 DOI 10.1002/eji.201141436 Eur. J. Immunol. 2011. 41: 1196–1202

Review

European Journal of Immunology

Mitochondria: Sovereign of inflammation?

Jürg Tschopp

Department of Biochemistry, University of Lausanne, Center for Immunity and Infection, Epalinges, Switzerland

NLRP3 inflammasome-dependent inflammatory responses are triggered by a variety of signals of host danger, including infection, tissue damage and metabolic dysregulation. How these diverse activators cause inflammasome activation is poorly understood. Recent data suggest that the mitochondria integrate these distinct signals and relay this information to the NLRP3 inflammasome. Dysfunctional mitochondria generate ROS, which is required for inflammasome activation. On the contrary, the NLRP3 inflammasome is negatively regulated by autophagy, which is a catabolic process that removes damaged or otherwise dysfunctional organelles, including mitochondria. In addition to the processing and secretion of pro-inflammatory cytokines such as IL-1β, NLRP3 inflammasome activation also influences cellular metabolic pathways such as glycolysis and lipogenesis. Mapping the connections between mitochondria, metabolism and inflammation is of great interest, as malfunctioning of this network is associated with many chronic inflammatory diseases.

Keywords: Immune regulation · Inflammation · Innate immunity

See accompanying reviews by Bevan and Dinarello, also winners of the 2010 Novartis Immunology Prizes, and the Forum article describing the Prizes

Detecting and repairing tissue damage

A consequence of multicellularity is the necessity for coordinated repair mechanisms to respond to damage induced by environmental, intracellular and extracellular insults. Examples include pathogens, DNA damage and metabolic stress. To mount an appropriate repair response to these dangerous situations, the organism requires mechanisms that identify the source of the problem and engage suitable effectors. Tissue damage, cellular stress and infection are sensed by the innate immune system through pattern recognition receptors (PRRs), which, upon activation, initiate defense and repair programs [1, 2]. If the cellular or tissue damage is extensive, these receptors trigger an acute inflammatory response. In the vast majority of cases, this response removes the injurious stimuli and repairs the damaged tissue within days of its initiation. In some situations, however, the source of tissue or cellular stress cannot be effectively resolved. Continued death of damaged cells leads to progressive destruction of the tissue, while the immune system initiates futile attempts to repair the damage. This simultaneous destruction and healing of tissue is characteristic of chronic inflammation, and is seen in a number of degenerative disease including arthritis, diabetes, inflammatory bowel diseases and many more (see accompanying review by Dinarello [3]).

The NOD-like receptor family and the NLRP3 inflammasome

The central role of the NOD-like receptor (NLR) family of cytoplasmic PRRs in the initiation of the inflammatory responses is becoming increasingly clear. NLRs typically have a tripartite architecture, consisting of a central and defining nucleotide binding and oligomerization (NACHT) domain, C-terminal leucine-rich repeats (LRRs) and an N-terminal effector domain (Fig. 1). There are 22 known human NLR family members, which

Correspondence: Dr. Jürg Tschopp
e-mail: jurg.tschopp@unil.ch

can be subgrouped in NLRP (NALP), NOD and IPAF clans (for recent reviews, see [1, 2]).

NLRs continuously monitor the cytosol for abnormal conditions. Upon activation, some NLR family members form multiprotein complexes, termed inflammasomes, which serve as platforms for caspase-1 activation and subsequent proteolytic maturation of the potent pro-inflammatory cytokine IL-1β (Fig. 1). So far, the NLR family members NLRP1, IPAF and NLRP3 and the HIN200 family member AIM2 have been demonstrated to nucleate inflammasomes. Due to its association with numerous inflammatory diseases, the NLRP3 inflammasome has drawn the most attention. Upon detecting cellular stress, NLRP3 oligomerizes and exposes its effector domain for interaction with the adaptor protein ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain), which in turn recruits pro-caspase-1. Pro-caspase-1 clustering leads to its activation via autoprocessing, and active caspase-1 proteolytically cleaves a variety of cytoplasmic targets, including IL-1β (Fig. 1).

Activation of the NLRP3 inflammasome

A wide variety of danger signals activate the NLRP3 inflammasome (for a review, see [4]). These include exogenous danger signals, such as pathogen-associated molecular patterns (PAMPs), and environmental irritants, as well as endogenous danger signals or damage-associated molecular patterns (DAMPs) such as ATP and gout-associated monosodium urate (MSU) crystals that are indicative of cellular malfunction. The mechanisms by which these activators trigger NLRP3 inflammasome activation are poorly understood. In light of the sheer number of NLRP3 inflammasome activators and their chemical and structural diversity, it seems unlikely that NLRP activation follows the same rules as proposed for Toll-like receptors (TLRs), namely that each of the receptors interacts directly with distinct PAMPs. Rather, it is more plausible that all agonists (or signals downstream of them) influence a common cellular structure or organelle that integrates the various activation signals and consequently initiates a signaling pathway that leads to inflammasome activation.

The first evidence for such an integrative organelle-mediated activation mechanism came from Latz and colleagues [5]. Their proposal, which is particularly attractive for large particulate activators such as monosodium urate, asbestos or silica, was that inefficient clearance of the particle following phagocytosis leads to lysosomal destabilization and rupture. The ensuing release of the lysosomal protease cathepsin B into the cytosol then triggers NLRP3 inflammasome activation either by direct cleavage of NLRP3, or by an uncharacterized pathway. However, macrophages from cathepsin B-deficient mice show minimal reduction of inflammasome activation [6]. This suggests that the other lysosomal cathepsins are redundant with cathepsin for inflammasome activation [7], or that lysosome-independent inflammasome activation mechanisms exist. The latter view is supported by the fact that lysosome rupture is not required for nonparticulate/small molecule NLRP3 activators such as nigericin and ATP [5].

The central role of mitochondria in NLRP3 inflammasome activation

All NLRP3 activators examined, including particulates, cause the generation of short-lived ROS. Furthermore, sequestration of ROS with chemical scavengers suppresses inflammasome activation in response to a range of agonists [6, 8]. Thus, similar

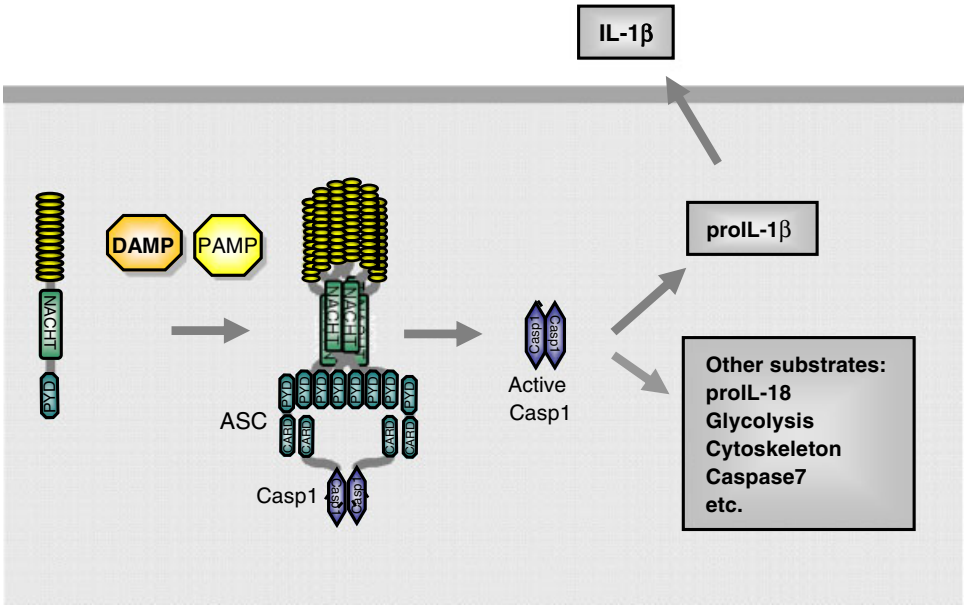

Figure 1. The NLRP3 inflammmasome. Upon sensing PAMPs or DAMPs, NLRP3 oligomerization and recruitment of ASC and procaspase-1 trigger autoactivation of caspase-1 and the maturation and secretion of pro-inflammatory cytokines, such as IL-1β. The NLRP3 inflammasome also directs caspase-1-mediated cleavage of a number of cytoskeletal proteins and glycolytic enzymes.

to the activation of the apoptosome [9], increased levels of ROS are essential for NLRP3 activation. The source of ROS was initially thought to be phagosome-associated NADPH oxidases, which are activated upon the engulfment of inflammasome-activating particulate agonists [6]. However, macrophages deficient in critical subunits of four (out of seven) NADPH oxidases complexes (NOX1, NOX2, NOX3 and NOX4) respond normally or have even slightly elevated inflammasome activity [10]. This suggests compensation by the remaining NADPH oxidase members, or the existence of an entirely different cellular source of inflammasome-activating ROS.

Indeed, recent evidence from two groups suggests that mitochondria are the main source of inflammasome-activating ROS, and as such may constitute the signal-integrating organelle for NLRP3 inflammasome activation [11, 12] (Fig. 2). NLRP3 inflammasome activation is highly impaired in macrophages in which mitochondrial activity is dampened by depletion of mitochondrial DNA [11] or by inactivation of the mitochondrial outer membrane protein VDAC (voltage dependent anion channel) [11, 12]. VDACs are the major channels for the exchange of metabolites and ions between the mitochondria and other cellular compartments including the endoplasmic reticulum (ER) [13]. As such, they are important regulators of mitochondrial metabolic activity, which is required for ROS production via the mitochondrial electron transport chain. In cells with diminished VDAC expression, caspase-1 activation is considerably impaired upon addition of all NLRP3 inflammasome activators tested. On the contrary, VDAC and thus mitochondria are not essential for the activation of the IPAF or the AIM2 inflammasomes [12]. VDAC activity is regulated by Bcl-2 family members. Overexpression of Bcl-2 leads to partial VDAC closure and a concomitant decrease of mitochondrial Ca^{2+} levels and ROS production [13]. Consistent with this, IL-1β levels are decreased in macrophages from *bcl2*-transgenic mice [12].

A link between NLRP3 and mitochondria is also suggested by the subcellular localization of NLRP3. Under resting conditions, most overexpressed and endogenous NLRP3 protein is associated with the ER [12]. Upon inflammasome stimulation, NLRP3 relocates to the perinuclear space and colocalizes with structures that stain positively for both the ER and the mitochondria. ASC,

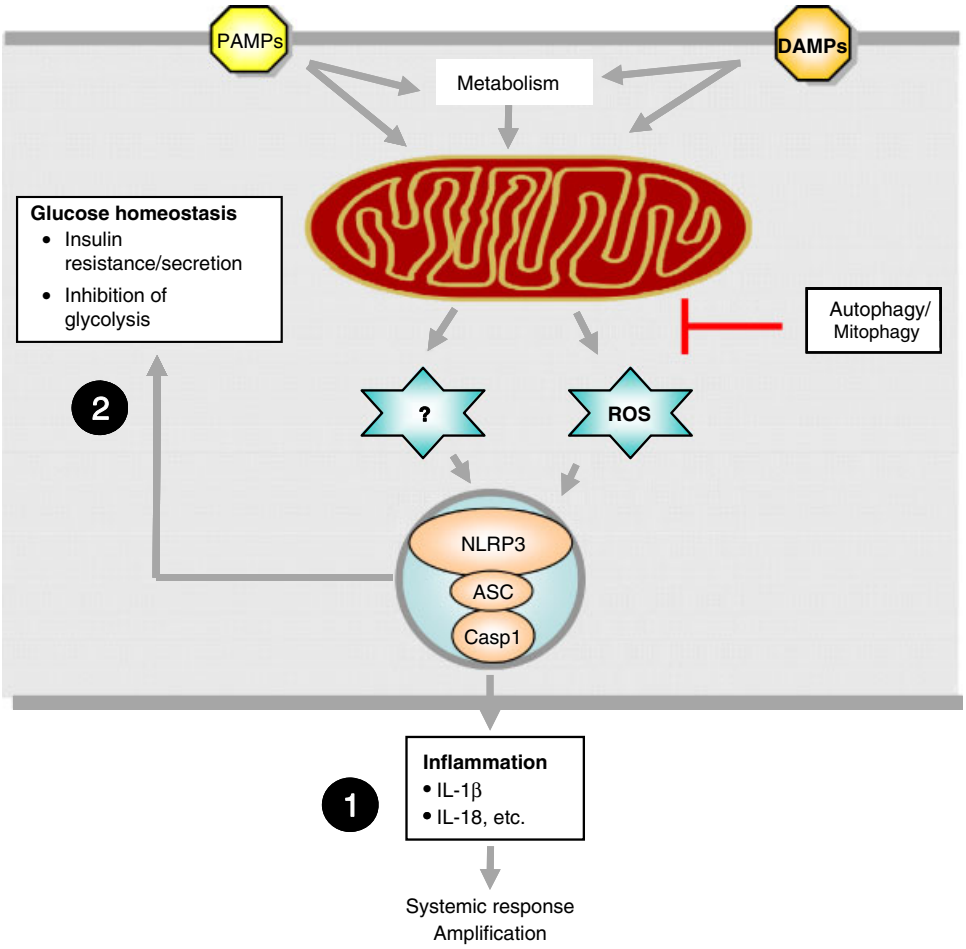

Figure 2. Mitochondria: integrators of metabolic stress and activators of the NLRP3 inflammasome. DAMPs and PAMPs, directly or via the alteration of cellular metabolism, induce partial mitochondrial dysfunction resulting in the elevated production of ROS. Via an unknown mechanism downstream of ROS, the NLRP3 inflammasome is activated and triggers (1) an extracellular inflammatory response through processing of the cytokines IL-1β and IL-18, and (2) an intracellular response that alters cellular glucose and lipid metabolism.

which is present in the cytoplasm of resting cells, also relocates to these perinuclear areas upon NLRP3 activation.

ER membranes can tightly associate with mitochondria forming mitochondria-associated ER membranes (MAMs). Among other functions, MAMs are important for the transfer of lipids and Ca^{2+} from the ER to the mitochondria [14]. Subcellular fractionation techniques reveal that the NLRP3-associated ER/mitochondria staining most likely corresponds to MAMs. Thus, by virtue of its ER/mitochondrial localization upon activation, the NLRP3 inflammasome is strategically located to receive signals emanating from mitochondria.

Two other observations provide support for a pivotal role of mitochondria in NLRP3 inflammasome activation. ROS production can be induced specifically in mitochondria by blocking key enzymes of the electron transport chain. Complex I is one of the main sites at which electrons can leak to oxygen [15]. Addition of the Complex I inhibitor rotenone results in the partial loss of mitochondrial membrane potential and strong ROS production. Complex III inhibition by antimycin A has a similar effect, and indeed both drugs lead to NLRP3 inflammasome activation [12]. To avoid cellular damage, ROS-generating mitochondria are constantly removed by a specialized form of autophagy termed mitophagy [16]. Inhibition of mitophagy/autophagy leads to the prolonged presence of ROS-producing damaged mitochondria, and, as a consequence, to the activation of the NLRP3 inflammasome [11, 12]. Taken together, these observations provide good evidence that ROS produced by dysfunctional mitochondria causes NLRP3 inflammasome activation.

What are the signals released from mitochondria that cause inflammasome activation?

It is rather unlikely that the mere increase of ROS from mitochondria directly induces NLRP3 inflammasome activation (for example, by causing a conformational change in NLRP3). For comparison, activation of the structurally related apoptosome requires not only increased ROS production, but also the release of cytochrome *c* from the mitochondrial inner membrane. Cytochrome *c* interacts with and subsequently activates Apaf-1 [17]. By analogy, it is tempting to speculate that an NLRP3-binding protein is released from partially damaged or dysfunctional mitochondria, though there is currently no experimental evidence for such a model (Fig. 2).

Nonetheless, increased ROS levels induce the association of NLRP3 with a nonmitochondrial protein, thioredoxin-interacting protein (TXNIP). Treatment with NLRP3 agonists triggers the interaction of NLRP3 with TXNIP in an ROS-dependent manner [18]. In unstimulated cells, TXNIP is constitutively bound to the oxidoreductase thioredoxin 1 (TRX1). Upon an increase in cellular ROS concentration, this complex dissociates and TXNIP binds to NLRP3 translocates from the cytoplasm to the mitochondria. Although the role of mitochondria-associated TXNIP is not clear at this point, there is evidence that it regulates mitochondrial events. In mitochondria, TXNIP binds to and oxidizes mitochondrial TRX2, thereby precluding association of TRX2 with apoptosis signal-regulated kinase 1 (ASK1), and allowing for ASK1 phosphorylation and activation. ASK1 activation can lead to cytochrome *c* release and the induction of apoptosis [19]. Consistent with these findings, TXNIP deficiency protects pancreatic β cells against apoptosis [20].

In addition to elevated ROS, a decrease in cytoplasmic K^+ levels is an obligatory event for NLRP3 inflammasome activation. Under resting conditions, high cytoplasmic K^+ concentrations (150 mM) prevent inflammasome activation. ATP, a potent activator of the NLRP3 inflammasome, reduces intracellular K^+ concentration by approximately 50% [21]. The bacterial toxin and K^+ ionophore nigericin similarly activates the NLRP3 inflammasome [22]. In keeping with this, a drop in cytoplasmic K^+ concentration to less than 70 mM is required for inflammasome activity in vitro [8]. Inhibition of K^+ efflux by high extracellular K^+ concentration, or by addition of the K^+ channel inhibitor glibenclamide [23–25] blocks inflammasome activation in response to all NLRP3 activators tested. Interestingly, low cytoplasmic K^+ levels are also required for the activation of the apoptosome, which indicates that "normal" intracellular concentrations of K^+ safeguard the cell against inappropriate formation of caspase-containing stress-responsive complexes [26]. Although the mechanism by which cytoplasmic K^+ concentration modulates NLRP3 inflammasome activation is unknown, it is possible that its effect is via the mitochondria. These organelles possess several K^+ channels that are important for their functioning [27].

Inflammasome and metabolism

Considering that mitochondrial dysfunction drives NLRP3 inflammasome activation, it is not surprising that the NLRP3 inflammasome reciprocally regulates cellular metabolism. Mitochondria generate chemical energy in the form of ATP from glucose, lipids, amino acids and nucleic acids with the assistance of molecular oxygen. The number of potential metabolic signaling molecules is astounding, and research is only starting to unravel how these metabolites influence cell proliferation, inflammation and cell death. Here are two examples that hint to a connection between the mitochondrial metabolic activity and the inflammasome.

A large body of literature implicates a pathogenic role for the inflammasome-interacting TXNIP in type 2 diabetes (T2D). TXNIP expression is induced by glucose in β cells [18, 28], repressed by insulin [29] and elevated in T2D [29]. TXNIP deficiency improves glucose tolerance, insulin sensitivity and insulin secretion in mice [30, 31]. NLRP3-deficient mice have a similar phenotype [18, 32, 33]. Furthermore, the NLRP3 inflammasome is activated by high glucose levels in β cells, leading to cleavage and secretion of IL-1β [18, 34]. In addition to cleaving IL-1β, caspase-1 also targets several key glycolytic enzymes, such as aldolase and pyruvate kinase, [35]. Together, these findings identify the NLRP3 inflammasome as an important regulator of glucose metabolism under stress conditions.

Obesity induces chronic inflammation that can lead to insulin resistance. In addition to regulating glucose metabolism, the

NLRP3 inflammasome may also influence obesity by regulating lipid metabolism [32, 33]. Caloric restriction and exercise-mediated weight loss in obese individuals with T2D are associated with a reduction in adipose tissue expression of NLRP3, as well as with decreased inflammation and improved insulin sensitivity. Deletion of NLRP3 in mice prevents lipid-induced inflammasome activation and decreases fat deposits in the liver [33]. This may represent a direct role of the inflammasome within adipocytes, as ex vivo differentiation of preadipocytes isolated from caspase-1- or NLRP3-deficient mice results in the production of fat cells that are more metabolically active than their wild-type counterparts [32]. These metabolic effects of the inflammasome may be mediated by IL-1β. Diet- and genetically-induced obese animals display increased levels of IL-1β in adipose tissue than lean mice [32]. Thus, the NLRP3 inflammasome may sense obesity-associated danger signals and contribute to obesity-associated metabolic changes and inflammation.

Collectively, these findings suggest that the NLRP3 inflammasome may have a key role in the etiology of T2D. This assertion is supported by the successful treatment of T2D patients with inhibitors of IL-1β (see accompanying review by Dinarello [3]).

Dysfunctional mitochondria in inflammatory diseases

If dysfunctioning mitochondria trigger the activation of the NLRP3 inflammasome, one would predict that some inflammatory diseases are associated with defects in these organelles or their removal by autophagy. This is indeed the case. Crohn's disease (CD), Parkinson's disease and T2D are discussed here, but there are many other inflammatory diseases that display mitochondrial dysnsfunction, including Huntington's and Alzheimer's diseases, cancer and cardiovascular disease [36]. Although it is tempting to propose that inflammasome activation downstream of dysfunctional mitochondria drives pathogenic inflammation in CD, Parkinson's disease and T2D, direct evidence is mostly lacking as little work has been done in this field. Nonetheless, evidence in support of this hypothesis is described below.

CD is a chronic relapsing and remitting inflammatory disease of the gastrointestinal tract. Although the precise etiology of CD remains elusive, epidemiological data conclusively point to a dysregulation of the immune response against the luminal flora in a genetically susceptible host [37]. The efficacy of inhibitors targeting TNF speaks to the importance of this pro-inflammatory cytokine in CD, but there is also evidence for a contribution of IL-1β, IL-18 [38, 39] and the inflammasome [40, 41]. Combined polymorphisms in Cardinal/CARD8 (a component of the NLRP3 inflammasome) and NLRP3 were found to be associated with the development of CD [42, 43]. Interestingly, defects in autophagy, the process that removes dysfunctional mitochondria, have also been linked to CD. In genome-wide association studies, three autophagy-related genes were found to be associated with CD: *IRGM, NOD2* and *ATG16L1* [37]. ATG16L1 interacts with ATG5 and is crucial for autophagic vacuole assembly, whereas IRGM is a putative GTPase associated with autophagy. NOD2 is an initiator of autophagy through its capacity to interact with ATG16L1. The autophagic activity is lost in NOD2 mutant forms found in patients with CD [38, 44]. Mice deficient in ATG16L1 display hyperactivation of the inflammasome leading to an exacerbated dextran sulphate sodium-induced colitis phenotype that can be alleviated by injection of IL-1β and IL-18 blocking antibodies [45]. This provides a direct link between autophagy defects and inflammasome activation. These findings are also in line with the observation that inhibition of autophagy by down-regulation of other autophagy genes (*Beclin1*, *ATG5* or *LC3*) leads to spontaneous activation of the NLRP3 inflammasome and subsequent IL-1β secretion [11, 12].

There is also a clear link between dysfunctional mitochondria and Parkinson's disease (for review, see [46]). PINK1, PARKIN and DJ-1 are frequent causes of recessive Parkinson's disease. Both PINK1 and PARKIN are crucial for the removal of damaged mitochondria by mitophagy, whereas DJ-1 is localized to the mitochondria and has a role in oxidative stress protection. HTRA2/Omi is a mitochondrial protease that is released as part of the apoptotic cascade. The Parkinson's-associated G399S mutation results in reduced protease activity and HTRA2/Omi knockout mice display a parkinsonian phenotype. Furthermore, there is emerging evidence for a role of IL-1β, perhaps downstream of mitochondrial dysfunction [47, 48]. Indeed, treatment with the mitochondrial Complex I inhibitor rotenone is used to induce parkinsonism in animal models. In addition to our finding that rotenone induces mitochondrial ROS production, NLRP3 inflammasome activation, and IL-1β secretion, there is also evidence for elevated IL-1β production in the hypothalamus of rotenone-treated parkinsonian animals [48].

Mitochondrial dysfunction may also play a role in the pathogenesis of T2D. It is well established that correct mitochondrial function is required for normal glucose-stimulated insulin secretion from β cells, and that the presence of dysfunctional mitochondria leads to β-cell dysfunction and impaired insulin secretion [49]. However, the most convincing proof for a crucial role of mitochondria comes from the observation that maternally inherited defects in mitochondrial DNA cause an insulin-deficient type of diabetes [50]. Support for the critical role of IL-1β in the pathogenesis of T2D comes from the finding that administration of inhibitors of IL-1β leads to dramatic normalization of glucose levels [51] (discussed in the accompanying review by Dinarello [3]).

Concluding remarks

In recent years, the evidence for a central role of inflammasomes in sensing abnormal situations in our body and in orchestrating a subsequent inflammatory and repair program has constantly increased. However, due to the multitude of danger signals sensed by the NLRP3 inflammasome, it has remained a mystery how a single molecule can achieve this almost impossible task. A plausible explanation is that instead of detecting each danger signal individually, the NLRP3 inflammasome monitors the

activity of the mitochondrion, which acts as an integrator of danger signals, including those of metabolic origin. Via a mechanism that remains elusive, excessive ROS production by mitochondria leads to activation of the inflammasome.

It is comprehensible that many situations that lead to altered cellular metabolism can trigger the NLRP3 inflammasome. It is even possible that viruses or bacteria invading a cell activate the inflammasome not via a PAMP, but also through altered cellular metabolism and mitochondrial activity. One example hints to this possibility. Although it was initially proposed that influenza virus is recognized by NLRP3 through specific recognition of its RNA [52], this was later shown to be due to the influenza virus M2 protein, a proton selective ion channel known to efficiently decrease the mitochondrial membrane potential [53, 54].

Despite accumulating evidence supporting the link between the NLRP3 inflammasome, mitochondria and metabolism, several important questions remain unanswered. What mitochondria-derived molecule(s) activate(s) the NLRP3 inflammasome? If mitochondria are involved in both the inflammatory and the apoptotic process, what mechanisms regulate the choice of the signaling pathway? How is activation of the NLRP3 inflammasome linked to the glucose and lipid metabolism? Answers to these questions may provide new pharmacological targets for the treatment of chronic inflammatory and metabolic diseases.

Acknowledgements: J. T. is supported by grants of the Swiss National Science Foundation, EU grant Apo-Sys and by the Institute of Arthritis Research. Many thanks to Christina Thomas for the careful reading of the manuscript.

Conflict of interest: The author declared no financial or commercial conflict of interest. This article is editorially independent of Novartis.

References

1 **Schroder, K. and Tschopp, J.,** The inflammasomes. *Cell* 2010. **140**: 821–832.

2 **Davis, B. K., Wen, H. and Ting, J. P.-Y.,** The inflammasome NLRs in immunity, inflammation, and associated diseases. *Annu. Rev. Immunol.* 2011. **29**: 707–735.

3 **Dinarello, C. A.,** A clinical perspective of interleukin-1β as the gatekeeper of inflammation *Eur. J. Immunol.* 2011. **41**: 1203–1217.

4 **Schroder, K., Zhou, R. and Tschopp, J.,** The NLRP3 inflammasome: a sensor for metabolic danger? *Science* 2010. **327**: 296–300.

5 **Hornung, V., Bauernfeind, F., Halle, A., Samstad, E. O., Kono, H., Rock, K. L., Fitzgerald, K. A. and Latz, E.,** Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. *Nat. Immunol.* 2008. **9**: 847–856.

6 **Dostert, C., Petrilli, V., Van Bruggen, R., Steele, C., Mossman, B. T. and Tschopp, J.,** Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science* 2008. **320**: 674–677.

7 **Newman, Z. L., Leppla, S. H. and Moayeri, M.,** CA-074Me protection against anthrax lethal toxin. *Infect. Immun.* 2009. **77**: 4327–4336.

8 **Pétrilli, V., Papin, S., Dostert, C., Mayor, A., Martinon, F. and Tschopp, J.,** Activation of the NALP3 inflammasome is triggered by low intracellular potassium concentration. *Cell Death Differ.* 2007. **14**: 1583–1589.

9 **Sato, T., Machida, T., Takahashi, S., Iyama, S., Sato, Y., Kuribayashi, K., Takada, K. et al.,** Fas-mediated apoptosome formation is dependent on reactive oxygen species derived from mitochondrial permeability transition in Jurkat cells. *J. Immunol.* 2004. **173**: 285–296.

10 **Latz, E.,** NOX-free inflammasome activation. *Blood* 2010. **116**: 1393–1394.

11 **Nakahira, K., Haspel, J. A., Rathinam, V. A., Lee, S. J., Dolinay, T., Lam, H. C., Englert, J. A. et al.,** Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. *Nat. Immunol.* 2011. **12**: 222–230.

12 **Zhou, R., Yazdi, A. S., Menu, P. and Tschopp, J.,** A role for mitochondria in NLRP3 inflammasome activation. *Nature* 2010. **469**: 221–225.

13 **Colombini, M.,** VDAC: the channel at the interface between mitochondria and the cytosol. *Mol. Cell. Biochem.* 2004. **256–257**: 107–115.

14 **Hayashi, T., Rizzuto, R., Hajnoczky, G. and Su, T. P.,** MAM: more than just a housekeeper. *Trends Cell Biol.* 2009. **19**: 81–88.

15 **Brookes, P. S., Yoon, Y., Robotham, J. L., Anders, M. W. and Sheu, S. S.,** Calcium, ATP, and ROS: a mitochondrial love-hate triangle. *Am. J. Physiol. Cell Physiol.* 2004. **287**: C817–C833.

16 **Zhang, J. and Ney, P. A.,** Role of BNIP3 and NIX in cell death, autophagy, and mitophagy. *Cell Death Differ.* 2009. **16**: 939–946.

17 **Riedl, S. J. and Salvesen, G. S.,** The apoptosome: signalling platform of cell death. *Nat. Rev. Mol. Cell Biol.* 2007. **8**: 405–413.

18 **Zhou, R., Tardivel, A., Thorens, B., Choi, I. and Tschopp, J.,** Thioredoxin-interacting protein links oxidative stress to inflammasome activation. *Nat. Immunol.* 2010. **11**: 136–140.

19 **Saxena, G., Chen, J. and Shalev, A.,** Intracellular shuttling and mitochondrial function of thioredoxin-interacting protein. *J. Biol. Chem.* 2010. **285**: 3997–4005.

20 **Shalev, A.,** Lack of TXNIP protects beta-cells against glucotoxicity. *Biochem. Soc. Trans.* 2008. **36**: 963–965.

21 **Perregaux, D. and Gabel, C. A.,** Interleukin-1 beta maturation and release in response to ATP and nigericin. Evidence that potassium depletion mediated by these agents is a necessary and common feature of their activity. *J. Biol. Chem.* 1994. **269**: 15195–15203.

22 **Mariathasan, S., Weiss, D. S., Newton, K., McBride, J., O'Rourke, K., Roose-Girma, M., Lee, W. P. et al.,** Cryopyrin activates the inflammasome in response to toxins and ATP. *Nature* 2006. **440**: 228–232.

23 **Muruve, D. A., Petrilli, V., Zaiss, A. K., White, L. R., Clark, S. A., Ross, P. J., Parks, R. J. and Tschopp, J.,** The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response. *Nature* 2008. **452**: 103–107.

24 **Lamkanfi, M., Mueller, J. L., Vitari, A. C., Misaghi, S., Fedorova, A., Deshayes, K., Lee, W. P. et al.,** Glyburide inhibits the Cryopyrin/Nalp3 inflammasome. *J. Cell Biol.* 2009. **187**: 61–70.

25 **Masters, S. L., Dunne, A., Subramanian, S. L., Hull, R. L., Tannahill, G. M., Sharp, F. A., Becker, C. et al.,** Activation of the NLRP3 inflammasome by islet amyloid polypeptide provides a mechanism for enhanced IL-1beta in type 2 diabetes. *Nat. Immunol.* 2010. **11**: 897–904.

26 **Cain, K., Langlais, C., Sun, X. M., Brown, D. G. and Cohen, G. M.,** Physiological concentrations of K+inhibit cytochrome c-dependent formation of the apoptosome. *J. Biol. Chem.* 2001. **276**: 41985–41990.

27 **Heinen, A., Camara, A. K. S., Aldakkak, M., Rhodes, S. S., Riess, M. L. and Stowe, D. F.,** Mitochondrial Ca2+-induced K+influx increases respiration and enhances ROS production while maintaining membrane potential. *Am. J. Physiol. Cell Physiol.* 2007. **292**: C148–C156.

28 **Minn, A. H., Hafele, C. and Shalev, A.,** Thioredoxin-interacting protein is stimulated by glucose through a carbohydrate response element and induces beta-cell apoptosis. *Endocrinology* 2005. **146**: 2397–2405.

29 **Parikh, H., Carlsson, E., Chutkow, W. A., Johansson, L. E., Storgaard, H., Poulsen, P., Saxena, R. et al.,** TXNIP regulates peripheral glucose metabolism in humans. *PLoS Med.* 2007. **4**: e158.

30 **Hui, S. T., Andres, A. M., Miller, A. K., Spann, N. J., Potter, D. W., Post, N. M., Chen, A. Z. et al.,** Txnip balances metabolic and growth signaling via PTEN disulfide reduction. *Proc. Natl. Acad. Sci. USA* 2008. **105**: 3921–3926.

31 **Oka, S., Yoshihara, E., Bizen-Abe, A., Liu, W., Watanabe, M., Yodoi, J. and Masutani, H.,** Thioredoxin binding protein-2/thioredoxin-interacting protein is a critical regulator of insulin secretion and peroxisome proliferator-activated receptor function. *Endocrinology* 2009. **150**: 1225–1234.

32 **Stienstra, R., Joosten, L. A., Koenen, T., van Tits, B., van Diepen, J. A., van den Berg, S. A., Rensen, P. C. et al.,** The inflammasome-mediated caspase-1 activation controls adipocyte differentiation and insulin sensitivity. *Cell Metab.* 2010. **12**: 593–605.

33 **Vandanmagsar, B., Youm, Y. H., Ravussin, A., Galgani, J. E., Stadler, K., Mynatt, R. L., Ravussin, E. et al.,** The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. *Nat. Med.* 2011. **17**: 179–188.

34 **Maedler, K., Sergeev, P., Ris, F., Oberholzer, J., Joller-Jemelka, H. I., Spinas, G. A., Kaiser, N. et al.,** Glucose-induced beta cell production of IL-1beta contributes to glucotoxicity in human pancreatic islets. *J. Clin. Invest.* 2002. **110**: 851–860.

35 **Shao, W., Yeretssian, G., Doiron, K., Hussain, S. N. and Saleh, M.,** The caspase-1 digestome identifies the glycolysis pathway as a target during infection and septic shock. *J. Biol. Chem.* 2007. **282**: 36321–36329.

36 **Neustadt, J. and Pieczenik, S. R.,** Medication-induced mitochondrial damage and disease. *Mol. Nutr. Food Res.* 2008. **52**: 780–788.

37 **Cho, J. H.,** The genetics and immunopathogenesis of inflammatory bowel disease. *Nat. Rev. Immunol.* 2008. **8**: 458–466.

38 **Maeda, S., Hsu, L. C., Liu, H., Bankston, L. A., Iimura, M., Kagnoff, M. F., Eckmann, L. and Karin, M.,** Nod2 mutation in Crohn's disease potentiates NF-kappaB activity and IL-1beta processing. *Science* 2005. **307**: 734–738.

39 **Ishikura, T., Kanai, T., Uraushihara, K., Iiyama, R., Makita, S., Totsuka, T., Yamazaki, M. et al.,** Interleukin-18 overproduction exacerbates the development of colitis with markedly infiltrated macrophages in interleukin-18 transgenic mice. *J. Gastroenterol. Hepatol.* 2003. **18**: 960–969.

40 **Dupaul-Chicoine, J., Yeretssian, G., Doiron, K., Bergstrom, K. S. B., McIntire, C. R., LeBlanc, P. M., Meunier, C. et al.,** Control of intestinal homeostasis, colitis, and colitis-associated colorectal cancer by the inflammatory caspases. *Immunity* 2010. **32**: 367–378.

41 **Zaki, M. H., Boyd, K. L., Vogel, P., Kastan, M. B., Lamkanfi, M., Kanneganti, T. D.,** The NLRP3 inflammasome protects against loss of epithelial integrity and mortality during experimental colitis. *Immunity* 2010. **32**: 379–391.

42 **Roberts, R. L., Topless, R. K. G., Phipps-Green, A. J., Gearry, R. B., Barclay, M. L. and Merriman, T. R.,** Evidence of interaction of CARD8 rs2043211 with NALP3 rs35829419 in Crohn's disease. *Genes Immun.* 2010. **11**: 351–356.

43 **Schoultz, I., Verma, D., Halfvarsson, J., Törkvist, L., Fredrikson, M., Sjöqvist, U., Lördal, M. et al.,** Combined polymorphisms in genes encoding the inflammasome components NALP3 and CARD8 confer susceptibility to Crohn's disease in Swedish men. *Am. J. Gastroenterol.* 2009. **104**: 1180–1188.

44 **Travassos, L., Carneiro, L., Ramjeet, M., Hussey, S., Kim, Y., Magalhães, J., Yuan, L. et al.,** Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. *Nat. Immunol.* 2009. **11**: 55–62.

45 **Saitoh, T., Fujita, N., Jang, M. H., Uematsu, S., Yang, B.-G., Satoh, T., Omori, H. et al.,** Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. *Nature* 2008. **456**: 264–268.

46 **Schapira, A. H.,** Mitochondrial dysfunction in Parkinson's disease. *Cell Death Differ.* 2007. **14**: 1261–1266.

47 **Pott Godoy, M. C., Tarelli, R., Ferrari, C. C., Sarchi, M. I. and Pitossi, F. J.,** Central and systemic IL-1 exacerbates neurodegeneration and motor symptoms in a model of Parkinson's disease. *Brain* 2008. **131**: 1880–1894.

48 **Yi, P.-L., Tsai, C.-H., Lu, M.-K., Liu, H.-J., Chen, Y.-C. and Chang, F.-C.,** Interleukin-1beta mediates sleep alteration in rats with rotenone-induced parkinsonism. *Sleep* 2007. **30**: 413–425.

49 **Lowell, B. B. and Shulman, G. I.,** Mitochondrial dysfunction and type 2 diabetes. *Science* 2005. **307**: 384–387.

50 **Luft, R.,** The development of mitochondrial medicine. *Proc. Natl. Acad. Sci. USA* 1994. **91**: 8731–8738.

51 **Larsen, C. M., Faulenbach, M., Vaag, A., Vølund, A., Ehses, J. A., Seifert, B., Mandrup-Poulsen, T. and Donath, M. Y.,** Interleukin-1-receptor antagonist in type 2 diabetes mellitus. *N Engl. J. Med.* 2007. **356**: 1517–1526.

52 **Allen, I. C., Scull, M. A., Moore, C. B., Holl, E. K., McElvania-TeKippe, E., Taxman, D. J., Guthrie, E. H. et al.,** The NLRP3 inflammasome mediates *in vivo* innate immunity to influenza A virus through recognition of viral RNA. *Immunity* 2009. **30**: 556–565.

53 **Ichinohe, T., Pang, I. K. and Iwasaki, A.,** Influenza virus activates inflammasomes via its intracellular M2 ion channel. *Nat. Immunol.* 2010. **11**: 404–410.

54 **Ilyinskii, P. O., Gabai, V. L., Sunyaev, S. R., Thoidis, G. and Shneider, A. M.,** Toxicity of influenza A virus matrix protein 2 for mammalian cells is associated with its intrinsic proton-channeling activity. *Cell Cycle* 2007. **6**: 2043–2047.

Abbreviations: **ASC:** apoptosis-associated speck-like protein containing a caspase recruitment domain · **ASK1:** apoptosis signal-regulated kinase 1 · **CD:** Crohn's disease · **DAMP:** damage-associated molecular pattern · **MAM:** mitochondria-associated ER membrane · **NLR:** NOD-like receptor · **PAMP:** pathogen-associated molecular pattern · **T2D:** type 2 diabetes · **TXNIP:** thioredoxin-interacting protein · **VDAC:** voltage dependent anion channel

See accompanying reviews, also written by winners of the 2010 Novartis Immunology Prizes, and the Forum article describing the Prizes
http://dx.doi.org/10.1002/eji.201041377
http://dx.doi.org/10.1002/eji.201041550
http://dx.doi.org/10.1002/eji.201141682

Received: 20/1/2011
Revised: 25/2/2011
Accepted: 11/3/2011
Accepted article online: 17/3/2011

Note added in proof

Sadly Jürg Tschopp passed away on 22 March 2011. An Obituary can be found in this issue (*Eur. J. Immunol.* 2011. **41**: 1189–1190). He will be greatly missed by all.

