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Treg Cells Survive and Thrive in Inhospitable Environments

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Immune responses are dangerous by nature and require regulation to prevent inflammatory and/or autoimmune sequelae and allow healing. $CD4^+Foxp3^+$ T cells (Treg cells) play a crucial role in this process, and in this edition of *Cell Metabolism*, Angelin et al. (2017) describe how these cells are metabolically adapted to the job.

Activated immune cells display fate-associated metabolic differences that are linked to function. Relevant to this discussion, effector $CD4^+$ T cells (Teff cells), which are at the sharp end of the immune response, primarily utilize aerobic glycolysis, whereas Treg cells, which are critical for immune homeostasis because they suppress immune responses, primarily use oxidative phosphorylation (OXPHOS). Broadly speaking, inhibition of the dominant metabolic pathways in these cells inhibits their specific functions.

As immune responses and associated inflammation progress, and/or as tumors grow or infections take hold, extracellular tissue conditions can change such that glucose is depleted and lactate accumulates, a direct consequence of increased aerobic glycolysis at the site. These conditions themselves tend to inhibit Teff function (Scharping et al., 2016), but what about Treg cells? In this issue of *Cell Metabolism*, Angelin et al. propose that the transcription factor Foxp3 reprograms the metabolic state of $CD4^+$ T cells, allowing them to maintain their suppressive functions in environments with low-glucose and high-lactate concentrations (Angelin et al., 2017). This report is one of three recent papers to show that Foxp3 plays a major role in regulating the expression of metabolic pathway genes in Treg cells (Gerriets et al., 2016; Howie et al., 2017).

Treg cells proliferate in inflammatory conditions, a process supported by glycolysis (Gerriets et al., 2016). However, during this proliferative phase Foxp3 expression is suppressed and Treg cells exhibit impaired regulatory function (Gerriets et al., 2016). Nevertheless, increased expression of Foxp3 leads to the inhibition of aerobic glycolysis and redirects cellular metabolism toward OXPHOS, ultimately restoring suppressive function (Gerriets et al., 2016). Here, Angelin et al. show that the ability of Treg cells to suppress the proliferation of Teff cells relies particularly on the intact function of complex I of the electron transport chain (ETC). In this context, they find that Treg cells deficient in *Nd6*, a component of Complex I, lose suppressive activity. In low glucose, Treg cells maintain redox balance by oxidizing NADH to NAD^+ through the coupled action of the tricarboxylic acid (TCA) cycle and the ETC (a process that is supported by their ability to oxidize fatty acids to support OXPHOS (Howie et al., 2017; Michalek et al., 2011). In contrast, Teff cells, which rely on aerobic glycolysis to regenerate NAD^+, face a redox imbalance in low glucose. This is critical for these cells since, among other things, GAPDH, a central regulator of glycolysis, is NAD^+ dependent. In the absence of sufficient NAD^+, glycolysis cannot proceed. The effects of such an event on Teff function are illustrated in the paper by the use of heptelidic acid, an inhibitor of GAPDH, which inhibits Teff proliferation but has no effect on Treg cell suppressive function. Thus the work by Angelin et al. adds weight to the consensus that OXPHOS is critical for Treg function and illuminates the process by adding details regarding the relative roles of different ETC components and of OXPHOS versus glycolysis in maintaining redox balance under glucose-restricted conditions.

How then does Foxp3 expression alter cellular metabolism and promote OXPHOS? Angelin et al. reveal that Foxp3 represses enactment of the c-Myc-dependent transcriptional response that underpins Teff cell development. Following activation in Teff cells, c-Myc expression is induced, allowing metabolic reprogramming for aerobic glycolysis and glutaminolysis (Preston et al., 2015; Wang et al., 2011). The authors show that Foxp3 effectively suppresses c-Myc expression through a process that correlates with its binding to the *Myc* TATA box. The study also hints at the fact that Foxp3 acetylation, a process that the authors have shown in previous reports promotes OXPHOS and Treg function, favors Foxp3 association with the *Myc* promoter, although this issue is left somewhat unresolved. These results begin to provide an explanation for how glycolysis is regulated in Treg cells and are in agreement with earlier studies showing that expression of the glucose transporter GLUT1 is downregulated by Foxp3 (Gerriets et al., 2016; Michalek et al., 2011).

Teff function is reduced within the tumor microenvironment, which often has higher lactate concentrations compared to lymph nodes due to the adoption of aerobic glycolysis by tumor cells (Scharping et al., 2016). Angelin et al. confirm that increased lactate concentrations are able to suppress Teff proliferation and show that they have no effect on Treg cell proliferation or function under conditions where glucose is limiting. In these conditions, both Treg and Teff cells oxidize lactate to pyruvate at similar rates. However, Treg cells sustain their proliferation, development, and

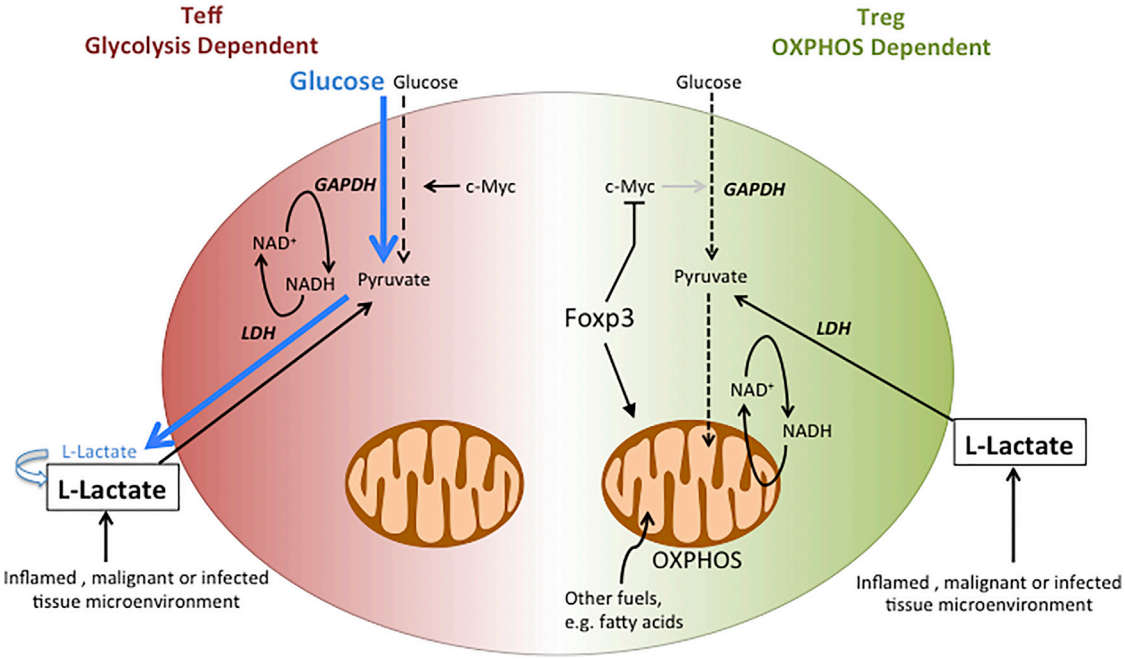

Figure 1. OXPHOS Maintains the Redox Balance Required for Treg Cells to Function in Low-Glucose/High-Lactate Environments
In Teff cells, Myc expression drives increased glycolysis, which supports cellular proliferation. Under glucose-limiting conditions, with high extracellular lactate concentrations (due to the use of aerobic glycolysis by other cells in the tissue as well as Teff cells), these cells become less proliferative due to a decline in aerobic glycolysis that is coupled to the redox imbalance associated with reversal of the pyruvate-to-lactate reaction. In Treg cells, Foxp3 suppresses Myc expression and therefore glycolysis, and promotes OXPHOS. These cells are able to maintain redox balance under low-glucose/high-lactate concentrations because NAD^+ is regenerated by the TCA cycle. Thus, in low-glucose/high-lactate conditions, continued Treg cell function coupled with impaired Teff proliferation results in amplified Teff response suppression.

importantly their suppressive functions under these high-lactate concentrations in vitro and in vivo. Further studies revealed that lactate dehydrogenase (LDH), an enzyme that reversibly catalyzes the conversion of pyruvate to lactate, plays a major role in suppressing Teff but not Treg cell functions. This is the result of LDH favoring the reduction of NAD^+ to NADH under low-glucose, high-lactate conditions, rather than the opposite under high-glucose, low-lactate conditions. In Treg cells this change in function related to redox balance has less of an effect because NAD^+ is being generated by strong NADH oxidation in mitochondria (Figure 1). The idea that OXPHOS allows Treg maintenance and function in tissues with limited glucose concentrations or high lactate concentrations is reinforced by other studies where Treg cells have been shown to play a crucial role in the repair of renal ischemia-reperfusion injury (Gandolfo et al., 2009) or muscle damage (Burzyn et al., 2013). Indeed, these findings may help explain why $CD8^+$ effector T cell dysfunction can be rescued in the tumor microenvironment by overexpression of PGC-1α within effector T cells and enforcement of OXPHOS (Scharping et al., 2016).

The findings from Angelin et al. provide an evolved view of how Treg cells are able to function within environmental conditions that negatively affect Teff metabolism. As befits an interesting new study, the work by Angelin et al. raises important questions that remain to be answered. For example, the paper leaves open the issue of how binding of Foxp3 to the TATA box of *Myc* suppresses transcription of this gene. Moreover, it raises interesting questions about the relative expression of c-Myc versus Foxp3 during Treg cell development. For example, is c-Myc expressed early and then suppressed by Foxp3, and is this process dynamic to allow the type of reciprocal cycling between glycolysis and OXPHOS that supports proliferative expansion followed by maximal suppressive function within Treg cell populations? Finally, how do the findings in mouse Treg cells described in the paper translate to human Treg cells, where glycolysis has been shown to be important for suppressive function (De Rosa et al., 2015)?

REFERENCES

Angelin, A., Gil-de-Gómez, L., Dahiya, S., Jiao, J., Guo, L., Levine, M.H., Wang, Z., Quinn, W.J., 3rd, Kopinski, P.K., Wang, L., et al. (2017). Cell Metab. *25*, this issue, 1282–1293.

Burzyn, D., Kuswanto, W., Kolodin, D., Shadrach, J.L., Cerletti, M., Jang, Y., Sefik, E., Tan, T.G., Wagers, A.J., Benoist, C., and Mathis, D. (2013). Cell *155*, 1282–1295.

De Rosa, V., Galgani, M., Porcellini, A., Colamatteo, A., Santopaolo, M., Zuchegna, C., Romano, A., De Simone, S., Procaccini, C., La Rocca, C., et al. (2015). Nat. Immunol. *16*, 1174–1184.

Gandolfo, M.T., Jang, H.R., Bagnasco, S.M., Ko, G.J., Agreda, P., Satpute, S.R., Crow, M.T., King, L.S., and Rabb, H. (2009). Kidney Int. *76*, 717–729.

Gerriets, V.A., Kishton, R.J., Johnson, M.O., Cohen, S., Siska, P.J., Nichols, A.G., Warmoes, M.O., de Cubas, A.A., MacIver, N.J., Locasale, J.W., et al. (2016). Nat. Immunol. *17*, 1459–1466.

Howie, D., Cobbold, S.P., Adams, E., Ten Bokum, A., Necula, A.S., Zhang, W., Huang, H., Roberts,

D.J., Thomas, B., Hester, S.S., et al. (2017). JCI Insight *2*, e89160.

Michalek, R.D., Gerriets, V.A., Jacobs, S.R., Macintyre, A.N., MacIver, N.J., Mason, E.F., Sullivan, S.A., Nichols, A.G., and Rathmell, J.C. (2011). J. Immunol. *186*, 3299–3303.

Preston, G.C., Sinclair, L.V., Kaskar, A., Hukelmann, J.L., Navarro, M.N., Ferrero, I., MacDonald, H.R., Cowling, V.H., and Cantrell, D.A. (2015). EMBO J. *34*, 2008–2024.

Scharping, N.E., Menk, A.V., Moreci, R.S., Whetstone, R.D., Dadey, R.E., Watkins, S.C., Ferris, R.L., and Delgoffe, G.M. (2016). Immunity *45*, 374–388.

Wang, R., Dillon, C.P., Shi, L.Z., Milasta, S., Carter, R., Finkelstein, D., McCormick, L.L., Fitzgerald, P., Chi, H., Munger, J., and Green, D.R. (2011). Immunity *35*, 871–882.