*Nature Reviews Immunology* | Published online 18 Jul 2016; doi:10.1038/nri.2016.84

S. Bradbrook/NPG

IMMUNOMETABOLISM

# Innate sensing role for metabolic enzyme

A new study from the Underhill laboratory has found that a metabolic enzyme that regulates glycolysis can also serve as an innate sensor during bacterial infection. The authors show that sugars from bacterial peptidoglycan are bound by hexokinase, leading to its dissociation from the mitochondrial outer membrane and triggering the downstream activation of the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome.

Peptidoglycan is found in the cell wall of Gram-positive bacteria, and its degradation leads to the release of muramyl dipeptide, which activates the cytosolic NOD2 receptor. However, peptidoglycan also activates the NLRP3 inflammasome in macrophages (leading to caspase 1 activation and interleukin-1β (IL-1β) secretion) in a NOD2-independent manner through an undefined pathway. The authors set out to explore the mechanistic basis of this response. They found that, unlike other activators of NLRP3, peptidoglycan activates the NLRP3 inflammasome in lipopolysaccharide (LPS)-primed macrophages independently of potassium efflux. Interestingly, and also unlike other NLRP3 activators, peptidoglycan-induced activation of caspase 1 did not induce macrophages to undergo pyroptosis, an inflammatory form of cell death. The authors tested various degradation products of peptidoglycan and found that N-acetylglucosamine (NAG) — a sugar subunit found in the backbone of peptidoglycan — is the minimal component of the polysaccharide that is responsible for activation of the NLRP3 inflammasome. In keeping with this, peptidoglycan from *Bacillus anthracis*, which contains little NAG owing to the deacetylation of NAG by a bacterial enzyme, did not activate the NLRP3 inflammasome. By contrast, *B. anthracis* peptidoglycan that had been re-acetylated to contain artificially high amounts of NAG induced IL-1β production in an NLRP3-dependent manner.

Previous studies have shown that NAG can block glycolysis by competing with glucose for binding to hexokinase, which catalyses the phosphorylation of glucose to glucose-6-phosphate (G6P) in the first stage of glycolysis. Hexokinase associates with the mitochondrial outer membrane through its interaction with the voltage-dependent anion channel (VDAC), an important regulator of mitochondrial function, and the authors hypothesized that NAG might interfere with this interaction. Indeed, they found that stimulation of macrophages with peptidoglycan or NAG led to dissociation of hexokinase from mitochondria but did not disrupt overall mitochondrial function. Of note, treatment of LPS-primed macrophages with HKVBD (a peptide that induces dissociation of hexokinase from VDAC) also induced activation of the NLRP3 inflammasome. These findings suggest that NAG activates the NLRP3 inflammasome by inducing the dissociation of hexokinase from mitochondria.

The authors found that metabolic conditions that lead to the release of hexokinase into the cytosol also trigger activation of the NLRP3 inflammasome. Glycolysis is partly regulated through feedback inhibition, and high levels of G6P block the first step of glycolysis by triggering the release of hexokinase from the mitochondria. Accordingly, treatment of primed macrophages with G6P promoted NLRP3 inflammasome activation and secretion of IL-1β. Similarly, treatment of primed macrophages with the tricarboxylic acid cycle intermediate citrate (which, when in excess, backs up glycolysis and elevates G6P levels) also triggered IL-1β production via activation of the NLRP3 inflammasome.

Phagocytosis originally evolved as a mechanism to obtain nutrients through the degradation of other microorganisms; the authors suggest that phagocytes in mammals have since adapted the metabolic machinery of the cell to sense microbial sugars or metabolic disturbances indicative of potential danger. Interestingly, *B. anthracis* seems to have evolved a strategy to evade the hexokinase-sensing pathway described in this study. It is still unclear exactly how the dissociation of hexokinase from mitochondria triggers inflammasome activation, but this study adds to our growing appreciation of the link between cellular metabolism and innate immune signalling.

*Yvonne Bordon*

**ORIGINAL ARTICLE** Wolf, A. J. *et al.* Hexokinase is an innate immune receptor for the detection of bacterial peptidoglycan. *Cell* http://dx.doi.org/10.1016/j.cell.2016.05.076 (2016)

> “a metabolic enzyme that regulates glycolysis can also serve as an innate sensor during bacterial infection”