Immunology & Cell Biology 2021; **99**: 122–125
www.wileyonlinelibrary.com/journal/icb

NEWS AND COMMENTARY

Bridging the gap – a new role for STAT3 in TLR4-mediated metabolic reprogramming

Hauke J Weiss & Luke AJ O'Neill

School of Biochemistry and Immunology, Trinity College Dublin, Trinity Biomedical Sciences Institute, Dublin 2, Ireland

Immunology & Cell Biology 2021; **99**: 122–125; doi: 10.1111/imcb.12414

In this article, we discuss a new study from the laboratory of Ashley Mansell that successfully links innate immune receptor signaling to metabolic changes in macrophages, establishing for the first time an uninterrupted pathway from ligand binding to mitochondrial reprogramming and subsequential effector function modulation in this cell type.[1]

Macrophages were first described by Ilya Metchnikoff as early as 1882 and have since been identified as critical players in innate immunity. The main function of macrophages is the clearance of microbes, cancer cells, cellular debris and other foreign components in a process called phagocytosis, in which the macrophage engulfs and digests its target. Macrophages detect foreign substances through an array of pattern-recognition receptors (PRRs), one of which is the lipopolysaccharide (LPS) sensing receptor Toll-like receptor 4 (TLR4). Activation of TLR4 on macrophages induces distinct metabolic changes, characterized by increased glycolysis, accumulation of the tricarboxylic acid (TCA) cycle metabolite succinate[2] and decreased oxidative phosphorylation (OXPHOS).[3] These changes facilitate the increased demand for energy and biomaterials and are crucial for macrophage cytokine production and effector function.[4] A direct result of succinate accumulation in macrophages is the stabilization of hypoxia-inducible factor-1α (HIF-1α), a key mediator in the expression of pro-glycolytic genes and the production of pro-inflammatory cytokines such as interleukin-1β (IL-1β).[5] Precise mechanistic details on how TLR4 shapes metabolic reprogramming, however, are lacking. TLR4 drives phosphorylation of the well-known TLR activated kinases TANK-binding kinase 1 (TBK-1) and IκB kinase-ε (IKKε)[6] and promotes glycolysis.[7] In an elegant study reported in this issue, Balic *et al.* link these kinases to the signal transducer and activator of transcription 3 (STAT3), which was known to impact mitochondrial metabolism[8] and to induce the generation of reactive oxygen species (ROS).[9] The study proposes a new non-canonical role for STAT3 in TLR-mediated signaling.

Tumor necrosis factor receptor (TNFR)-associated factor 6 (TRAF6) is a well-known mediator of signaling events downstream of TLR activation.[10] Due to its structure, Balic *et al.* expected TRAF6 to be a binding partner of activated STAT3, a hypothesis they were able to confirm by identifying TRAF6 binding motifs on STAT3, and subsequently pulling down STAT3 together with TRAF6 in LPS-activated macrophages, thus presenting the first link between STAT3 activation and TLR signaling. STAT3 phosphorylation on Tyr705 is known to induce translocation to the nucleus and to activate the expression of several genes; however, TLR4 stimulation induced phosphorylation at a different site, Ser727, rapidly enough to be considered part of direct downstream signaling events, rather than an autocrine secondary effect. The group and others had previously shown that phosphorylation at this particular site translocates STAT3 to the mitochondria. Here, STAT3 alters mitochondrial metabolism by driving the activity of complex I and II of the electron transport chain, ultimately enhancing mitochondrial respiration.[8] STAT3 phosphorylation was not detected in the myeloid differentiation primary response 88 (MyD88) deficient cells. As MyD88 is a well-known adaptor protein downstream of TLR signaling, these results integrate STAT3 into TLR signaling for the first time (Figure 1).

At this point, the relationship between Ser727 and Tyr705 phosphorylation of STAT3 should be mentioned, as these events are not subject to two completely independent functioning pathways. Both phosphorylation events seem to influence each other under certain conditions; however, details are

Correspondence
Hauke J Weiss, School of Biochemistry and Immunology, Trinity College Dublin, Trinity Biomedical Sciences Institute, Dublin 2, Ireland.
E-mail: weissh@tcd.ie

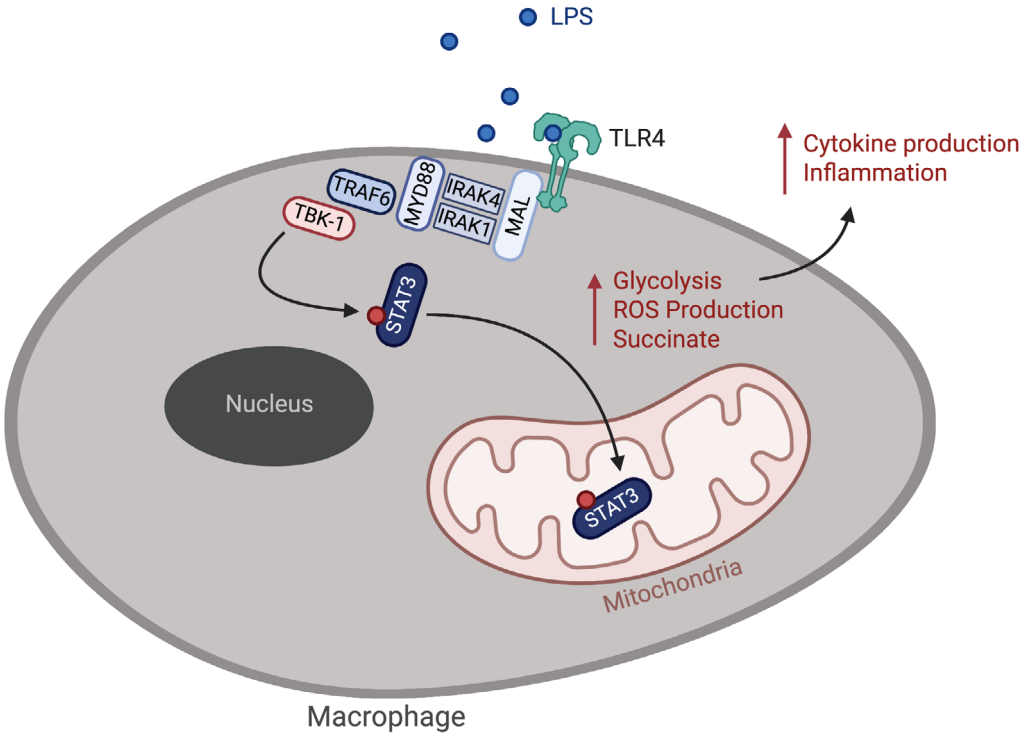

Figure 1. Lipopolysaccharide-induced activation of TLR4 results in TRAF6-mediated recruitment of the serine kinase TBK-1. TBK-1 subsequently phosphorylates STAT3 at Ser727, which is then translocated to the mitochondria. Here, STAT3 induces drastic metabolic reprogramming, resulting in increased glycolytic activity, succinate accumulation and cytokine production, driving a pro-inflammatory phenotype.

subject to debate. Regardless, the authors next aimed to investigate whether STAT3 Ser727 phosphorylation was indeed critical for TLR-induced metabolic reprogramming. Mutating the relevant serine in mouse peritoneal macrophages decreased both resting glycolysis and the glycolytic burst upon TLR4 stimulation, confirming phosphorylation of Ser727 to be a critical step in the pathway. The mutant cells exhibited a drastically reduced oxygen consumption rate (OCR) and maximal respiratory capacity compared with wild type cells. These cells were viable, but unable to respond to LPS stimulation, as respiratory capacity could not be upregulated above basal levels. Other than changes in the respiratory chain, LPS-stimulated macrophages also exhibit characteristic breaks in the TCA cycle, leading to accumulation of the pro-inflammatory metabolite succinate. Succinate production not only deprives the respiratory chain of its substrate, it is also required for ROS pro-inflammatory IL-1 production.[5] The authors found that in the above mentioned STAT3 mutants, LPS-induced succinate production was reduced compared with wild type cells. Conversely, the mutants exhibited increased the production of lactate, suggesting that due to defective mitochondrial metabolism, they rely more on aerobic glycolysis than wild type macrophages. These data suggest that Ser727 phosphorylation of STAT3 is not only required for induction of OXPHOS, but also for LPS-induced accumulation of succinate.

The authors now asked what kinases might phosphorylate STAT3? As the group had previously shown mitochondrial STAT3 to be responsible for the generation of ROS,[11] mitochondrial ROS concentrations were used as a readout while screening a large library of kinase inhibitors. They pointed to TBK-1, which was then shown to interact with both TRAF6 and STAT3 upon LPS stimulation. Deletion of TBK-1 confirmed this kinase to be responsible for Ser727 phosphorylation of STAT3. The related kinase IKKε was subsequently shown to be required for full STAT3 phosphorylation as well. Both kinases were previously mainly known for being involved in TLR-induced IRF3 phosphorylation and induction of IFNβ, another pro-inflammatory cytokine.[12] TBK-1 dysregulation has been found to be involved in multiple inflammatory diseases such as colitis, rheumatoid arthritis, hepatitis, and atherosclerosis,[13] while an interesting study published this year has

implicated IKKε in tumor formation in breast cancer.[14] These data elegantly explain the immunomodulatory properties of these kinases by revealing a new substrate and completing the signaling pathway below. Furthermore, this discovery reveals differences between metabolic reprogramming in myeloid cell lines. While dendritic cells for example rely on phosphoinositide 3-kinase (PI3K) and Akt activity for TBK-1/IKKε induced glycolysis, macrophages increase glycolytic activity independent of these mediators.

Lastly, it only remained to be investigated whether the metabolic changes associated with TLR4-induced STAT3 signaling result in actual functional consequences in macrophages. A key effector function in LPS-stimulated macrophages is the production of IL-1β. Generation of this pro-inflammatory cytokine is critically dependent on TCA cycle remodeling and the subsequent succinate accumulation and should therefore be mediated by the pathway described in this study. When stimulated with LPS, macrophages with mutated STAT3 were impaired in IL-1β mRNA generation and protein production compared with wild type cells. The production of IL-6, IL-10 and tumor necrosis factor (TNF) upon LPS challenge was also reduced in the mutants, while basal IL-10 expression was slightly increased. STAT3 mutant mice were also analyzed *in vivo* in a model of LPS-induced sepsis, and, in line with the *in vitro* experiments, the production of IL-1β and IL-6, as well as chemokine (C-C motif) ligand 2 (Ccl2) were decreased in the serum of mutant mice following LPS challenge, while TNF production was unchanged. This is surprising as TNF dependent macrophage functions have been postulated to require mitochondrial STAT3,[15] suggesting the possibility of surrogate pathways in physiologically relevant conditions. The roles of TNF and also of the anti-inflammatory cytokine IL-10 in this pathway could indeed be a subject for further investigation.

Together, these findings demonstrate a critical role of STAT3 Ser727 phosphorylation in LPS-induced cytokine production in macrophages and therefore effector function. The authors have unraveled a new process in LPS-induced metabolic reprogramming in macrophages. LPS induces TRAF6-mediated phosphorylation of STAT3 by TBK-1, resulting in localization of STAT3 to the mitochondria, shaping metabolic remodelling and subsequently effector functions. One interesting question for further exploration is precisely how STAT3 regulates mitochondrial functions. It is well established that mitochondrial STAT3 can regulate ATP synthesis, increase mitochondrial Ca^{2+} influx and decrease ROS release in T and B lymphocytes. Here, STAT3 associates with various components of the electron transport chain (ETC), enhancing ETC complex activity.[16] Interestingly, a recent study has found that mitochondrial STAT3 phosphorylated at Ser727 induced autophagy in human gastric epithelial cells when infected with *Helicobacter pylori*,[17] expanding the list of the potential functions of mitochondrial STAT3. As a well-described transcription factor, STAT3 could possibly also directly regulate mitochondrial gene expression. The study presented in this article elegantly pieces together established pathways to increase our understanding of immune cells to explain cell fate and effector function. It is a great example of how expanding one's horizon and interpreting previous observations can lead to important new discoveries.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Hauke J Weiss: Conceptualization; Writing-original draft; Writing-review & editing. **Luke O'Neill:** Conceptualization; Writing-original draft; Writing-review & editing.

REFERENCES

1. Balic JJ, Albargy H, Luu K, *et al*. STAT3 serine phosphorylation is required for TLR4 metabolic reprogramming and IL-1β expression. *Nat Commun* 2020; **11**: 3816.
2. Mills EL, Kelly B, Logan A, *et al*. Succinate dehydrogenase supports metabolic repurposing of mitochondria to drive inflammatory macrophages. *Cell* 2016; **167**: 457–470.
3. Garaude J. Reprogramming of mitochondrial metabolism by innate immunity. *Curr Opin Immunol* 2019; **56**: 17–23.
4. O'Neill LAJ, Kishton RJ, Rathmell J. A guide to immunometabolism for immunologists. *Nat Rev Immunol* 2016; **16**: 553–565.
5. Tannahill GM, Curtis AM, Adamik J, *et al*. Succinate is an inflammatory signal that induces IL-1β through HIF-1α. *Nature* 2013; **496**: 238–242.
6. Everts B, Amiel E, Huang SC-C, *et al*. TLR-driven early glycolytic reprogramming via the kinases TBK1-IKKε supports the anabolic demands of dendritic cell activation. *Nat Immunol* 2014; **15**: 323–332.
7. Tan Y, Kagan JC. Innate immune signaling organelles display natural and programmable signaling flexibility. *Cell* 2019; **177**: 384–398.
8. Wegrzyn J, Potla R, Chwae Y-J, *et al*. Function of mitochondrial Stat3 in cellular respiration. *Science* 2009; **323**: 793–797.

9. Mantel C, Messina-Graham S, Moh A, *et al*. Mouse hematopoietic cell–targeted STAT3 deletion: stem/progenitor cell defects, mitochondrial dysfunction, ROS overproduction, and a rapid aging–like phenotype. *Blood* 2012; **120**: 2589–2599.
10. Verstak B, Stack J, Ve T, *et al*. The TLR signaling adaptor TRAM interacts with TRAF6 to mediate activation of the inflammatory response by TLR4. *J Leukoc Biol* 2014; **96**: 427–436.
11. Garama DJ, Harris TJ, White CL, *et al*. A synthetic lethal interaction between glutathione synthesis and mitochondrial reactive oxygen species provides a tumor-specific vulnerability dependent on STAT3. *Mol Cell Biol* 2015; **35**: 3646–3656.
12. Fitzgerald KA, McWhirter SM, Faia KL, *et al*. IKKε and TBK1 are essential components of the IRF3 signaling pathway. *Nat Immunol* 2003; **4**: 491–496.
13. Yu T, Yi Y-S, Yang Y, Oh J, Jeong D, Cho JY. The pivotal role of TBK1 in inflammatory responses mediated by macrophages. *Mediators Inflamm* 2012; **2012**: 1–8.
14. Wilcz-Villega E, Carter E, Ironside A, *et al*. Macrophages induce malignant traits in mammary epithelium via IKKε/TBK1 kinases and the serine biosynthesis pathway. *EMBO Mol Med* 2020; **12**: e10491.
15. Shulga N, Pastorino JG. GRIM-19-mediated translocation of STAT3 to mitochondria is necessary for TNF-induced necroptosis. *J Cell Sci* 2012; **125**: 2995–3003.
16. Rincon M, Pereira F. A new perspective: mitochondrial Stat3 as a regulator for lymphocyte function. *Int J Mol Sci* 2018; **19**: 1656.
17. Piao J-Y, Kim S-J, Kim D-H, *et al*. *Helicobacter pylori* infection induces STAT3 phosphorylation on Ser727 and autophagy in human gastric epithelial cells and mouse stomach. *Sci Rep* 2020; **10**: 15711.