DENDRITIC CELLS

TLR agonists trigger rapid metabolic changes

The transition of dendritic cells (DCs) from a quiescent into an activated state requires metabolic changes to support the increased anabolic demands of cellular activation, but exactly what this metabolic reprogramming entails is poorly understood. Now, Pearce and colleagues show that the glycolytic rate in DCs increases within minutes after stimulation with a Toll-like receptor (TLR) agonist, and that this response is crucial for the *de novo* synthesis of fatty acids and consequently for DC activation.

Previous studies have shown that the survival of DCs that are differentiated from bone marrow cells *in vitro* — in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) — depends on a metabolic transition that is characterized by an increased rate of glycolysis 24 hours after activation. In this study, the authors investigated the early metabolic changes that occurred after stimulation with TLR agonists, as it was hypothesized that these events would be important to meet the energetic and anabolic demands of TLR-driven DC activation.

Using GM-CSF-induced bone marrow-derived DCs, the authors monitored real-time changes in glycolytic metabolism by determining the rate of extracellular acidification after stimulation with lipopolysaccharide (LPS); there was a rapid increase in the rate of glycolysis within minutes of DC activation. Furthermore, global profiling of metabolites revealed

changes in the abundance of metabolic intermediates of glycolysis and the tricarboxylic acid (TCA) cycle. Stimulation of DCs with LPS in the presence of the hexokinase inhibitor 2-deoxyglucose resulted in blocked glycolysis, and these cells had impaired cell surface expression of the co-stimulatory molecules CD40 and CD86, and of MHC class I and class II molecules; consequently, these cells showed an impaired T cell priming capacity. Thus, an early increase in the rate of glycolysis is important for TLR-induced DC activation and function.

Next, the authors determined whether the increased rate of glycolysis is required to provide extra ATP or to supply carbon for anabolic processes that are crucial for DC activation. As their experiments could not discern a role for glycolytic ATP in DC activation, the authors hypothesized that the increased rate of glycolysis generates more pyruvate to feed into the TCA cycle, which would lead to altered mitochondrial activity. Indeed, the mitochondrial membrane potential and respiratory capacity increased rapidly after LPS stimulation. In addition, glucose-derived carbon was more rapidly incorporated into TCA cycle intermediates in LPS-stimulated DCs compared with unstimulated cells, and this increase was prevented by 2-deoxyglucose. Hence, the improved mitochondrial fitness was mediated by the increased flux of glycolytic carbon into the TCA cycle.

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So, what is the effect of the increased entry of glucose-derived carbon into the TCA cycle? The authors found that the intracellular concentration of citrate and isocitrate — but not that of downstream intermediates in the TCA cycle — decreased after LPS stimulation. As citrate is the main source of carbon for the *de novo* synthesis of fatty acids, the authors speculated that the rapid increase in the glycolytic rate was required to support this. Indeed, the authors found that inhibition of the citrate shuttle or of fatty acid synthase impaired the LPS-induced activation of DCs. Furthermore, using transmission electron microscopy the authors discovered that the endoplasmic reticulum and Golgi were expanded in stimulated DCs in a manner that was dependent on *de novo* fatty acid synthesis, which suggests that the increased rate of glycolysis promotes this expansion to support the increase in protein synthesis, transport and secretion that is required for DC activation.

Finally, the authors investigated the signalling pathways mediating the TLR-induced increase in the rate of glycolysis. They found that AKT, which is activated by the two upstream kinases TBK1 and I κ B kinase- ϵ , promoted the association of the glycolytic enzyme hexokinase 2 with mitochondria, which was crucial for the increased rate of glycolysis.

In summary, this study shows that activation of TLRs results in an early and rapid increase in the rate of glycolysis in DCs, and that this metabolic transition is essential to meet the anabolic requirements of DC activation and function.

Elisabeth Kugelberg

ORIGINAL RESEARCH PAPER Everts, B. *et al.* TLR-driven early glycolytic reprogramming via the kinases TBK1-I κ B ϵ supports the anabolic demands of dendritic cell activation. *Nature Immunol.* <http://dx.doi.org/10.1038/ni.2833> (2014)