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REVIEW PAPER

Mitochondrial DNA and inflammatory diseases

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Abstract Increasing experimental evidence supports a connection between inflammation and mitochondrial dysfunction. Both acute and chronic inflammatory diseases course with elevated free radicals production that may affect mitochondrial proteins, lipids, and mtDNA. The subsequent mitochondrial impairment produces more reactive oxygen species that further reduce the ATP generation, increasing the probability of cell death. Mitochondrial impairment in now considered a key factor in inflammation because (1) there are specific pathologies directly derived from mtDNA mutations, causing chronic inflammatory diseases such as neuromuscular and neurodegenerative disorders, (2) there are neurodegenerative, metabolic, and other inflammatory diseases in which their progression is accompanied by mitochondrial dysfunction, which is directly involved in the cell death. Recently, a direct implication of mitochondrial reactive oxygen species and, particularly, mtDNA in the innate immune response has been reported. Thus, the mitochondria should be considered targets for new therapies related to the treatment of acute and chronic inflammatory diseases, including the auto-inflammatory ones.

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Introduction: inflammatory clues and mtDNA

Although inflammation constitutes a defensive response of the organism against noxious stimuli, occasionally it can become in a deleterious reaction responsible for tissue injury in a variety of inflammatory diseases including the so-called auto-inflammatory diseases (McMorrow and Murphy 2011). Tissue damage, infection, and stressor signals are sensed by the innate immune system through pattern recognition receptors (PRRs), which initiate defense and repair programs (Tschopp 2011). Normally, the acute inflammatory response should be able to repair the damage, preventing further injury to the cell or tissue. However, when the acute inflammatory response is excessive, there is a progressive damage and multiorgan failure. A third condition, i.e. chronic inflammation, takes place when a progressive cell death cannot be counteracted by the acute response of the innate immune system. The latter underlies a number of diseases, including neurodegenerative and metabolic diseases, and it is also applicable to aging itself.

Inflammatory diseases course with increased reactive oxygen (ROS) and nitrogen (RNS) species production that may affect mitochondrial proteins, lipids, and DNA (Crimi et al. 2006). Moreover, inducible nitric oxide synthase (iNOS) induction during inflammation led to increased mitochondrial iNOS (i-mtNOS) enzyme activity, producing high levels of nitric oxide ($NO^{\cdot}$) responsible for the respiratory chain (RC) inhibition, ATP reduction (Escames et al. 2003; Lopez et al. 2006b), and mtDNA damage (Bartz et al. 2011), all of them related to the severity of the inflammatory process (Brealey et al. 2002). In these conditions, mitochondrial impairment produces ROS and RNS that in turn reduces mitochondrial bioenergetics, favoring cell damage and death (Escames et al. 2007; Lopez et al.

2006b). On the other hand, there are specific pathophysiological conditions directly derived from mtDNA mutations, causing chronic inflammatory diseases including neuromuscular and neurodegenerative disorders (Acuna et al. 2011; DiMauro 2010; Muller et al. 2010; Reddy 2008). There are also multiple neurodegenerative, metabolic, and other inflammatory diseases that course with progressive mitochondrial dysfunction and cell death (Reddy 2008). Finally, a direct implication of mitochondrial ROS and, specifically, mtDNA in the innate immune response has been recently showed (Nakahira et al. 2011; Zhou et al. 2011). Thus, the mitochondria-inflammation interplay in these diseases will be revised here.

Mitochondrial DNA

Mitochondrion, the main energetic source of the cell, is the unique mammalian organelle that contains its own genetic material, i.e. mtDNA. Human mtDNA is a 16.569-kb circular, double-stranded molecule ([H]eavy and [L]ight strands), which contains 37 genes: 2 rRNA genes, 22 tRNA genes, and 13 structural genes encoding subunits of the mitochondrial respiratory chain complexes (Anderson et al. 1981). The genetics bases of mtDNA differ from Mendelian genetics in some factors: (1) the mtDNA is polyploid; (2) normal cells harbor homoplasmic mtDNA copies but mutated cells harbor heteroplasmic mtDNA copies; (3) at cell division, the proportion of mutant mtDNAs in daughter cells may shift and the regulation of this process (if any) is misunderstood. This phenomenon is called mitotic segregation; and (4) the mtDNA is exclusively inherited from the mother (Chen et al. 1995; DiMauro 2004; Van 2004).

Because mtDNA only codes 13 structural proteins, its maintenance, replication and transcription totally depend on nuclear DNA (nDNA)-encoded proteins that are imported into mitochondria. Some of these proteins are packaged together with mtDNAs in a macrocomplex called nucleoid (Iborra et al. 2004; Legros et al. 2004). It has been described that each mammal nucleoid is composed of 1–10 copies of mtDNA (Holt et al. 2007) and associated proteins, which at least include major mtDNA binding proteins involved in mtDNA maintenance, such as TFAM, mtSSB, polymerase γ, the mitochondrial helicase Twinkle, mitochondrial RNA polymerase, TFBM1, TFBM2, Terf1 and mitochondrial topoisomerase I (Bogenhagen 2010; Chen and Butow 2005; Wang and Bogenhagen 2006). Other proteins with helicase motifs and some factors involved in protein folding and quality control have been also identified as components of the mitochondrial nucleoids (Mootha et al. 2003; Wang and Bogenhagen 2006). In addition, an antioxidant system composed by manganese-dependent superoxide dismutase (Mn-SOD) and glutathione peroxidase (GPx) seems to be an integral constituent of mitochondrial nucleoids (Kienhofer et al. 2009). These antioxidant enzymes may protect mtDNA from oxidative damage.

Using the nucleoid machinery, mtDNA is replicated in a different way that nDNA. Two different models have been purposed to explain the mtDNA replication mechanism: (1) an asynchronous strand displacement model, and (2) a strand-coupled bidirectional replication model (Brown et al. 2005). In both models, the DNA polymerization reaction is performed by polymerase γ, which needs a mitochondrial deoxynucleotide triphosphates (dNTPs) pool for the mtDNA synthesis. Interestingly, mitochondria have their own biochemical pathway to produce the necessary dNTPs for mtDNA replication. This biochemical pathway, which is called *salvage pathway*, is independent of the cell cycle because mtDNA is continuously replicating even in postmitotic cells (Marti et al. 2003). Interestingly, defects in the enzymes involved in the *salvage pathway* cause mitochondrial diseases (Mandel et al. 2001; Nishino et al. 1999; Saada et al. 2001).

Besides mtDNA replication machinery, mtDNA also needs a transcription process perfectly regulated by nDNA-encoded transcription factors. This mtDNA transcription has a particular characteristic because transcription from both light strand promoter and heavy strand promoter is polycistronic and produces near genome-length transcripts (no individual gene transcription), which are processed to release the individual RNA molecules. Several transcription factors can regulate the initiation of the transcription, including PGC1α, NRF-1, NRF-2, TFAM, TFB1M and TFB2M; but also some important transcription factors can regulate the mtDNA transcription termination, including MTERF-1, -2, -3 and -4 (Falkenberg et al. 2007).

Together with mtDNA, all proteins and factors that regulate mtDNA replication and transcription are modulated to respond to the cell energy demand and pathophysiological conditions including inflammatory disease and oxidative stress related disorders.

The NLRP3 inflammasome-mtDNA connection

In the process of energy generation by the oxidative phosphorylation system, mitochondria generate ROS in such way that they are considered the main ROS-generating organelle in the cell. Besides ATP/heat production, last years have provided evidences that imply mitochondria in calcium homeostasis and apoptosis/necrosis regulation. With the recent finding showing that NLRP3 (NLR-related protein 3 nucleotide-binding domain leucine-rich repeat containing receptors-related protein 3) inflammasome

(Mariathasan et al. 2004; Martinon et al. 2002) can be activated by mitochondrial ROS/mtDNA, an unexpected new function for these organelle in controlling the innate immune response is now emerging.

A particular case of inflammation: cryopyrinopathies

Cryopyrinopathies are a group of autoinflammatory diseases different from autoimmune diseases that include familial cold auto-inflammatory syndrome, Muckle–Wells syndrome and chronic infantile neurologic cutaneous articular syndrome (they are also grouped under CAPS, cryopyrin-associated periodic syndromes). They are characterized by recurrent episodes of fever and systemic inflammation of tissues, such as joints and skin (Neven et al. 2008).

Cryopyrinopathies are caused by inherited mutations in the NLRP3 gene that encodes NLRP3/NALP3 protein or cryopyrin (Hoffman et al. 2001), which belongs to the family of NLR (nucleotide-binding domain and leucine-rich-repeat containing) proteins (Ting et al. 2006). As a difference with the NF-kB pathway activation, which is initiated by stimulation of the Toll-like membrane receptors (TLRs), the NLRP3 proteins act as intracellular sensors of stressors to form a complex called the inflammasome (Martinon et al. 2002). NF-kB and NLRP3 constitute part of the innate immune responses and work together to activate inflammatory cytokines, such as IL-1β, IL-6, IL-18, and IL-33. Mutations in the NLRP3 gene result in an inappropriate activation of the inflammasome, leading to excessive IL-1β release and manifestation of CAPS symptoms (Aganna et al. 2002; Hoffman et al. 2001; Kubota and Koike 2010). Although there are IL-1β inhibitors able to counteract most of the symptoms of these diseases (Gabay et al. 2010), the patients display symptoms, probably dependent on other inflammatory mediators activated by NLRP3, which remain after the treatment. From a preventive point of view, the development of successful therapies for cryopyrinopathies should include, besides IL-1β antagonists, molecules targeting mitochondria to reduce their production of ROS and, thus, disabling the inflammasome activation.

A view of the NLRP3–mtDNA relationship

Pathogen-associated molecular patterns (PAMPs) such as bacterial lipopolysaccharides (LPS), and damage-associated molecular patterns (DAMPs), such as uric acid and extracellular ATP, bind and activate specific pattern recognition membrane (TLRs) or cytosolic (NLRs) receptors. One of the most characterized NLRs is NLRP3, which acts as a cytosolic receptor sensing stressor signals, including ROS, ATP and mtDNA (Dunne 2011;Masters et al. 2009), and its activation leads to the inflammasome formation and caspase-1 activation (Fig. 1).

NLRP3 or NALP3 contains an amino terminal pyryn domain, a carboxyl terminal LRR domain (ligand binding leucine-rich repeat domain), and a central NACHT domain (nucleotide-binding and oligomerization) (Agostini et al. 2004; Cassel et al. 2009). When activated, NLRP acts as a multiprotein complex named NLRP3 inflammasome, constituted by NLRP3 protein, the ASC coupling protein (apoptosis-associated speck-like protein containing CARD, caspase activation and recruitment domain) and caspase-1 (Agostini et al. 2004). Once constituted, the inflammasome activates caspase-1 that cleavages pro-IL-1β, pro-IL-18, and pro-IL-33, yielding the corresponding active forms of these cytokines (Mariathasan 2007). IL-18 induces TNFα and INFγ, which enhance the NF-κB inflammatory pathway (Nakanishi et al. 2001; Takeda et al. 1998). The combination of the NF-κB and NLRP3 inflammasome pathways yields to a overstimulation of the inflammatory response (Tsutsui et al. 2010).

Although the causative effect of ROS on NLRP3 inflammasome activation was recognized some years ago (Schroder et al. 2010), it was not until recently when Zhou et al. (2011) and Nakahira et al. (2011) demonstrated that mitochondria can modulate the innate immunity through the direct activation of the NLRP3 inflammasome. Nakahira et al. (2011) reported that autophagy inhibits the NLRP3 inflammasome activation preventing the accumulation of dysfunctional mitochondria. Zhou et al. showed that mitochondrial ROS trigger the NLRP3 inflammasome. Together, these data suggest that autophagy/mitophagy inhibit the NLRP3 inflammasome by removing ROS-producer dysfunctional mitochondria. A link between NLRP3 inflammasome and mitochondria is also supported by the subcellular location of the organelle. Under resting conditions, NLRP3 and ASC proteins are associated with the endoplasmic reticulum (Zhou et al. 2011). Once activated, the NLRP3 inflammasome moves to a perinuclear distribution together with mitochondria (Zhou et al. 2011) (Fig. 2).

The inflammasome activation also occurs in response to cytosolic bacterial, viral and mammalian DNA, although the sensing molecule is ASC and not NLRP3 (Muruve et al. 2008). mtDNA can act as a DAMP (Zhang et al. 2010) because the activation of the NLRP3 inflammasome is impaired in macrophages depleted from mtDNA (Nakahira et al. 2011). Besides IL-1β, cytosolic mtDNA also increases IL-18 secretion. Before mtDNA release to the cytosol, mitochondrial ROS open the mitochondrial permeability transition pore (MTP). The mtDNA copy number in the cytosolic fraction is increased with LPS (and ATP) treatment, whereas antioxidants such as Mito-TEMPO, or cyclosporine A, which closes the MTP, reduce the presence

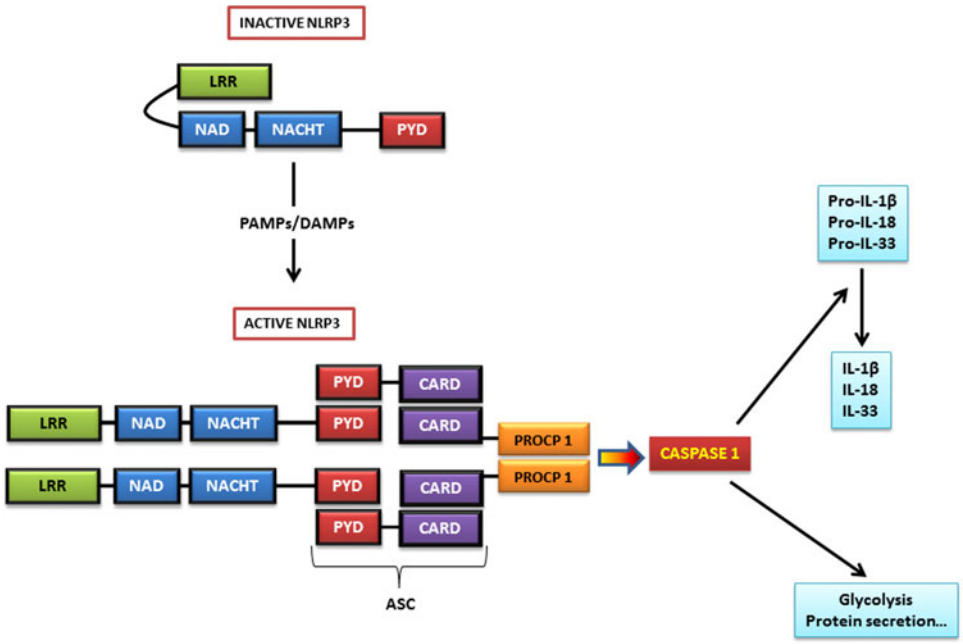

Fig. 1 Schematic representation of the NLRP3 components assembly during the inflammasome activation. In its inactive form, NLRP3 is constitutively expressed in the cytoplasm (*upper*). A series of PAMPs or DAMPs may induce conformational modifications in NALP3, which rapidly interacts with ASC through their respective pyrin domains. Then, ASC can interact with procaspase 1 through their caspase recruitment domains, activating caspase 1. In turn, active caspase 1 activates the pro-inflammatory cytokines pro-IL-1β and pro-IL-18 to their active IL-1β and IL-18 forms, respectively. In addition, caspase 1 also can mediate several other cell functions, including glycolysis, protein secretion, etc. *ASC* apoptosis-associated speck-like protein containing a caspase recruitment domain, *CARD* caspase recruitment domain, *DAMPs* danger-associated molecular patterns, *IL* interleukins, *LRR* leucine-rich-repeat domain, *NACHT* *N*ucleotide-binding oligomerization domain, leucine-rich-repeat family, *A*poptosis inhibitory protein, *C*lass II, major histocompatibility complex transactivator, *H*et-E incompatibility locus protein from *Podospora anserina*, *T*elomerase-associated protein 1, *NAD* NACHT-associated domain, *NALP3* NACHT leucine-rich repeat and pyrin domains containing protein 3, *PAMPs* pathogen-associated molecular pattern, *PROCP 1* procaspase 1, *PYD* pyrin domain

of mtDNA in the cytosol. The activation of caspase-1 was directly related to the amount of mtDNA in the cytosol, suggesting a role of mtDNA as a coactivator of caspase-1. Interestingly, deficiency in NLRP3 suppresses the mtDNA release into the cytosol in response to LPS, although the generation of ROS by mitochondria was not affected by NLRP3 deficiency. Together with additional experiments, Nakahira et al. (2011) suggested that the NALP3 inflammasome is required for MPT opening and mtDNA translocation to the cytosol in response to LPS plus ATP. In any case, deficiency in the autophagic/mitophagic process leads to the accumulation of dysfunctional mitochondria and increased NARLP3 inflammasome activation. Therefore, autophagy/mitophagy represents a survival mechanism for the cell, removing damaged mitochondria and preventing ROS/mtDNA-induced NLRP3 activation.

Mitochondrial DNA alterations in chronic inflammatory diseases

The relationships between mtDNA and NLRP3-inflammasome activation suggest the participation of this innate immune activation pathway in pathologies coursing with mitochondrial dysfunction, but different from the autoinflammatory diseases. In this regards, we focus here on some conditions involving chronic inflammation and mitochondrial hyperoxidative status, including neurodegenerative disorders, and metabolic diseases. Specifically, the NLRP3 inflammasome–mitochondria interplay as a common inflammatory pathway in these diseases will be analyzed. Other pathologies that may involve inflammation, such as primary mitochondrial disorders (DiMauro and Schon 2008) and autoimmune disorders (Roifman et al. 2011), will be not considered here.

Neurodegenerative diseases

A common feature in neurodegenerative diseases is the presence of oxidative/nitrosative stress, inflammation, and mitochondrial impairment. Neurodegenerative disorders include diseases that display mtDNA and nDNA mutations, although in some of them it is yet unclear whether these mutations are cause or consequence of the disease. Some examples include Alzheimer's disease (AD) and Parkinson's disease (PD).

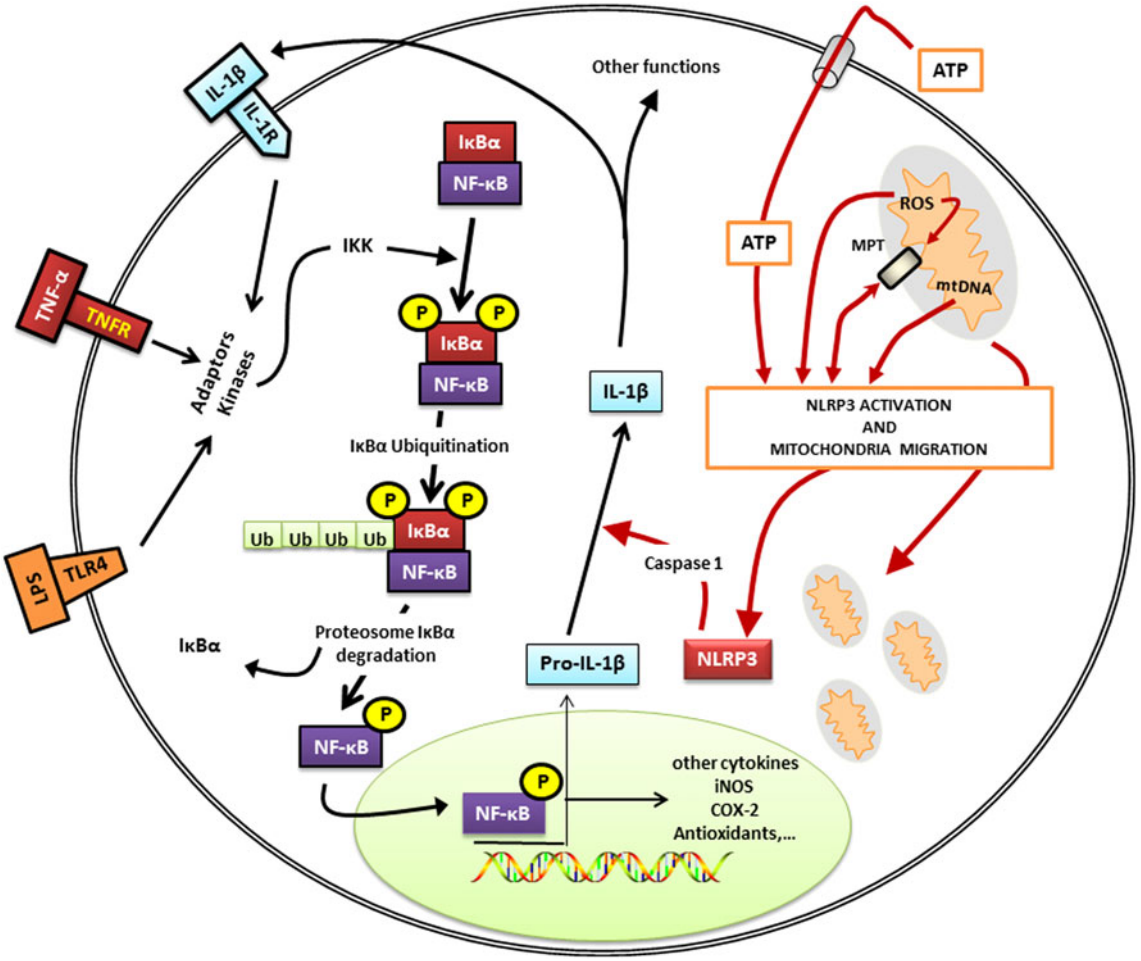

Fig. 2 Cooperation between NF-kB and NLRP3 inflammasome in the innate immune response. In canonical NF-κB (*left*), stimulation of the TLR4 or TNFR receptors lead to activation of a series of adaptor proteins and kinases that activate IKK complexes. IKKs phosphorylate IκB proteins, targeting them for polyubiquitination and posterior degradation by the proteasome. As a result, the p50/p65 heterodimer of the NF-κB is released, and enters the nucleus, activating gene transcription. Among others, NF-κB induces the expression of pro-IL-1β that, after activated to IL-1β, may act through its specific membrane receptor to further increase the NF-κB pathway. In parallel, the inflammatory condition that triggers the NF-κB pathway also may induce mitochondrial damage and ROS production, which in turn induces mtDNA damage and open the MPT pore. The releases of ROS and mtDNA to the cytoplasm activate the NLRP3 inflammasome, activating caspase 1 that is responsible for the production of IL-1β from the pro-IL-1β induced by NF-κB. Once activated, NLRP3 inflammasome also participates in the MPT opening. Other factors, including LPS and mediated NLRP3 activation. Interestingly, activated NLRP3 inflammasome moves with mitochondria to a perinuclear location. *IκBα* NF-κB inhibitor α, *IL-1β* interleukin 1β, *IL-1R* IL-1β receptor, *LPS* lipopolysaccharide, *MPT* mitochondrial permeability transition pore, *NF-κB* nuclear factor kappa B, *ROS* reactive oxygen species, *TLR4* toll-like receptor 4, *TNF-α* tumor necrosis factor α, *TNFR* TNF-α receptor, *Ub* ubiquitination

The mitochondria participate in the pathophysiology of AD, although the exact mechanism is yet unclear (Reddy and Beal 2008). Typically, AD shows accumulation of extracellular β-amyloid (Aβ), and hyper-phosphorylated forms of the microtubule-associated protein tau (Twig et al. 2008). Mitochondrial accumulation of Aβ induces ROS generation and that may explain the early mitochondrial failure and cell death in AD (Caspersen et al. 2005; Chen and Yan 2010; Devi et al. 2006; Manczak et al. 2006). Inversely, mitochondrial inheritance was related to the risk of AD (Chen et al. 2000; Swerdlow et al. 2010). Anyway, attempts to identify mtDNA mutations in AD brains have not given conclusive results (Aliev et al. 2008). Recent data support a role of mitochondrial fusion/fission impairment in the pathogenesis of AD (Manczak et al. 2011; Wang et al. 2009). Mitochondrial dynamics change mitochondrial morphology and, depending on the energy demand of the cell, mitochondria can move through the cytosol (Frazier et al. 2006). Interestingly, it was reported the accumulation of mitochondria in the perinuclear area of hippocampal neurons over-expressing APP (Wang et al. 2008), which resembles the mitochondrial redistribution after NLRP3 inflammasome activation. These data aims in studying the participation of

mitochondrial ROS and mtDNA in the NLRP3 inflammasome activation during AD.

The mitochondrial involvement in Parkinson's disease (PD) is related to the deficiency of mitochondrial complex I (C–I) activity in substantia nigra (Schapira 1999), with a reduction in GSH levels, suggesting the existence of oxidative stress and subsequent nDNA and mtDNA damage. Together, these pathogenic mechanisms may lead to the dopaminergic degeneration and death (Swerdlow et al. 2010). The existence of mtDNA and POLG mutations in early-onset Parkinsonism in different families (Davidzon et al. 2006) support the mitochondria connection in PD. Anyway, there are no currently convincing proofs for a primary role of mtDNA mutations in this disorder (Howell et al. 2005). Mutations in a series of nuclear genes (PARK2, PARK7, PINK1, SNCA, LRRK2 and HTRA2) have been found to be associated with the familial form of PD (DiMauro and Schon 2008). These genes coded for a series of proteins involved in mitochondrial function, oxidative stress damage, and control of MPT (Hardy et al. 2006). Following to dopaminergic death in substantia nigra, there is an inflammatory reaction depending on the induction of cytosolic iNOS (Liberatore et al. 1999) and mitochondrial iNOS (i-mtNOS), yielding high amounts of $NO^{\cdot}$ responsible for the inhibition of the respiration (Acuna-Castroviejo et al. 2007; Tapias et al. 2009). The consequence is an overproduction of mitochondrial ROS, mtDNA damage and MPT opening that maintains the inflammatory process. Any treatment of PD, from pharmacological therapy to stem cell transplantation, lacks efficacy due to the sustained hyperoxidative status and inflammation in the substantia nigra. Moreover, no beneficial effects were found after administration of anti-inflammatory drugs to PD or AD patients (Driver et al. 2011; Sonnen et al. 2010). Consequently, inflammation in these neurodegenerative diseases should be beyond from the usual targets of the anti-inflammatory drugs. In this regard, the participation of NLRP3 inflammasome in the pathogenesis of PD should be now seriously considered.

Neurogenesis and mtDNA

Neurogenesis continues throughout life, with thousands of new neurons generated daily in the dentate gyrus subgranular zone (Cameron and McKay 2001; Gage 2000) and in the subventricular area (Alvarez-Buylla and Lois 1995). It is known that neural cells have higher aerobic metabolic rate than the neural stem cells (NSCs) (Siggins et al. 2008). Stem cells have few mitochondria with poorly developed cristae, and low mtDNA content (Baharvand and Matthaei 2003; Cho et al. 2006; St John et al. 2005), and tend to localize in perinuclear groups. When cells differentiate, they show larger mitochondria with distinct cristae. It has been proposed that the mitochondrial perinuclear arrangement might be a cellular marker for stemness (Lonergan et al. 2006) whereas an elevation in ATP content may reflect a loss of stemness and subsequent onset of differentiation (Lonergan et al. 2007). Increased aerobic activity required for ATP production produces ROS that can potentially influence the differentiation process, rendering mitochondrial macromolecules vulnerable to oxidation damage (Wang et al. 2010). Thus, the differentiation process may damage mtDNA, whereas mtDNA integrity is required for the initial development of RC complexes I, III, and IV (Stuart et al. 2005). Consequently, equilibrium between mitochondrial maturation and ROS production should be strictly maintained during neurogenesis.

Neural precursor cells die within a short period of time after mtDNA depletion (Fike et al. 2009). Inhibition of mitochondrial RC promotes rapid loss of mitochondrial membrane potential in neurons (Bolanos and Almeida 2006), opening the MPT and initiating apoptosis. Complex I defects are enough to decrease differentiation of ES cells into the functional neurons (Kirby et al. 2009). Therefore, mitochondrial inactivation has been suggested to be a critical mechanism of cellular damage in neurodegenerative diseases (Fukui and Moraes 2008). Volobouéva et al. (2010) observed that 24 h of mitochondrial inhibition produced no significant changes in astrocytes or oligodendrocyte viability, but reduced viability of mature neurons by 30%, and reduced survival of immature neurons by 60%. These authors showed that protecting the mitochondrial function reverses the inflammation-associated impairment on neurogenesis.

These results suggest that mitochondrial function might play an important role in neurogenesis. NSCs may play a therapeutic role in some neurodegenerative diseases, such as PD (Kim et al. 2002), as well as in the repair and regeneration process after neurological damage secondary to ischemia or injury (Johansson et al. 1999). But mitochondrial ROS and mtDNA damage produced during neuronal differentiation, resulting in defective mitochondrial maturation and inflammation, limits the beneficial therapy with NSCs. Therefore, the connection between dysfunctional mitochondria, which produce ROS and release mtDNA to the cytosol after MPT opening, and the NLRP3 inflammasome, should be explored during NSCs differentiation. If this connection exists, we will get a new therapeutical strategy to improve the NSC survival.

Metabolic diseases

The metabolic syndrome, comprising obesity, insulin resistance, and type-2 diabetes mellitus (Lim et al. 2010), is closely associated with chronic inflammation (Hotamisligil et al. 1993). Insulin resistance is also related to the

production of ROS (Goldstein et al. 2005), and mitochondrial dysfunction (Frojdo et al. 2009; Semple et al. 2004). Some data suggest that reduced OXPHOS and ATP production following mtDNA reduction, and increased ROS formation, precede the development of type-2 diabetes mellitus (Lee et al. 1998; Morino et al. 2005; Stienstra et al. 2010).

Alteration in the expression of PGC-1α during the inflammatory process of metabolic syndrome further supports a role of mtDNA in this disease. PGC-1α is an activator of mitochondrial biogenesis and OXPHOS, and its expression is reduced during type-2 diabetes. Moreover, the OXPHOS genes that are dysregulated in type-2 diabetes are under transcriptional regulation of PGC-1α (Handschin et al. 2007). Skeletal muscle-specific PGC-1α knockout mice showed increased expression of pro-inflammatory genes, which in turn suppresses glucose-stimulated insulin secretion (Handschin et al. 2007). The deficiency of PGC-1α may be directly related with decreased mtDNA and elevated production of ROS, both of them associated with the NLRP3 inflammasome (Zhou et al. 2011), which acts as a sensor of metabolic stress (Schroder et al. 2010). NLRP3 agonists stimulate the association of NLRP3 with thioredoxin-interactin protein (TXNIP). TXNIP, which is normally bound to thioredoxin, is dissociated in the presence of ROS. Once dissociated from thioredoxin, TXNIP interacts with NLRP3 in a ROS-dependent manner (Zhou et al. 2010). A connection between TXNIP, NLRP3 and IL-1β is now stated (Schroder et al. 2010). In this sense, TXNIP expression is induced by glucose and repressed by insulin, and it is elevated in type-2 diabetes (Chen et al. 2008). Therefore, ROS induce TXNIP dissociation and binding to NLRP3, activating the inflammasome that triggers IL-1β, and leading to pancreatic β-cell death.

These data suggest that glucose excess drives ROS production by increasing the activity of the mitochondrial RC (Nishikawa and Araki 2007), and the expression of TXNIP. The activation of NLRP3 inflammasome and the reduction in PGC-1α further favor mitochondrial dysfunction and mtDNA depletion, facilitating IL-1β release and pancreatic β-cell death. This vicious cycle contributes to type-2 diabetes mellitus progression and suggests that mitochondria ROS/mtDNA and NLRP3 inflammasome are new targets for therapeutic intervention in metabolic syndrome.

Mitochondrial DNA alterations in acute inflammatory diseases

The recognition of pathogen-derived molecules by the innate immune system is mediated by a number of receptors, including members of the TLR (Toll-like receptor) and of the NLR (NOD-like receptor) families. Whereas Toll-like membrane receptors are linked to the recognition of bacterial lipopolysaccharides (LPS) and other PAMPs, NLRP3, a subtype of NLRs, acts as a cytosolic receptor sensing stressor signals, including ROS, ATP and mtDNA (Dunne 2011; Masters et al. 2009). As revised above, activation of NLRP3 receptors leads to the assembly of the inflammasome, activating caspase-1 that in turn activates a series of pro-inflammatory cytokines. But before caspase-1 could act, the pro-cytokines should be produced through the NF-kB pathway. Thus, the NF-kB-NLRP3 connection is of great importance to understand the innate immune response during inflammation.

Sepsis: a typical acute inflammatory disease

Sepsis constitutes the main cause of death in the intensive care units across the world and consists in a pathological process produced by the presence of bacteria in blood (Angus and Wax 2001). NF-κB is a transcription factor that plays a critical role in the innate immune response during sepsis, controlling the expression of multiple genes. Primarily described in 1986 (Sen and Baltimore 1986), cytosolic NF-κB is expressed in most of the cells in an inactive form associated with the inhibitory protein I-κB. Once released from its inhibitor, NF-κB translocate to the nucleus, where it regulates the expression of a series of genes related with the immune and inflammatory responses (Zingarelli 2005). Among others, these NF-κB-regulated genes comprise cytokines, such as IL-1β, IL-6, IL-18, and TNFα (Sen and Baltimore 1986; Zingarelli et al. 2003; Zingarelli 2005), and the pro-inflammatory enzymes iNOS and COX-2 (Fig. 2). Once produced, IL-1β and TNFα can also activate NF-κB, constituting a vicious cycle that increases the severity and duration of the inflammatory response. The NF-κB-dependent transcription of I-κBα, yields a negative feedback circuit that limits the activation of the former (Sen and Baltimore 1986; Zingarelli et al. 2003; Zingarelli 2005).

The initial steps in the sepsis induced by LPS depend on the neutrophil activation, yielding high amounts of ROS and inflammatory mediators, such as TNFα, the latter under NF-κB control (Janssen-Heininger et al. 2000; Zhou et al. 2001). Moreover, ROS produced during sepsis also activate antioxidant enzymes, such as SOD, GPx and CAT, attempting to control the hyperoxidative status (Asehnoune et al. 2004; Biolo et al. 2007; Delerive et al. 2001; Harris 1992).

The pro-inflammatory mediators as well as ROS produced during sepsis, directly impair the mitochondrial RC causing lipids, proteins and mtDNA damage, and ATP depletion (Suliman et al. 2003, 2005). The impairment of mitochondrial function in sepsis is well documented

(Escames et al. 2003, 2007; Lopez et al. 2006a, b), and the degree of mitochondrial impairment and mortality during sepsis are well correlated (Svistunenko et al. 2006). Together with ROS, iNOS/i-mtNOS induction in sepsis enhances the production of mitochondrial $NO^{\cdot}$ (Escames et al. 2003, 2007; Lopez et al. 2006a, b), causing irreversible damage to mitochondrial macromolecules (Brown 2001; Poderoso et al. 1996). The parallel failure of the RC and OXPHOS leads to energy depletion and cell death (Brealey et al. 2002; Callahan and Supinski 2005).

Structural alterations in mitochondria can be observed in different tissues during sepsis. These alterations, include swelling, cristae disruption, and elongation, reflecting the opening of the MPT caused by ROS/RNS. Septic mice show oxidative damage to mtDNA with reduced density of mtDNA (Bartz et al. 2011). As a consequence of the sepsis-induced oxidative damage, mtDNA copy number is reduced in the early phase of experimental sepsis (Haden et al. 2007). After this initial phase, mtDNA copy number is restored, suggesting an increase in mtDNA replication and mitochondrial biogenesis (Haden et al. 2007). In fact, septic mice also show high tissue levels of nuclear respiratory factor-1 (NRF-1) and NRF-2, PGC1a and TFAM (Haden et al. 2007; Suliman et al. 2004; Sweeney et al. 2010). These changes may reflect mitochondrial biogenesis and energy homeostasis recovery that takes place during the resolution phase, which is activated by innate immunity (Sweeney et al. 2010).

The signal transduction pathway that link sepsis-induced inflammation to the upregulation of mitochondrial biogenesis involves the TLR receptors (Sweeney et al. 2010). This response constitutes a pro-survival event that further protects mtDNA from oxidative damage during sepsis, whereas it recovers the energy demands of the cell. However, the relationships between mtDNA and sepsis are not reduced to the mtDNA damage by ROS/RNS during the response of the innate immune system. In fact, as it was discussed above, mitochondrial damage by ROS results in MPT opening and mtDNA release to the cytosol, that may be sensed by the mechanisms involved in the NLRP3 inflammasome activation. This is another link between canonical NF-κB activation by LPS, mitochondria damage during sepsis, and inflammasome activation (Fig. 2). Pharmacological interventions in sepsis should now address not only the NF-κB pathway, but also the control of the NLRP3 inflammasome activation.

Melatonin, a disruptor of the NLRP3 inflammasome-mtDNA connection?

From the data revised here, we know that the dysfunctional mitochondria trigger the activation of the NLRP3 inflammasome, which depends on the mitochondria ROS production and mtDNA release after MPT opening. Thus, one would predict that targeting antioxidants to mitochondria may be useful in reducing the overstimulation of the innate immune response during inflammation (Galley 2010).

Whereas experimental evidence suggest that manipulating autophagy mechanisms and/or mitochondrial ROS production can reduce the NLRP3 inflammasome activation (Nakahira et al. 2011), the discovery of new pharmacological tools against this inflammatory pathway becomes essential. From a pharmacological point of view, we can act on mitochondria, reducing ROS production and/or closing MPT, or impeding NLRP3 recruitment and activation. Herein, we revise some aspects of the antioxidant melatonin as a type of molecule physiologically relevant in mitochondrial homeostasis, and with important pharmacological properties in inflammation. Although there is not information regarding a direct role of melatonin on the NLRP3 inflammasome activation, the participation of melatonin in the control of mitochondrial ROS production and autophagy (Acuna et al. 2011; Acuna-Castroviejo et al. 2007; Lopez et al. 2009; Martin et al. 2000), and its potent anti-inflammatory action (Crespo et al. 1999; Escames et al. 2003, 2006a), support its involvement in the control of the NLRP3 inflammasome activation.

Melatonin is a phylogenetic old molecule, present from unicellular organisms to mammals (Tan et al. 2010). In mammals is synthesized from tryptophan in many organs and tissues besides the pineal gland (Halberg et al. 2005; Rensing and Ruoff 2002). Whereas the pineal melatonin is related to the synchronization of endogenous rhythms (Kennaway and Wright 2002; Reiter 1980), extrapineal melatonin is related to the antioxidant and anti-inflammatory protection of the cell (Acuna et al. 2011; Acuna-Castroviejo et al. 2001).

Melatonin is a potent-free radical scavenger with antioxidant and anti-inflammatory properties (Crespo et al. 1999; Escames et al. 2003; Tan et al. 1993, 1998, 2001, 2007; Tang et al. 1998). Melatonin is a special class of antioxidant because when scavenging free radicals, it becomes in a series of metabolites that are also free radical scavengers (Hardeland et al. 2009). Melatonin is taken up by mitochondria (Lopez et al. 2009), providing an in situ protection against oxidative damage (Acuna et al. 2002, 2011; Acuna-Castroviejo et al. 2007; Jou et al. 2010; Lopez et al. 2009; Paradies et al. 2010). Under physiological and pathological conditions, the ability of melatonin to maintain mitochondrial homeostasis has been reportedly showed (Acuna et al. 2011; Acuna-Castroviejo et al. 2001; Escames et al. 2007; Lopez et al. 2006a, b). Melatonin, but not other antioxidants including vitamins C and E, and *N*-acetylcysteine, was highly efficient to maintain

mitochondrial GSH homeostasis in extremely oxidative conditions, closing the MPT (Paradies et al. 2010) and promoting mitochondrial survival (Martin et al. 2000, 2002). In normal isolated mitochondria, melatonin increased the activity of the RC complexes, reduced oxygen consumption and decreased slightly the mitochondrial inner membrane potential, decreasing ROS (Lopez et al. 2009). In the presence of t-butyl hydroperoxide (t-BHP), which depletes the mitochondrial GSH pool and blunts both GPx and GRd activities (Martin et al. 2002), 100 nM melatonin restored the GSH pool and the normal activity of the RC and ATP production. In these mitochondria, however, *N*-acetyl cysteine (NAC) and vitamins E and C had any effect despite the doses of these compounds used were 10,000 higher than melatonin (Martin et al. 2000). In vivo, melatonin also restored the normal mitochondrial function impaired in different inflammatory status, including neurodegenerative diseases (Acuna et al. 2011; Cardinali et al. 2010; Khaldy et al. 2003; Olcese et al. 2009; Tapias et al. 2009), aging (Carretero et al. 2009; Escames et al. 2010), neuromuscular disorders (Chahbouni et al. 2010), metabolic dysfunction (Cardinali et al. 2011; Kozirog et al. 2011), and sepsis (Escames et al. 2006a). In these conditions, melatonin reduces mitochondrial ROS production to normal levels, recovers the normal RC activity and ATP production, and maintains the GSH homeostasis, closing the MTP. Melatonin also blunted iNOS/i-mtNOS expression during sepsis, recovering full mitochondrial function (Crespo et al. 1999; Escames et al. 2003, 2006b, 2007; Lopez et al. 2006a, b). Although the initial mechanism proposed for these effects of melatonin therapy was related to the inhibition of the NF-κB pathway activation, the capability of mitochondria to concentrate melatonin, and the ability of the latter to reduce mitochondrial ROS production, suggest that the indoleamine may also inhibits the mitochondrial ROS-dependent NLRP3 inflammasome activation. The relationship between melatonin and autophagy (Coto-Montes and Tomas-Zapico 2006; Garcia et al. 2010; Yoo and Jeung 2010), which seems to involve a reduction in the oxidative stress by the indoleamine, further support this hypothesis.

Concluding remarks and future direction

Apart from sepsis, there are a series of diseases coursing with inflammation and mitochondrial impairment, with or without mtDNA mutations, in which the exact mechanism connecting mitochondria with inflammation is yet a matter of debate. Interestingly, the common pathophysiological events in these disorders include oxidative/nitrosative stress, mitochondrial dysfunction and ROS generation, which may in turn damage the mtDNA and/or release it to the cytosol. In the light of new data, the connection between ROS-generating mitochondria and NLRP3 inflammasome activation may explain the inflammatory process in these pathologies, opening new perspectives for therapeutical approaches.

Thus, NLRP3 inflammasome and/or mitochondria are now major targets of interest from the pharmacological management of these diseases. Herein, melatonin becomes in a class of drug that fulfills these criteria, and it may also constitute a template for the development of new anti-inflammatory molecules. These new drugs may be of utility in those diseases, such as PD and AD, in which the classical anti-inflammatory drugs have not effects (Driver et al. 2011; Sonnen et al. 2010).

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