purposes. Although well-characterized small-molecule inhibitors are available that could be used to specifically inhibit mitochondrial respiratory complexes, we could also make use of patients with mitochondrial

complex III deficiency. Several mutations leading to mitochondrial complex III deficiency have been identified in humans including *MT-CYB* (which encodes CIII subunit in the mitochondrial DNA), *BCS1L* (which encodes assembly factor), and UQCRB (a nuclear-encoded structural subunit) (Fernández-Vizarra and Zeviani, 2015). Therefore, investigation of Tregs in those patients should validate the data obtained with mice.

In cancer cells, mutated isocitrate dehydrogenase (IDH)1 and 2 contribute to elevated D-2-HG (Yang et al., 2012). However, in Tregs, mitochondrial complex III deficiency (with normal IDH2) also leads to increased 2-HG (Weinberg et al., 2019). It is worth mentioning that in many autoimmune patients, Tregs are normal in number but are defective in their functions. The data of Weinberg et al. (2019) raise a prospect that elevated 2-HG could be responsible for Treg defects in those patients and 2-HG levels could be used as a potential biomarker of Treg dysfunction. Not much is known regarding the mechanisms and enzymes that enhance 2-HG in the cells lacking IDH mutations. Increased transamination, mainly catalyzed by GOT1, has been reported to increase the levels of 2-HG (Xu et al., 2017). Identification of pathways that promote elevated 2-HG in Tregs should incite identification of inhibitors targeting those enzymes/pathways to prevent Treg dysfunction and to treat autoimmune and inflammatory diseases.

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