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Mitochondria in autoinflammation: cause, mediator or bystander?

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People suffering from autoinflammatory disease (AID) have recurring sterile inflammation due to dysregulated inflammasome activation. Although certain genes have been associated with several AIDs, the molecular underpinnings of seemingly spontaneous inflammation are not well understood. Emerging data now suggest that mitochondrial reactive oxygen species (ROS), mitochondrial DNA (mtDNA), and autophagy might drive key signaling pathways towards activation of the inflammasome. In this review, we discuss recent findings and highlight common features between different AIDs and mitochondrial (dys)function. Although it is still early to identify clear therapeutic targets, the emerging paradigms in inflammation and mitochondrial biology show that mitochondria play an important role in AIDs, and understanding this interplay will be key in the development of new therapies.

Importance of mitochondria in autoinflammatory disease

Mitochondria are best known for their role as the energy producers of the cell. In addition to supplying cellular energy, they play pivotal roles in various cellular processes including cell cycle progression, cellular differentiation and growth, and inflammation. Mitochondria are positioned near the endoplasmic reticulum (ER) [1], where they supply energy for protein production and ROS for disulfide-bond formation [2], and contribute to lipid biosynthesis [3]. They are also master regulators of apoptosis [4], and contribute to the activation of the inflammasome and thereby to directing immune responses [5] (Figure 1). The concept that mitochondria contribute to inflammasome activation is relatively new, with data gathered mostly during the last decade. Unsolicited inflammasome activation is now known to cause sterile inflammation [6]. Indeed, AID involves recurrent sterile inflammation due to inappropriate inflammasome activation [7]. In this review, we focus on the role of mitochondria in initiation and propagation of AID. We examine various aspects of mitochondrial signaling in the activation of the inflammasome and the possible consequences for AID development. Finally, we discuss possible mechanistic links between distinct AID types.

Autoinflammation and AID

The innate immune system is mobilized for activation upon sensing an external agent associated with pathogen infection. In autoinflammation (see Glossary), however, innate immune activation occurs upon perception of not external but internal cues [8]. A beneficial role for autoinflammation lies in responsiveness to injuries, supporting wound healing [9,10], through a process called sterile inflammation [6]. Autoinflammation can also turn pathological, presenting as AID. The signaling routes contributing to AID are not clear, although key molecules in immune activation pathways are deduced by clarification of genes that cause

Glossary

Autoinflammation: refers to the activation of the inflammation pathway without the presence of foreign or pathogenic triggers. The immune response is indistinguishable from a normally triggered response.
Autoinflammatory disease (AID): autoinflammatory disease is characterized by periodic fever and inflammatory symptoms followed by complete resolution of the inflammation. It is mostly caused by monogenetic hereditary mutations.
Inflammasomes: are large intracellular multiprotein complexes that play central role in innate immunity. They contain a cytosolic receptor for molecular patterns and, when triggered, activate caspase-1. Most known inflammasomes contain a member of the NOD-like receptor (NLR) family.
Mitochondria associated membrane (MAM): refers to the interface between mitochondria and the endoplasmic reticulum (ER). MAM are part of the ER and are reversibly tethered to mitochondria. This site is important for the exchange of metabolites, lipid biosynthesis, protein folding and calcium homeostasis.
Mitochondrial fission and fusion: Mitochondria undergo fission and fusion to ensure their proper function. This process is regulated by the master regulator proteins mitofusin 1 and 2 (MFN1 and MFN2), mitochondrial fission protein 1 (FIS1), dynamin-related protein 1 (DRP1), Optic atrophy 1 (OPA1), and the ER.
Reactive oxygen species (ROS): synthesis of ATP in mitochondria occurs through a process called respiration in which oxygen is used, and which simultaneously produces reactive byproducts. A foremost product is the superoxide radical O_2^-, which can cause cellular damage if it is not neutralized. Under normal conditions such radicals are contained and neutralized in the mitochondria. There are other sources of ROS in the cell, but oxygen metabolism is the main contributor.
Mitophagy: mitochondria undergo continuous quality control to ascertain overall health of cellular mitochondria. Damaged mitochondria are, degraded by the cell by a specialized form of autophagy, called mitophagy.
NLR family, pyrin domain containing 3 (NLRP3): also called cryopyrin, is a Pyrin-like protein containing a Pyrin domain, a nucleotide-binding domain (NBD), and a leucine-rich repeat (LRR) motif. NLRP3 functions as cytosolic sensor and is activated by numerous stimuli. When activated it oligomerizes and forms a complex with the adaptor protein ASC and caspase-1. The mature complex is called an inflammasome and processes the inactive pro-forms of IL-1β and IL-18 into their mature bioactive forms.
Sterile inflammation: Inflammation that occurs without the presence of pathogens or foreign material. This can be triggered by endogenous danger signals, such as IL-1β, or by inappropriate activation of inflammasomes, as is the case in AID.

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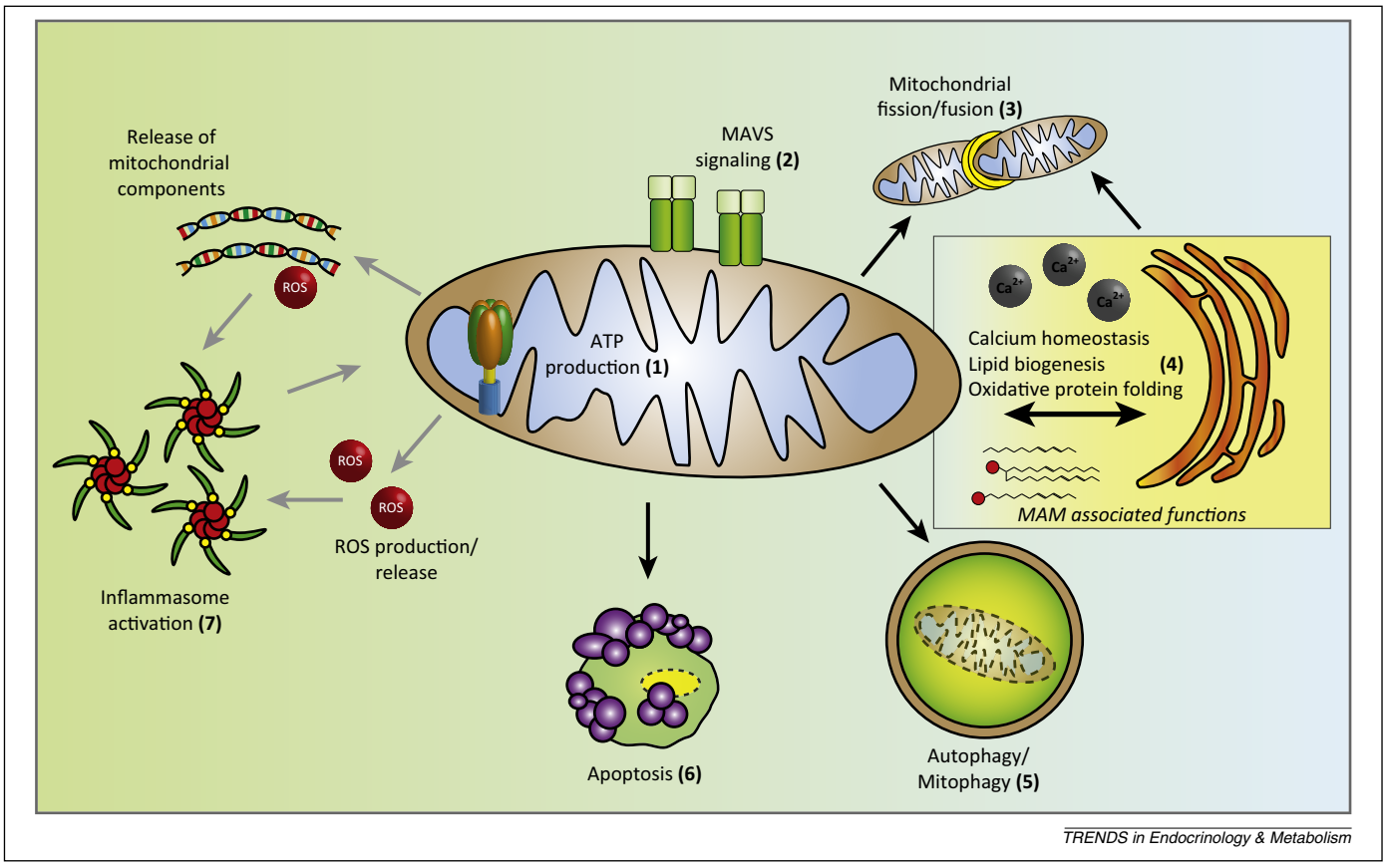

Figure 1. General overview of mitochondrial functions of the cell. The primary function of mitochondria is the generation of ATP (1). In addition, MAVS is expressed on the mitochondrial surface, and provides a signaling platform for immune activation, in particular for antiviral pathways (2). Mitochondrial fission and fusion can be adjusted to meet the demands of the cell or as response to mitochondrial damage (3). The MAM comprises the interface between the mitochondria and the endoplasmic reticulum. In this specialized compartment, mitochondria supply energy and metabolites for lipid synthesis and oxidative protein folding. In addition, calcium homeostasis is tightly regulated at the MAM (4). Released mitochondrial ROS can induce autophagy of cellular components, including mitochondria. Mitophagy degrades damaged or superfluous mitochondria (5). Release of mitochondrial components on large scale is a potent trigger for apoptosis (6). Mitochondria also play an important role in the induction of the inflammasome (7). There is no consensus on the exact order of events (gray arrows). Two main possibilities exist; either the mitochondria become damaged and release their content, leading to activation of the inflammasome. Or the inflammasome becomes activated and induces release of mitochondrial components, creating a feedback loop. Abbreviations: MAM, mitochondria-associated membrane; MAVS, mitochondria antiviral signaling; ROS, reactive oxygen species.

hereditary AID [11]. The result is the inadvertent triggering at subthreshold stimulation levels, for more or less periodic systemic activation of the innate immune response. Presentation of AID features includes: recurrent fever, joint inflammation, erythema, gastric inflammation, and mucosal inflammation [7]. Diagnosis of AID is often a long and difficult process. Because of the low incidence and the fact that activation of the inflammatory response in AID is indistinguishable from pathogen-induced immune activation, it usually takes multiple cycles before the possibility of autoinflammation is recognized. Final diagnosis of hereditary AID can only be made by DNA analysis, such as by next-generation sequencing [12].

We think it is relevant to emphasize here the mechanistic distinction between AID and autoimmune diseases. Autoimmune diseases are disorders of the adaptive, B and T lymphocyte-mediated branch of the immune system, notwithstanding that innate pathways also contribute. In autoimmunity, an important feature is chronic adaptive immune activation, while in autoinflammation, the innate immune system is activated in a recurrent manner that is followed by complete resolution of the inflammation until the next episode of activation. Clinically, autoinflammation is separated from autoimmunity through the lack of adaptive features, autoantibodies and autoreactive T cells [13].

Inflammasomes

Inflammasomes commonly consist of three constituents: a nucleotide-binding oligomerization domain (NOD)-like receptor (NLR), the adaptor protein apoptosis-associated Speck like protein containing a CARD (ASC), and caspase-1. The NLR, which forms the core, determines the type of inflammasome. The exogenous signal that triggers activation determines which type of inflammasome is formed, although the NLRP3 inflammasome responds to several stimuli (including even endogenous stimuli) [9,10], and is therefore special amongst NLRs. It has been suggested that NLRP3 inflammasomes may be activated by a single messenger or an intermediate one, but no consensus has been reached to date [14,15]. Interestingly, of the three general activators that have been suggested (potassium efflux, calcium ions, and ROS) (Figure 1), two are closely related to mitochondria [16–18]. The identification of a single conserved signal is complicated by the existence of a noncanonical pathway, which also activates NLRP3 via caspase-11 [19]. Multiple intermediate signaling pathways may therefore exist that all drive NLRP3 activation. Of note,

polymers of ASC resembling inflammasomes were recently described as being secreted mediators, thereby triggering the extracellular propagation of inflammation [20].

The process of inflammasome assembly is not fully understood [21]. It is however clear that the activation of the NLR can induce self-oligomerization via its NACHT domain; an evolutionarily conserved domain often found in proteins involved in apoptosis [22]. This domain contains sites for protein–protein interaction and a nucleotide-binding domain (NBD). The induced conformational changes during oligomerization recruit ASC to the exposed Pyrin (PYR) domains. ASC, through its N-terminal PYR domain binds to the PYR domain of NLRP3. Consequently, the C-terminal region of ASC, containing a caspase recruiting domain (CARD), recruits caspase-1 to the inflammasome complex. The recruitment of several caspase-1 molecules results in self-dimerization and perpetuated self-activation of caspase-1. The activated caspase-1 cleaves the pro-interleukin (IL)-1β to generate the mature inflammatory cytokine (Figure 2) [23].

Although the crystal structure of the NLRP3 NACHT domain is yet to be resolved, sequence alignments predict that the NACHT region is highly conserved throughout the NLR family [24]. The binding and hydrolysis of triphosphate nucleotides, which appears to be required for NLRP3 oligomerization, induces conformational changes, thereby facilitating protein oligomerization, to induce inflammasome activation [25].

One recurrent question is whether nucleotides are bound to the NACHT domain when NLRP3 is inactive. One possibility is that the NBD in resting cells is blocked, only to become available after activation, or alternatively,

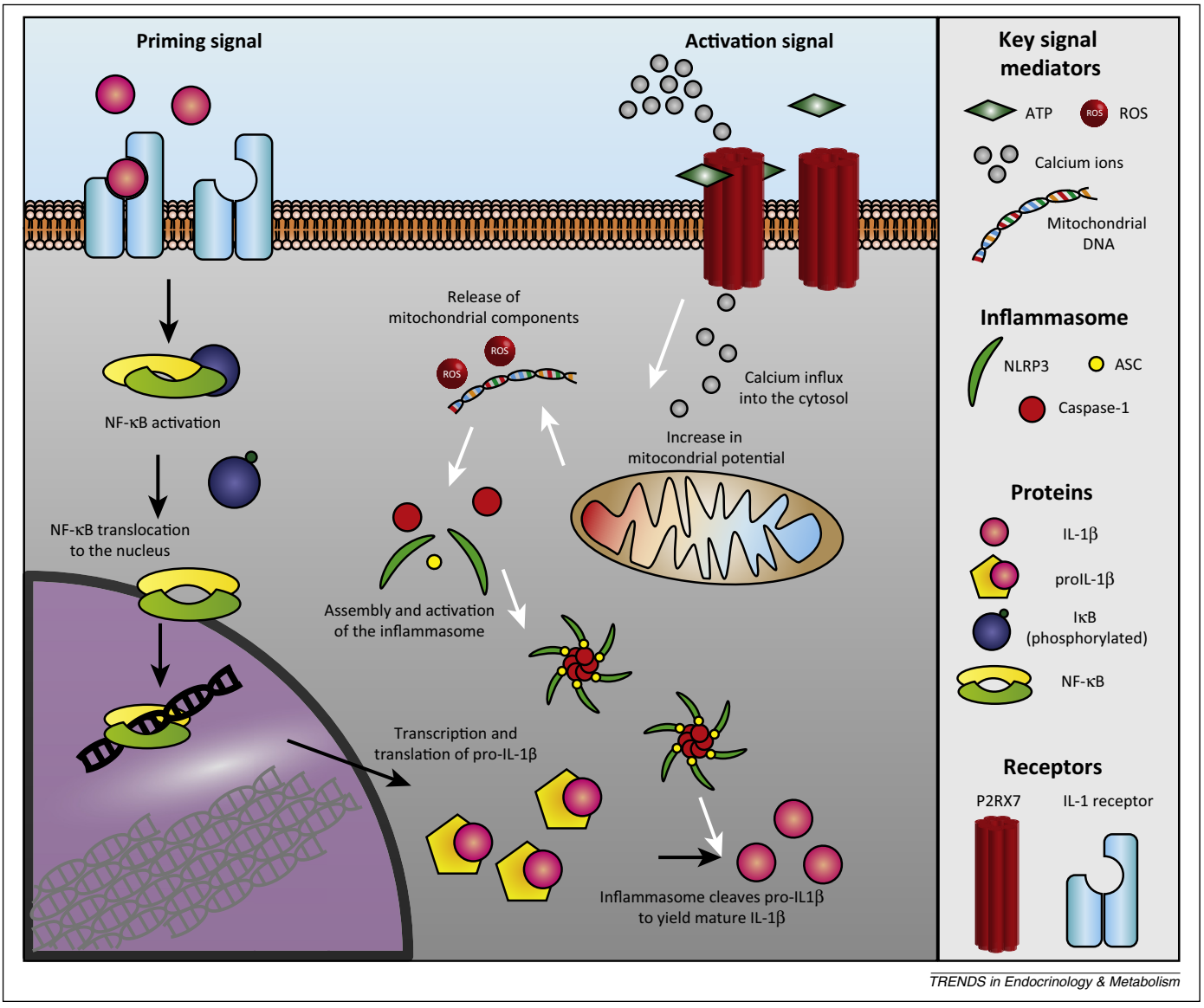

Figure 2. Two-signal system for activation of the cellular immune response. To evoke the cellular immune response two signals are required, a priming signal (black arrows) and an activation signal (white arrows). The priming signal is an initial danger signal like IL-1β, tumor necrosis factor, or a pathogen-associated molecule such as lipopolysaccharide. The priming signal leads to the activation of the NF-κB pathway and induces the production of inflammatory molecules like proIL-1β and proIL-18. The second signal causes the activation of the inflammasome. In this example this is ATP, but other signals (for instance type 1 interferons) can act as activation signal. ATP activates cellular receptors leading to an influx of calcium. The calcium damages the mitochondria, leading to the release of ROS and mitochondrial DNA into the cytosol. These mitochondrial components induce the assembly of the inflammasome and thereby activate caspase-1. The activated caspase-1 cleaves the proIL-1β and proIL-18 to yield their mature and bioactive forms. Abbreviations: IL, interleukin; NF, nuclear factor; ASC, apoptosis-associated speck-like protein containing a CARD; NLRP3, NACHT, LRR and PYD domains-containing protein 3; ROS, reactive oxygen species.

that the catalytic activity of the pocket is increased after activation. In resting cells, NLRP3 is ubiquitinated, and deubiquitination needs to take place for NLRP3 to become active [26]. The site of ubiquitination has not yet been identified and it is therefore possible that ubiquitin blocks the NBD. Another possibility was reported by Zhou and colleagues; they identified thioredoxin-interacting protein (TXNIP) as an activator of NLRP3, and showed that TXNIP interacts directly with NLRP3 [18]. TXNIP is normally bound to thioredoxin (TRX); a regulator of the redox potential in the cell. TRX can scavenge ROS directly or reduce oxidized proteins. Zhou and colleagues proposed that under increasing redox stress conditions (for example through increased ROS production by mitochondria) TXNIP is released from TRX, for activation of NLRP3 [18]. If the model is correct, mitochondria would be activated to produce increased amounts of mitochondrial ROS (mtROS) to facilitate NLRP3 oligomerization, placing inflammasome formation downstream of mitochondrial activation. The finding however was challenging to replicate [27]. Yet in 2014, several rodent-based studies reported involvement of TXNIP in NLRP3 activation [28–32], suggesting that at least in those organisms, but not necessarily humans, TXNIP may contribute to NLRP3-inflammasome activation. In the next sections, we will discuss how inflammasome-mediated activation of caspase-1 is crucial to the ultimate secretion of IL-1β, and we will discuss its interactions with the mitochondria.

AIDs

Innate immune activation

Innate immune activation is considered the first step of the immune response, triggered by the detection of pathogens, foreign material, or endogenous danger signals by dedicated receptors. The cell responds with the release of proinflammatory cytokines such as IL-1β, IL-18 and tumor necrosis factor (TNF) α. The innate immune response is a potent and rapid defense system by virtue of the enormous variety of stimuli that can trigger a conserved set of cellular effector functions. However, the detection of foreign materials, that can abundantly express multiple innate stimuli, necessitates a safety guard to restrain unwarranted stimulation. This safety guard is built in, as cells require a combination of two signals for immune activation: first, a priming signal needs to be sensed that activates NF-κB and induces transcription of inflammasome components and proIL-1β. The second signal activates the inflammasome and mediates the maturation and release of bioactive IL-1β [15] (Figure 2). In AID, the activation of the innate response is altered and responds to subthreshold levels of activation, causing full-blown activation of the response even when the requirement of two signals is not fully fulfilled.

Known genetic associations

For several AIDs that are relatively common (although all with estimated incidences below 1 in 100-000), the affected proteins were identified allowing genetic tests to confirm the clinical diagnosis. The identification of disease-associated gene variants triggered investigations into underlying molecular mechanisms that converge into the activation of the inflammasome, triggering sterile inflammation. We here discuss four examples, familial Mediterranean fever (FMF), mevalonate kinase deficiency (MKD), TNF receptor associated periodic syndrome (TRAPS), and cryopyrin-associated periodic syndrome (CAPS).

FMF is caused by mutations in the Mediterranean fever *MEFV* gene encoding the protein Pyrin [33,34]. Pyrin is a cytoskeleton-associated protein produced by immune cells and serves as an intracellular sensor for inactivation of Rho GTPases. Certain bacterial toxins can inactivate Rho GTPases by glucosylation. This leads to the activation of Pyrin, through an unknown mechanism. Pyrin accordingly acts as a pathogen recognition receptor (PRR) of the NLR family, and oligomerizes with ASC and caspase-1 to form an inflammasome [35].

Mutations in the mevalonate kinase (MVK) gene can lead to MKD [36]. Low activity of MVK blocks the cholesterol biosynthetic pathway, resulting in shortage of nonsterol isoprenoids and decreased post-translational isoprenylation of selected proteins [37], such as small GTPases. Lack of prenylation can cause dysregulation of small GTPase activity and subcellular localization to membranes [38–40], resulting in defective autophagy and priming of the IL-1β response.

Mutations of the TNF receptor 1 (*TNFR1*) gene can cause TRAPS [41]. The mutated receptor is retained in the ER, thus escaping clearance. This results in increased cellular stress and dysregulation of several processes including autophagy, as well as activation of the inflammasome [42,43].

CAPS is caused by mutations in the *NLRP3* gene, many of which are found in the NDB domain [44]. Some mutations are predicted to modulate the hydrolytic activity of the inflammasome-associated NBD. Others are predicted to contribute to protein–protein interactions [24]. Regardless, these mutations presumably facilitate the oligomerization of NLRP3 inflammasomes spontaneously or at subthreshold levels of activation.

Mitochondria-derived signals

Mitochondrial damage

Induced mitochondrial damage can trigger apoptosis through the release of apoptotic factors; most notably cytochrome C [45]. Indirect damage to mitochondria can, for example, be triggered by cellular exposure to inert particles such as monosodium urate (MSU) crystals or silica. Such particles can temporarily be enclosed by endosomes and lysosomes followed by lysosomal rupture. Accordingly, cathepsin B activation and phagosomal acidification contribute to inflammasome activation via this route, with cathepsin B eventually damaging the mitochondrial membrane, leading to loss of potential and release of its content [46]. The mitochondrial content further stimulates activation of the inflammasome. Activation of the inflammasome through mitochondrial content is a major factor in AID and will be discussed in the next sections.

ROS

The release of ROS, and in particular, mitochondria-derived ROS (mtROS) can facilitate NLRP3 inflammasome

activation. However, mtROS is a normal byproduct of mitochondrial respiration. Deviation of the mitochondrial biochemical status quo is therefore sensed as a trigger for the activation of the inflammasome [47]. One possibility of how mtROS may be made available for inflammasome activation pertains to mitochondrial damage, causing mtROS to escape mitochondria and enter the cytosol. A second possibility is increasing mitochondrial metabolism, which would increase mtROS levels. The third option is a regulated release of mtROS, both in quantity and subcellular location [48]. How mtROS in AID is released is not always known, yet for both TRAPS and MKD, it has been shown that inhibition or neutralization of mtROS reduces inflammasome activity [43,49]. The release of mtROS is an important intermediate in inflammasome (Figure 1) activation and therefore in AID.

Disruption in mitophagy [50] also might lead to mtROS leaking into the cytosol. Cells deficient in autophagy components can increase secretion of IL-1β [51]. Such mechanisms can however work in opposite directions, as some autophagy proteins are also necessary for IL-1β release. Recently, a feedback loop was reported suggesting that inflammasome activation leads to mitochondrial damage and inhibition of mitophagy, further complicating the issue. It has also been reported that accumulation of damaged mitochondria can be responsible for increased IL-1β secretion [52] and defects in mitophagy have been suggested to play a role in MKD [49].

Finally, mtROS appearance in the cytosol may be consequential to increased mitochondrial activity. Mitochondrial activity is closely linked to calcium levels, and mitochondrial activity can be increased by calcium uptake [53,54]. High calcium levels can however damage and rupture the mitochondria [55]. The cytosolic levels of calcium are low, but high in the ER and the extracellular environment. Activation of 1,4,5-triphosphate-receptor (IP3R), a membrane glycoprotein complex acting as calcium channel on ER membranes, releases calcium into the cytosol that can now be taken up by mitochondria. Alternatively, lysosome rupture might cause calcium release into the cytosol, for entry into mitochondria [54]. This would have to occur in close proximity, as calcium is readily chelated in the cytosol [56]. Furthermore, binding of ATP to the transmembrane ionotropic receptor P2X purinoceptor 7 (P2RX7) can increase the intracellular calcium levels, causing a drop in mitochondrial potential and damaged mitochondria (Figure 2) [57]. Another and more regulated exchange of calcium with the mitochondria is direct contact with the ER via the mitochondria-associated membrane (MAM). At the MAM, calcium levels can be altered and thereby adjust mitochondrial metabolism [58]. It is suggested that potassium efflux, as associated with inflammasome activation, is coupled to calcium influx to balance charges over the plasma membrane. Yet, it has been shown that they occur relatively independent from one another, with both being necessary for inflammasome activation [59]. The role of mtROS in AID is well accepted, but the mechanisms of mtROS release are not completely understood and might differ between AID types.

mtDNA

Mitochondria maintain their own genome, which under normal conditions is shielded from cellular receptors. Extensive cellular damage can however cause mtDNA to be released into the extracellular environment. Indeed, patients suffering from systemic inflammatory response syndrome (SIRS) (the stage leading up to sepsis) have increased levels of mtDNA in their blood plasma. Here, mtDNA provides a proinflammatory signal by binding the PRR Toll-like receptor (TLR)9 [60]. Release of mtDNA in the cytosol through loss of mitochondrial integrity [51], can also initiate inflammasome activation. Prior to, or during mitochondrial release, mtDNA is oxidized by ROS, transforming it into a direct ligand for NLRP3 (Figure 1) [61].

Cytosolic release of mtDNA may contribute to the pathogenesis or propagation of AID. In support, experiments using MKD as a disease model showed that increasing mitochondria with loss of membrane potential was associated with mtDNA accumulation in the cytosol [49]. Similarly, in TRAPS, autophagy becomes defective when TNFR1 is retained for a prolonged period within the ER [42]. The authors detected elevated levels of mtROS, but unfortunately did not report mtDNA measurements. The study did not address whether damaged mitochondria contribute directly to pathogenesis of TRAPS, or are merely a consequence. Altogether, there is now credible support that mitochondrial components, mtROS, and mtDNA, are involved in inflammasome activation and subsequent immune responses. Although loss of mitochondrial membrane potential and integrity is a component of normal signaling pathways, we propose that dysregulation of mitochondrial integrity may tip the scale towards excess immune activation in AID.

MAMs

Calcium and ER stress

Mitochondria exist as separate cellular organelles, however, a fraction is associated with the ER [3]. At this interface, known as the MAM, mitochondria and the ER are in close proximity, which allows functional crosstalk [3,62]. At the MAM, the ER contributes to tight regulation of calcium levels, while vice versa, mitochondria provide the ER with the oxidizing environment required for oxidative protein folding [3]. In addition, several lipid-synthesizing enzymes are present at the MAM interface, which benefit from the exchange of metabolites between the ER and mitochondria (Figure 1) [3]. The physical connection between ER and mitochondria is vital for proper functioning of the cell and is involved in several immune signaling pathways [63].

The regulation of calcium exchange by calcium transporters is pivotal to the MAM, and essential for both ER and mitochondria [3]. Several chaperones depend on calcium and oxidation for their function in assisting nascent proteins to achieve their native folding [64,65]. Faulty chaperone function can cause accumulation of incorrectly folded proteins in the ER, and induction of the unfolded protein response (UPR) [66]. The UPR is a dedicated program to restore proper ER function, by reducing general protein translation, and increasing the expression of chaperone proteins [67]. In addition, UPR induces localized autophagy of the ER parts where the protein translation

and folding is hampered [66,67]. The UPR is accompanied by calcium efflux from the ER, which is taken up by mitochondria. Prolonged duration of ER stress, however, can cause mitochondrial damage and even apoptosis through excessive calcium uptake by the mitochondria.

Most AIDs are caused by SNPs that result in amino acid substitutions affecting the protein structure. Such mutations may disrupt protein stability and folding efficiency. The mutation in TNFR1 in TRAPS causes retention of the receptor in the ER, thus inducing ER stress [42]. In the case of MKD, there is little MVK protein detectable in patient cells. Experiments aimed to increase MVK protein folding efficiency had limited success, even though higher activity of the protein was achieved [68]. This suggests that mutations might disturb protein folding and stability, thereby contributing to ER stress. ER stress can prime cells for the expression of pro-IL-1β via NF-κB activation, contributing to IL-1β secretion [69]. Thus, ER stress, induced by misfolding of mutant proteins, may contribute to the pathogenesis of AID. This example clarifies that AID may not always involve mutations in obvious protein suspects such as NLRP3 or caspase-1. ER stress can also be induced by mitochondrial dysfunction [70], so one could speculate that the protein folding problems seen in TRAPS and MKD could originate from mitochondrial dysfunction, rather that the ER. The maturation of IL-1β following ER stress is intact in ASC deficient cells. The maturation occurs through an alternative pathway and requires caspase-8 [71]. Thus, compensatory mechanisms do exist to ensure IL-1β production upon stress, emphasizing the difficulty in separating cause from consequence in AID.

GTPases and scaffolds

The MAM is a highly organized membrane structure and many small GTPases are involved in the formation and regulation of membrane shape and protein contacts [72]. The small GTPase Rab32 can regulate important protein contacts in the MAM as well as influencing the calcium levels by disrupting calnexin retention at the ER. Calnexin, a marker for MAM, is a protein chaperone and a regulator of the sarcoendoplasmic reticulum Ca^{2+} ATPase (SERCA) 2b [73]. Perhaps more importantly, Rab32 has a predicted prenylation site, which would be affected in MKD, as here the biochemical synthesis route for prenylation motifs is diminished [74]. One may therefore deduce that dysregulated calcium homeostasis contributes to AID through induction of mitochondrial stress, as proposed in MKD.

The induced oligomerization of NLRP3 multiprotein complexes, via NACHT domains, is key to activation of the inflammasome. This process probably takes place at the MAM [47]. In CAPS, the mutations in NLRP3 are within the oligomerization domain [75]. Some affect the NBD, while others affect the predicted protein–protein interaction sites required for oligomerization. It is assumed that this interaction site is important for self-oligomerization, but it might also involve interaction with MAM-localized proteins. One such example, the MAM-associated protein mitochondria antiviral signaling (MAVS) is involved in antiviral signaling, and is mostly found on the outer mitochondrial membrane (Figure 1). Activated MAVS triggers the assembly of a macromolecular signaling complex that forms at the MAM and activates NF-κB [76]. A handful of studies describe the crosstalk between MAVS and NLRP3, with one report indicating that MAVS physically associates with NLRP3 and facilitates oligomerization [77]. There is no consensus on how exactly NLRP3 inflammasome activation relates to MAVS signaling, although MAVS signaling can also induce active caspase-1 without the need for NLRP3 [77–79].

A recent study identified another candidate protein that mediates activity of the NACHT domain, guanylate binding protein (GBP) 5. GBP proteins are induced by interferon (IFN)γ and induce defenses against intracellular pathogens [80]. GBP5 binds NLRP3 and assists with oligomerization [81]. Here however, it was not reported if the NBD activity of NLRP3 was required for oligomerization during inflammasome activation, but other studies have supported this possibility [75,82]. Of note, the GTPase activity of GBP5 was not required [81]. A trigger for GBP5 activation is unknown, although it has been suggested that GBP5 dimerization is induced upon nucleotide binding. GBP5 itself is induced by (IFN)γ [80], but such induction may be relevant to propagation of autoinflammation rather than initial immune activation. Although little is known about the cellular localization of GBP5, considering its role in NLRP3 oligomerization, localization to the MAM is a possibility. Finally, GBP5 shows interesting links to MKD. GBP5 contains a C-terminal motif that is normally prenylated with the lipid building moieties that MKD patients lack [37]. Dysregulation of small GTPases related to GBP5 has been seen in MKD, and could therefore contribute to the dysegulated IL-1β secretion in this AID.

Mitochondrial dynamics

Mitochondria are dynamic organelles. They continuously undergo fission, fusion, can migrate to different parts of the cell, respond to cellular signals, and become degraded (Figure 1). Although the study of fission and fusion has intensified recently, much remains unknown regarding mitochondrial morphology and how healthy mitochondria contribute to immune homeostasis [83,84].

Extensive stress or apoptosis causes mitochondria to disintegrate [85]. Proteins such as parkin, a component of the E3 ubiquitin ligase complex, protect against excessive fragmentation by the induction of mitophagy [86]. In addition, parkin protects against mitochondrial damage induced by ER stress. Parkin also provides protection against calcium transfer inhibitors, such as thapsigargin, that induce mitochondrial damage by disrupting calcium homeostasis between the ER and the mitochondria [87]. Caspase-1 can, however, cleave parkin and thereby diminish its protective properties. These findings suggest the existence of a possible positive feedback loop whereby ER stress or mitochondrial damage elicits caspase-1 activation, and caspase-1 activation in turn leads to more mitochondrial damage [52].

During mitophagy, inflammatory content such as mtROS, mtDNA, and cytochrome-C, are neutralized through engulfment within double-membrane vesicles

[86]. Although some dedicated proteins regulate mitophagy, the majority are also involved in autophagy. Knockout models of autophagy have shown that proteins, such as Beclin-1, autophagy-related protein (ATG) 16L and microtubule-associated proteins 1A/1B light chain 3B (LC3B) play nonredundant roles in reducing inflammasome activation [51]. Indeed, experiments using cells deficient in these proteins showed significantly elevated IL-1β secretion upon stimulation, due to defective mitochondrial clearance. These findings further support the notion that mitochondrial stability is an important determinant in inflammasome activation [51].

Several examples are now reported in which deficiency in mitophagy is a feature of AID. It was recently shown that there is increased accumulation of defective mitochondria and a lower number of autophagosomes after induction of autophagy in MKD [49]. In TRAPS, defective autophagy fails to remove the mutant TNFR [42]. Coincidentally, an increase in mtROS is observed which promotes an increase in IL-1β [43]. Although the authors of the latter study argue that the mechanism is more general than the inhibition of mitophagy, a contributing role for mitophagy would be expected. The processes of mitochondrial fission and fusion rely heavily on small GTPases. Indeed, lack of prenylation in MKD can affect the localization and activity of GTPases. In addition, studies using an MKD model of monocytic cells have revealed cells containing mitochondria with altered shapes [49]. Experiments that will further dissect the mechanistic links that connect prenylation of small GTPases to mitochondrial fission or fusion and their contribution to AID pathogenesis is warranted.

Concluding remarks and future perspectives

The role of mitochondria in AID has turned out to be a surprising one thus far. None of the known mutations in the AID affect mitochondria directly. However, their involvement in the activation of inflammasomes is now recognized, and implies that mitochondrial malfunction may contribute to various AID subtypes, as we have discussed. Clarification of the effects of increased activity, reduced stability, altered shape or localization of mitochondria may thus provide important insights in the molecular pathways and the development and pathogenesis of AID. Another crucial issue for investigators is to untangle the sequence of events in inflammation and AID. Feedback loops exist and sometimes it is unclear whether reported findings are cause or consequence. We believe that combined research efforts of investigators in metabolic disease and in immunology are optimally geared to elucidate the roles played by mitochondria in AID development and propagation. Although they may not constitute a direct target for therapeutic intervention in AID, such understanding will help elucidate how mitochondria contribute to dysregulation of innate immunity (Box 1). Finally, such outcomes will help identify new avenues for the development of interventions, not only for AID but also other inflammation-related pathologies.

Box 1. Outstanding questions

- How does mitochondrial morphology influence inflammasome activation, and is mitochondrial turnover altered in AID?
- How does the MAM contribute to AID and is it crucial for the assembly of the inflammasome?
- Does a common mitochondria-centered pathway contribute to different AIDs that could be used as a potential therapeutic target?

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