COMMENTARY
A critical role for citrate metabolism in LPS signalling
Luke A. J. O’NEILL
School of Biochemistry and Immunology, Trinity College Dublin, Dublin, Ireland

Macrophage activation is a key event in the inflammatory process, since these cells produce a range of pro-inflammatory molecules, including ROS (reactive oxygen species), prostaglandins, cytokines and nitric oxide. These factors promote inflammation by causing vasodilation and recruitment of neutrophils, monocytes and lymphocytes, which ultimately clear infection and repair damaged tissue. One of the most potent macrophage activators is the Gram-negative-derived bacterial cell wall component LPS (lipopolysaccharide). LPS is sensed by TLR4 (Toll-like receptor 4) and triggers highly complex signalling pathways that culminate in activation of transcription factors such as NF-kB (nuclear factor kB), which in turn increases transcription of genes encoding proteins such as COX2 (cyclo-oxygenase 2, a key enzyme in prostaglandin biosynthesis), nitric oxide synthase and cytokines such as TNF (tumour necrosis factor). Recently, a role for metabolic pathways in the regulation of LPS signalling has become a focus of research in inflammation. A notable example is LPS promoting the so-called Warburg effect—aerobic glycolysis. This allows for an up-regulation in ATP production, and also for the production of biosynthetic intermediates to meet the demands of the activated macrophages. In this issue of the Biochemical Journal, Infantino et al. add a new finding to the role of metabolism in LPS action. They demonstrate a requirement for the mitochondrial citrate carrier in the induction of ROS, nitric oxide and prostaglandins by LPS. The knockdown of the carrier with siRNA (small interfering RNA), or the use of an inhibitor BTA (benzene-1,2,3-tricarboxylate), abolishes these responses. Although no mechanism is provided, the authors speculate that acetyl-CoA generated could be required for phospholipid biosynthesis, the phospholipids being the source of arachidonic acid for prostaglandin production. Another product of citrate metabolism, oxaloacetate, will indirectly generate nitric oxide and ROS. This finding places citrate, transported from the mitochondria, as a key player in LPS signalling, at least for ROS, nitric oxide and prostaglandin production. This somewhat unexpected role for citrate in LPS action adds to a growing literature on the role for metabolism in the regulation of signalling in inflammation.

Key words: citrate carrier, lipopolysaccharide (LPS), metabolism, mitochondrion, Toll-like receptor 4 (TLR4).

To most immunologists and inflammation biologists, the word ‘metabolism’ conjures up memories of being forced to learn about glycolysis and the Krebs cycle (tricarboxylic acid cycle) as undergraduates. However, this perception is starting to change, as more and more papers are published demonstrating how during inflammatory cell signalling, including in macrophages and inflammatory T-lymphocytes such as Th17 cells, there is a profound alteration in the metabolic profile of the cell [1,2]. Such changes can have a determining role in the activation status of the cell, or indeed its developmental fate. Rather than being a background event which generates ATP or biosynthetic intermediates, metabolic pathways are beginning to be seen as more dynamic, with the metabolic changes in activated macrophages, for instance, having a determining role in macrophage responses.

There are currently two clear examples of these phenomena [2–4]. First, in different T-cell subsets there is a marked difference in glucose metabolism [2]. Th17 cells are a subtype of helper T-cell which has been shown to largely rely on glycolysis for ATP production. These helper T-cells are highly inflammatory since they produce the pro-inflammatory cytokine IL (interleukin)-17, which will induce neutrophil recruitment into the inflamed site. The basis for this shift in metabolism is induction of the transcription factor HIF-1α (hypoxia-inducible factor-1α), by the cytokines IL-23 and IL-6, well-known differentiators of Th17 cells. Remarkably, T-cells induced to become Tregs (regulatory T-cells) by TGF-β (transforming growth factor β), which are largely anti-inflammatory because they produce the anti-inflammatory cytokine IL-10, show no shift to glycolysis and have low levels of HIF-1α [2]. Most importantly, blocking glycolysis with 2-deoxyglucose reciprocally alters Th17 and Treg differentiation, reducing Th17, but promoting Treg differentiation. However, the mechanism linking glycolysis to HIF-1α is not fully known.

In the second example, and in an analogous fashion, LPS (lipopolysaccharide) will promote glycolysis in macrophages [3,4]. This is similar to the situation in Th17 cells, both cell types therefore having the classic ‘Warburg effect’ of aerobic glycolysis, a phenomenon first observed by Otto Warburg in the 1920s in tumour cells [5]. Metabolically, it is possible that inflammatory cells therefore have profiles highly similar to those of tumour cells [6], and this has been interpreted as being due to the increased demand for ATP and biosynthesis. Unlike the Krebs cycle, glycolysis can be strongly up-regulated and generate more ATP per unit time, even though the breakdown of glucose is not as efficient in terms of ATP generation as the process of oxidative phosphorylation in mitochondria. Boosting glycolysis will also generate biosynthetic intermediates from the pentose phosphate pathway and activated macrophages have a major requirement for such intermediates because of their prodigious capacity to generate a whole range of inflammatory products, notably

Abbreviations used: CIC, citrate carrier; HIF-1α, hypoxia-inducible factor-1α; IL, interleukin; LPS, lipopolysaccharide; ROS, reactive oxygen species; siRNA, small interfering RNA; TNF, tumour necrosis factor; Treg, regulatory T-cell.

1 email laoneill@tcd.ie
cytokines such as TNF (tumour necrosis factor). The mechanism whereby LPS increases glycolysis has been worked out to some extent, and involves induction of glycolytic enzymes including hexokinase and phosphofructokinase-2, as well as induction of pyruvate dehydrogenase kinase, which inhibits pyruvate dehydrogenase, thereby preventing the generation of acetyl-CoA for the Krebs cycle [1]. This ultimately leads to lactate production, a hallmark of activated macrophages. Another notable feature of the effect of LPS on metabolism is the inhibition of the expression of a range of mitochondrial enzymes involved in the Krebs cycle, an effective shut-down in mitochondrial metabolism therefore occurring in LPS-treated macrophages [7].

Why there would be a shift to glycolysis in inflammatory cells is not fully known, although it has been suggested that it might be a way to preserve ATP levels during an immune response [3]. One function for this ATP has been to maintain the mitochondrial membrane potential and prevent apoptosis [4]. The second reason is to generate the biosynthetic intermediates needed for production of inflammatory cytokines. Both activated macrophages and Th17 cells will be very reliant on extracellular glucose concentrations, and so, if this should become limiting, this could decrease the inflammatory process somewhat, in order to prevent possible over-activation of inflammation which could be damaging to the host.

Into this complex area, we now have the findings by Infantino et al. [8] in this issue of the Biochemical Journal. Their data indicate a key role for the mitochondrial CIC (citrate carrier) in the induction of nitric oxide, ROS (reactive oxygen species) and prostaglandins by LPS in macrophages. The carrier will deliver citrate from the lumen of the mitochondria (where it is generated as an intermediate in the Krebs cycle) to the cytosol. The functional evidence for this conclusion comes from the specific ablation of CIC using siRNA (small interfering RNA). What stimulated these authors to examine the CIC in LPS action? As the authors state, fatty acids are known to modulate macrophage function in various ways. Fatty acid biosynthesis requires CIC, since it transports citrate to the cytosol, where it is cleaved by citrate lyase into acetyl-CoA and oxaloacetate. Acetyl-CoA is directly used for fatty acid biosynthesis, whereas oxaloacetate produces NADPH + H⁺. These cofactors are required for the generation of nitric oxide (via conversion of L-arginine into citrulline) and also ROS by the NADPH oxidase. The authors therefore addressed whether CIC was required for induction of nitric oxide and ROS by LPS, and also prostaglandins, which are derived from the fatty acid arachidonic acid.

The first observation was a marked induction of CIC expression in response to LPS in macrophages, with a 28-fold induction evident. This was ultimately explained by the demonstration that the CIC promoter contains two functional NF-κB-response elements. Then using either siRNA to silence expression of CIC, or a CIC inhibitor termed BTA (benzene-1,2,3-tricarboxylate), the authors demonstrated inhibition of induction of nitric oxide, ROS and prostaglandins in response to LPS. These data indicate that CIC is critical for these particular LPS responses, and the plausible explanation is that citrate must be transported into the cytosol to allow for production of these inflammatory factors.

These findings indicate a key role for intermediary metabolism, in this case of citrate, in LPS action. However, a number of questions arise. First, if LPS is able to down-regulate enzymes in the Krebs cycle, how are citrate levels maintained? Second, what about other LPS responses such as induction of TNF? Although there is no obvious role for citrate metabolism in the induction of TNF, it could be that citrate export from the mitochondria is an important signal for LPS action in general. Thirdly, does LPS promote expression of other mitochondrial carriers, which would deliver other intermediates to the cytosol which could then signal? It is intriguing to speculate that in a manner analogous to cytochrome c release from the mitochondria in apoptosis, intermediate metabolites from the mitochondria might be delivered to the cytosol by carriers induced by LPS. This could in turn signal a response needed for LPS to induce inflammatory mediator production. Such metabolites could have an impact on transcription or, as is the case here, on biosynthesis.

As we delve deeper using newer technologies into the metabolic world of a cell activated during inflammation, it may be that the induction of the Warburg effect in tumour cells by inflammatory stimuli could be tumorigenic, providing another link between inflammation and cancer. Might there be genetic alterations in enzymes in metabolic pathways or mitochondrial carriers that could enhance or limit these pathways and associate with inflammatory diseases, as has been shown for example in the case of succinate dehydrogenase in tumours [9]? New targets for metabolites in signalling might also be uncovered. Finally, careful attenuation of these responses (e.g. partial inhibition of CIC) could have a therapeutic potential in the treatment of inflammatory diseases.

REFERENCES