Toll-like receptors hit calcium

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Mitochondrial Ca^{2+} uptake is a multifarious signal that controls both the activity of matrix dehydrogenases and sensitivity to apoptotic and necrotic challenges. Recent evidence indicates that mitochondria also play a role in triggering inflammation, as mitochondrial DNA, when released by the cell, is an important DAMP. There was no evidence so far that toll-like receptors (TLRs) close the loop, by affecting in turn mitochondrial activity. Two papers by Shintani et al. [1,2] reveal a new transduction mechanism of TLRs that impinges directly on mitochondrial function. Upon binding of CpG oligodeoxynucleotides, TLR9, which in non-immune cells is retained in the ER, inhibits SERCA2, thus reducing Ca^{2+} transfer to the mitochondria and stimulation of aerobic metabolism.

A few years ago, putting together inflammation and mitochondrial Ca^{2+} handling would have been quite a bizarre idea. The complex signaling network downstream of cytokine receptors was accepted to lead to the nucleus, and minimally affect mitochondria, and in inflammatory responses more attention was placed on oxygen consumption by NADPH oxidases than on the housekeeping machinery of aerobic respiration. Then, some surprising, novel information gradually set the stage for the heterodox association.

Indeed, Ca^{2+} accumulation by energized mitochondria, an old notion of bioenergetics, has entered a glittering phase. Numerous examples now highlight the concept that cellular Ca^{2+} signals, evoked by a variety of physiological or pathological challenges, are decoded within mitochondria into effects as diverse as increase of ATP production, release of apoptotic cofactors or bioenergetic collapse in necrosis. Moreover, in a variety of human diseases, ranging from neurodegenerative and metabolic disorders to cancer, alterations in mitochondrial Ca^{2+} handling plays a role in pathogenesis [3]. Then, mitochondria directly stepped into the mechanisms of inflammation, as they were shown not only to be a target of a toxic and/or immune damage, but also to directly promote the initiation and/or potentiation of inflammatory reactions by triggering Toll-like receptor (TLR) signaling. TLRs are a family of receptors, initially identified in immune cells, that includes 10 and 12 paralogues in humans and mice, respectively. Upon binding of specific ligands of bacterial, viral or fungal source (pathogen-associated molecular patterns, PAMPs), a signalling cascade is activated which culminates in the transcription of genes for inflammatory mediators, such as TNF-α and IL-6. In addition to microbial PAMPs, TLRs can sense also endogenous molecules released from infected or stressed cells (damage-associated molecular pattern, DAMPs). These ligands include nuclear structural components (HMG-B1), heat-shock proteins (HSP60 and HSP70), and also components of mitochondria, such as mtDNA [4]; the latter is released extracellularly upon tissue damage and is rich in unmethylated CpGs.

Finally, in the recent past the paradigm that TLRs are invariably associated with pro-inflammatory effects has been amended by the evidence that small doses of PAMPs may result in an attenuated inflammatory response to subsequent larger doses of PAMPs or to injury. This phenomenon is thought to be due to the transcription of genes coding for inhibitors of the TLR-NFκB signalling pathway [5]. Moreover, evidence that TLRs are not exclusively expressed in immune cells, but also in several other types of cells, including neurons and cardiomyocytes [5], suggested that this anti-inflammatory mechanism might operate directly in the potential targets of the inflammatory damage. Among the TLRs ligands able to trigger an anti-inflammatory response, unmethylated CpG-oligodeoxynucleotide (CpG-ODN), ligands of TLR-9, were shown to be very potent. Indeed, their administration, which is well tolerated clinically, attenuates the acute inflammatory cardiac dysfunction induced by both LPS and ischemia-reperfusion, by inhibiting the NFκB-pathway in ventricular myocytes [6].

Shintani and colleagues [2] clarify an alternative TLR9 signalling pathway that, in addition to the canonical TLR-NFκB axis, accounts for the activation of an anti-inflammatory mechanism within the parenchymal cells of an inflamed tissue. The alternative route stems from a different intracellular sorting of TLR9 in immune and non-immune cells. In immune cells, the chaperone Unc93b1 cargoes TLR9 from the ER to the endo/lysosomal compartment, where processing of the receptor and binding to CpG-ODN initiates the canonical MyD88-dependent pro-inflammatory signalling pathway [7]. In neurons or cardiomyocytes, at high risk of permanent damage by inflammation also due to their poor regenerative capacity, Unc93b1 is expressed at low levels [8], and TLR9 is mainly retained in the ER, where engagement by CpG-ODN triggers a different, hitherto unknown signalling route. Through biochemical studies, Shintani et al. identify SERCA2 (isoform 2 of the sarco-endoplasmic reticulum Ca^{2+} ATPase) as a protein directly interacting with TLR9. In particular, they show that in cardiomyocytes (but not in cardiac fibroblasts) TLR9, when engaged by CpG-ODN, interacts with the Ca^{2+} pump, reducing its activity and lowering [Ca^{2+}] in the ER lumen. As to the downstream consequences, the authors appropriately draw their attention to the emerging link between mitochondrial [Ca^{2+}] and pro-survival mechanisms, such as autophagy. Genetic ablation of the inositol 1,4,5 trisphosphate receptor, IP_3R, the Ca^{2+} release channel of the ER, was previously shown to dramatically increase autophagic rates, by impairing Ca^{2+} transfer to mitochondria and Ca^{2+}-dependent stimulation of aerobic metabolism. Hence, ATP levels were decreased and AMPK signaling to autophagy stimulated [9]. Accordingly, also Shintani et al. [1,2] observe in DAMP-challenged cells decreased mitochondrial [Ca^{2+}] levels and ATP production, and increased AMPK signaling. Although the authors did not directly address this issue, it is expected that decreased mitochondrial [Ca^{2+}] loading also correlates with lower sensitivity to apoptotic and necrotic challenges, thereby increasing cell resistance in the inflamed area. Indeed, numerous examples of pathology-related changes of mitochondrial Ca^{2+} homeostasis are available, and provide a coherent picture [2]. Oncogenes reduce ER Ca^{2+} levels and cancer-related miRNAs reduce the expression of the mitochondrial Ca^{2+} uniporter, thus reducing sensitivity to apoptosis, while tumor suppressor genes and viral proteins have the opposite effect. Thus, the observation that TLR9 protects parenchymal cells from death, operating through a different component of the Ca^{2+} signaling machinery, is an important addition to a well established conceptual framework.

The involvement of mitochondrial function as target of TLR activity, however, also opens the possibility that other processes, different from ATP production but still strictly dependent on mitochondrial bioenergetics, are involved in signal transduction. In particular, increased feeding of electrons to the respiratory chain due to the stimulation of Ca^{2+}-dependent matrix dehydrogenases, is expected to increase the production of ROS; interestingly, the latter are known to contribute to the stabilization of HIF-1, a transcription factor that activates the expression of a number of genes involved in the adaptation of tissues to hypoxia [10]. A reduction of mitochondrial Ca^{2+} loading should be paralleled by a decrease in ROS-dependent signaling, therefore it can be envisioned that the cellular change downstream of mitochondrial involvement could be very complex, and include both rapid changes in sensitivity to cell death pathways and a global change of the proteomic profile, that will be worth to analyze in detail.

Overall, the papers by Shintani et al. tie together the role of mitochondria in the initiation of inflammation and in the regulation of cell sensitivity to the inflammatory environment, by placing the focus on the Ca^{2+}-mediated signaling liaison between the ER and the mitochondria. It is tempting to think that the recent explosive advancement in the molecular understanding of mitochondrial Ca^{2+} transport [2] will now allow not only to rapidly expand these novel concepts, but also to develop new therapeutic approaches in the broad area of inflammatory diseases.

Conflict of interest

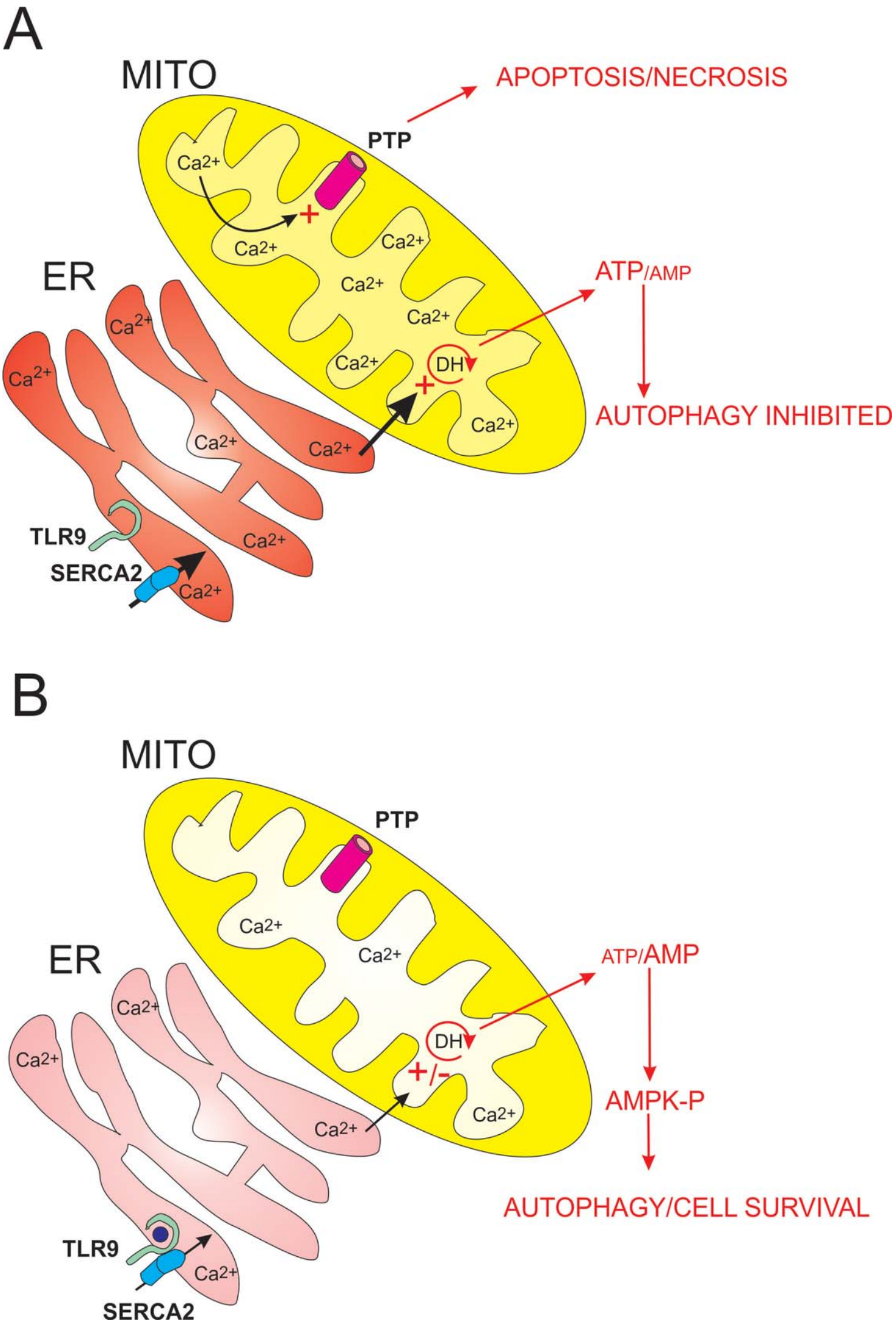
The authors declare that they have no conflict of interest.

References

1. Shintani Y, Kapoor A, Kaneko M, Smolenski RT, D'Acquisto F, Coppen SR, Harada-Shoji N, Lee HJ, Thiemermann C, Takashima S, *et al* (2013) Proc Natl Acad Sci USA 110: 5109 – 5114
2. Shintani Y, Drexler HC, Kioka H, Terracciano CM, Coppen SR, Imamura H, Akao M, Nakai J, Wheeler AP, Higo S *et al* (2014) EMBO Rep. DOI: 10.1002/embr.201337945
3. Rizzuto R, De Stefani D, Raffaello A, Mammucari C. (2012) *Nat Rev Mol Cell Biol* 13: 566 – 578
4. Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, Brohi K, Itagaki K, Hauser CJ. (2010) *Nature* 464: 104 – 107
5. Boyd JH (2012) *Curr Infect Dis Rep* 14: 455 – 461
6. Mathur S (2011) *Shock* 36: 478 – 483
7. Kim YM, Brinkmann MM, Paquet ME, Ploegh HL (2008) *Nature* 452: 234 – 238
8. Ramaiah SK, Günthner R, Lech M, Anders HJ (2013) *Int J Mol Sci* 14: 13213 – 13230
9. Cárdenas C, Miller RA, Smith I, Bui T, Molgó J, Müller M, Vais H, Cheung KH, Yang J, Parker I, *et al* (2010) *Cell* 142: 270 – 283
10. Zepeda AB, Pessoa A Jr, Castillo RL, Figueroa CA, Pulgar VM, Farías JG (2013) *Cell Biochem Funct* 31: 451 – 459

Legend to figure

Schematic view of the effect of TLR9 on mitochondrial Ca^{2+} signalling. **A.** In the absence of TLR9 engagement, Ca^{2+} transfer from the ER to mitochondria stimulates the Ca^{2+}-sensitive matrix dehydrogenases (DH) of the Krebs cycle, thus increasing electron feeding to the respiratory chain and stimulates ATP production. The increase in the ATP/AMP ratio decreases AMPK stimulation and suppresses autophagy. In addition, matrix [Ca^{2+}] favors the opening of the permeability transition pore (PTP), thus triggering the mitochondrial morphological and functional alterations of mitochondria which occur in apoptosis and necrosis. **B.** Upon engagement by CpG-ODN, TLR9 binds to SERCA2 and inhibits its activity, thus reducing both DH stimulation (and hence autophagy suppression) and sensitivity to apoptotic/necrotic challenges.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