PHAGOCYTOSIS

Mitochondria and phagosomes: better together

Phagocyte destruction of engulfed microorganisms depends on the production of bactericidal reactive oxygen species (ROS) by both the phagosomal NADPH oxidase pathway and mitochondria, but the mechanisms by which ROS production is coordinated spatially and temporally have been unclear. This study describes a new signalling pathway downstream of Toll-like receptor (TLR) stimulation by which the kinases MST1 and MST2 regulate the trafficking of mitochondria to phagosomes to deliver ROS.

Deficiency of MST1 in humans results in a severe immunodeficiency syndrome, and mice with haematopoietic cell-specific knockout of *Mst1* and *Mst2* have multiple, recurrent bacterial infections, which is indicative of an innate immune defect. Indeed, mice generated for this study with double knockout of *Mst1* and *Mst2* in myeloid cells (referred to as cDKO mice) were more susceptible to bacterial peritonitis than wild-type controls despite an enhanced inflammatory response, and cDKO phagocytes were defective in their clearance of intracellular bacteria. Furthermore, stimulation of cell-surface TLRs (TLR1, TLR2 and TLR4) was shown to activate MST1 and MST2 in a MYD88-dependent manner. Together, the results indicate that innate immune activation of phagocytes through TLRs leads to MST1 and MST2 activation that is required for intracellular killing of bacteria.

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In the absence of MST1 and MST2, the induction of mitochondrial ROS production after bacterial infection was markedly impaired, despite the normal ability of cDKO mitochondria to generate ROS in response to electron-transport inhibitors. The authors showed that cDKO macrophages had defective recruitment of mitochondria to phagocytosed bacteria as a result of disrupted F-actin cytoskeletal organisation downstream of defective activation of the RAC GTPase proteins. Mice expressing a constitutively active form of RAC1 in cDKO myeloid lineages had normal F-actin organisation, normal mitochondrion–phagosome juxtaposition and normal production of ROS after TLR stimulation.

Next, the authors looked at the mechanism by which TLR-stimulated MST1 and MST2 activate RAC proteins to positively regulate phagosome-mediated bacterial killing. MST2 co-precipitated with and phosphorylated protein kinase Cα (PKCα), which in turn interacted with and phosphorylated the RHO–GDP dissociation inhibitor LYGDI (also known as ARHGDIB), thereby disrupting its interaction with RAC–GDP to enable RAC protein activation by GTP binding.

Downstream of RAC1–GTP, it was shown that the E3 ubiquitin ligase TRAF6 catalyses the Lys63-linked polyubiquitylation and further activation of RAC1. A constitutively active form of RAC1 was ubiquitylated to a greater extent than wild-type RAC1, whereas RAC1–GDP was resistant to ubiquitylation. In keeping with this, the impaired activation of RAC1 in TLR-stimulated cDKO macrophages correlated with less ubiquitylation of RAC1. As TRAF6 binds selectively to inactive RAC1, the polyubiquitylation and further activation of RAC1 by TRAF6 resulted in the release of TRAF6 to bind the mitochondrial complex I assembly factor ECSIT. The TRAF6–ECSIT complex in turn mediates mitochondrion–phagosome juxtaposition and ROS production.

As a constitutively active form of RAC1 fully rescued the phenotype of cDKO mice, the authors conclude that RAC1–GTP — downstream of MST1 and MST2 activated by TLR signalling — has a crucial role in coordinating mitochondrial ROS production with its already established role in the activation of NADPH oxidase.

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ORIGINAL RESEARCH PAPER Geng, J. *et al.* Kinases Mst1 and Mst2 positively regulate phagocytic induction of reactive oxygen species and bactericidal activity. *Nat. Immunol.* http://dx.doi.org/10.1038/ni.3268 (2015)