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Reprogramming of mitochondrial metabolism by innate immunity

Johan Garaude

The reprogramming of cellular metabolism has emerged as a major aspect of innate immune cell activation. Mitochondria, which are well known for their critical functions in cellular bioenergetics and metabolism, also serve innate immune purposes by providing specific signaling platforms. Latest advances in our understanding of innate immune receptor-mediated metabolic reprogramming have unraveled specific immune functions of mitochondrial metabolites that place mitochondrial metabolism and particularly the mitochondrial respiratory chain at the center of innate immunity. This review highlights some recent studies that support mitochondrial metabolism as major immune signaling rheostat upon microbe recognition by innate immune cells.

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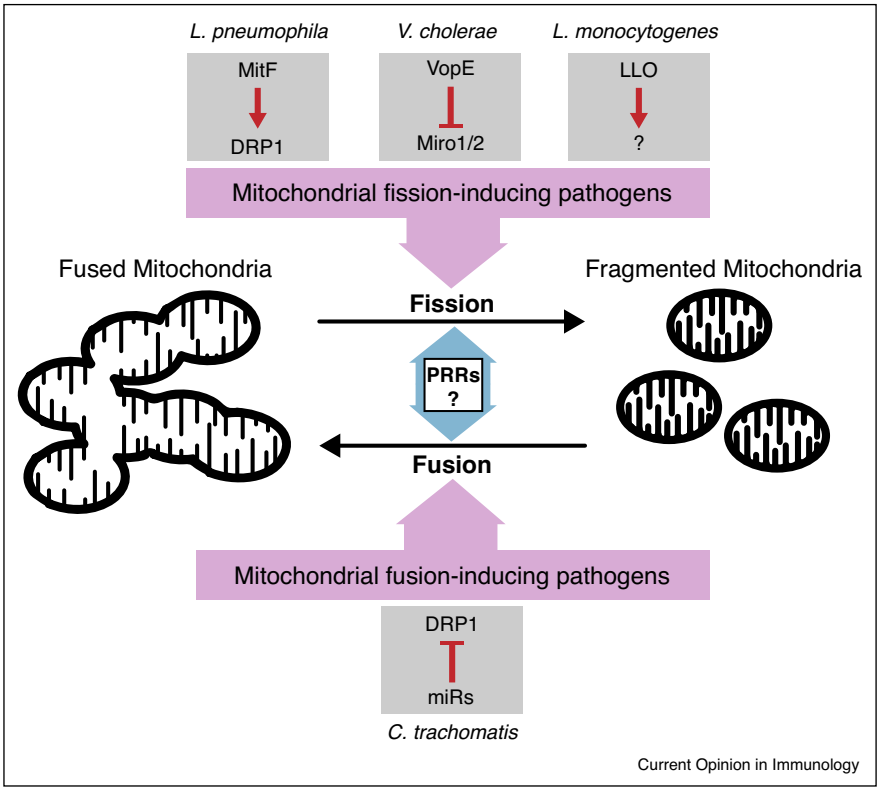
Introduction

Innate immune cells express pattern recognition receptors (PRRs) that sense microbial components to initiate different immune signaling pathways thereby promoting host defense [1]. Building on seminal experiments on phagocyte metabolism characterization [2,3] and on the observations by Otto Warburg that many cancer cells have an increased glycolytic flux and lactic acid fermentation in presence of high oxygen levels (the so-called Warburg effect) [4], recent research in immunology has placed cellular metabolism reprogramming at the center of signal transduction pathways that emerged from PRRs [5]. Advanced metabolomics and genetics tools have allowed to establish a comprehensive picture of the global metabolic reprogramming engaged by PRRs such as Toll-like receptors (TLRs) and some of these are now recognized as important aspects in innate immune signaling. Because they lie at the crossroads of cellular bioenergetics and metabolism, mitochondria are of great interest of course. Strikingly, mitochondria are also central to host defense by providing a pedestal for the assembly of supramolecular protein complexes that relay innate immune signals including those formed by mitochondrial antiviral signaling protein (MAVS) [6] or NOD-like receptor family, pyrin domain containing 3 (NLRP3) [7]. Thus, mitochondria have naturally appeared as an important hub that could interconnect cell metabolism to innate immunity. This idea has gained a lot of credibility since various mitochondrial metabolites have been found to contribute to innate immune cell fate or to transduce innate immune signals. Here I will focus on recent studies that decipher how innate immune receptors and microbial encounter trigger mitochondrial reprogramming to sustain innate immunity.

Microbial infection governs mitochondrial dynamics

Mitochondria are dynamic organelles that can connect to each other to form a long network (a process called fusion) or individualize thereby acquiring a spot-like aspect in the cytoplasm when observed by confocal microscopy (a process called fission). This fission/fusion equilibrium is closely associated to the metabolic status of mitochondria and tightly regulated by a set of well-defined proteins [8]. Mitochondrial fission is mainly governed by the recruitment of the GTPase dynamin-like protein 1 (DRP1) to specific constriction sites. DRP1 then oligomerizes to recruit Dynamin 2 and terminate fission. Mitochondrial fusion is ensured by a multi-step process that starts with outer membrane fusion mediated by mitofusin 1 and 2. Inner mitochondrial membranes subsequently fuse through the action of specific proteases such as fusion-promoting optic atrophy-1 (OPA1) [8]. It is striking that various bacteria induce mitochondrial fission although the molecular mechanisms accounting for such fragmentation of the mitochondrial network seems to differ depending on the nature of the microbes encountered (Figure 1). *Legionella pneumophila* uses its type 4 secretion system (T4SS) effector MitF to induce the accumulation of a fission-inducing large GTPases at the mitochondria [9]. Similarly, *Vibrio cholera* uses its T3SS effector VopE to interfere with Miro 1 and 2, two Rho GTPase implicated in the clustering of mitochondria [10]. *Listeria monocytogenes* induces mitochondrial fission through the secretion of the pore-forming listeriolysin O (LLO). Surprisingly, this is independent of the fission-promoting DRP1 and of the fusion-promoting OPA1 [11]. Contrary to the above

Figure 1



Microbial infection regulates mitochondrial dynamics. The mitochondrial network is dynamically regulated through fission and fusion events. Various bacteria were shown to target different proteins of fission or fusion machineries. Whether pattern recognition receptors (PRRs) can regulate mitochondrial dynamics is still elusive. It is nevertheless possible that PRR-induced ROS production triggers the fission-promoting protein DRP1. DRP1 (DNML1), dynamin-1-like protein; MFN1, mitofusin-1; Miro1/2, mitochondrial Rho-GTPase 1; miRs, micro RNAs.

mentioned pathogens, the obligate intracellular bacterium *Chlamydia trachomatis* maintains mitochondrial network integrity by inhibiting DRP1-induced mitochondrial fragmentation [12]. Although not discussed here, it is important to note that similar to bacteria, some viruses such as the influenza A virus or the hepatitis C virus induce mitochondrial fission through specific viral proteins while other viruses such as human immunodeficiency virus or Dengue virus rather preserve or promote mitochondrial network [13]. Whether the finality of microbe-mediated alterations of mitochondrial dynamics is to fulfill pathogen's metabolic requirements [14], to disrupt mitochondria-mediated innate immune signaling pathways [15] or, at the contrary, to promote host defense remains poorly understood. Interestingly, the internalization of apoptotic cell by phagocytes (efferocytosis) induces mitochondrial fission, which is required for further uptake of apoptotic cells [16•] providing a functional link between mitochondrial dynamics and phagocyte functions and raising the possibility that such regulation may also contribute to pathogen engulfment. Unfortunately, whether receptors involved in sensing of apoptotic cell are implicated in the control of mitochondrial dynamics is still poorly defined. Finally, because many PRRs induce ROS production that was shown to trigger DRP1-mediated mitochondrial fission, it is likely that innate immune receptors contribute to mitochondrial dynamics. Nevertheless, future studies should determine the exact contributions of PRRs in such processes and how mitochondrial dynamics transduce at the metabolic level in order to delineate new therapeutic strategies.

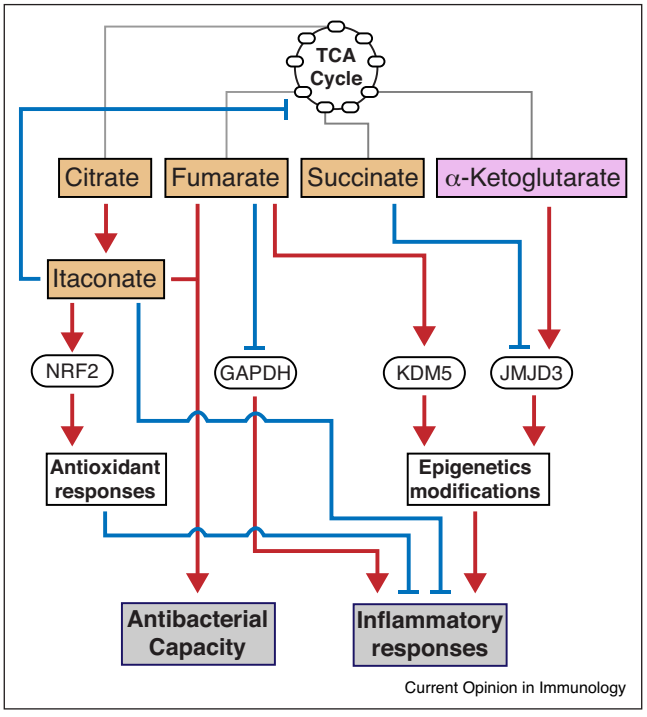
Innate immune receptors induce tricarboxylic acid cycle reprogramming

The mitochondrion houses a multitude of catabolic and anabolic reactions including those composing the tricarboxylic acid (TCA) cycle — also known as Krebs cycle — and the oxidative phosphorylation (OXPHOS) system, which together form the so-called central metabolism. In resting phagocytes, the TCA cycle is largely replenished by glucose catabolism, which eventually leads to pyruvate entry in the mitochondrion and to its conversion to acetyl-CoA. This is thought to provide sufficient fuel for ATP synthesis by the OXPHOS. However, activation of phagocytes diverts pyruvate from its entry into the mitochondrion towards lactate production in the cytosol implying

the replenishment of the TCA cycle through alternative catabolic pathways [17–20]. In a cornerstone study, Artyomov *et al.* combined computational and experimental transcriptomics and ^{13}C-tracing metabolomics approaches to determine several specificities of the TCA cycle reprogramming in activated bone marrow-derived macrophage (BMDMs) [21••]. They notably showed that the TCA cycle of BMDMs activated by the TLR4-agonist lipopolysaccharides (LPS) and interferon-γ present a breakpoint at the isocitrate dehydrogenase, which convert isocitrate to a-ketoglutarate. This breakpoint leads to increase level of citrate that is diverted from the Krebs cycle towards fatty acid synthesis and itaconate synthesis [21••] (Figure 2). Itaconate has since emerged as an important immunoregulatory metabolite. It can directly inhibit the succinate dehydrogenase (SDH) in the TCA cycle that metabolizes succinate into fumarate thereby decreasing hallmarks of inflammatory macrophages (IL-1β and IL-12p70, IL-6 and ROS production) [22]. Importantly, two recent studies demonstrated that itaconate regulates inflammatory genes expression through Nrf2-dependent and Nrf2-independent pathways thereby providing mechanistic details on immune functions of mitochondria-derived metabolites [23•,24]. Despite this role in dampening inflammatory pathways in macrophages, itaconate can also contribute to host defense due to its anti-bacterial properties. It limits bacterial growth probably through the inhibition of the isocitrate lyase, a key enzyme of the glycoxylate shunt, which is an important metabolic pathway of fatty acid-consuming bacteria [25,26]. Consequently, macrophages deficient for IRG1, the enzyme that produces itaconate from aconitate, have impaired antimicrobial functions [27•].

The study by Artyomov *et al.* also suggested the existence of a second breakpoint in the TCA cycle through a block at the SDH level, which can account for the accumulation of succinate observed in LPS/IFN-γ-activated BMDMs. The replenishment the TCA cycle by fumarate was then explained by an aspartate-arginosuccinate shunt [21••]. However, later studies found that SDH activity is induced by the stimulation of TLR3 or by the detection of viable bacteria [15,28••] and is required for the respiratory burst induced by LPS [29] and zymosan [30] suggesting that IFN-γ may provide additional signals for the regulation of SDH and TCA cycle reprogramming. Despite this discrepancy on SDH behavior and on the origin of fumarate in inflammatory macrophages, this metabolite appears to be of particular interest for host defense since it can directly prevent bacterial growth *in vitro* while its precursor succinate cannot [28••]. Furthermore, dimethyl-fumarate, a derivate used to treat multiple sclerosis and psoriasis, can directly modifies GAPDH through the 'succination' of the cysteine in its catalytic domain. This in turn downregulates aerobic glycolysis in LPS-activated phagocytes and dampens inflammatory pathways [31••] suggesting that, similar to itaconate, fumarate may contribute to a regulatory loop to limit inflammation and initiate the return to homeostasis.

Figure 2



Immune effects of TCA cycle-derived metabolites. Upon activation of innate immune cells, TCA cycle is reprogramed leading to the accumulation of different metabolites. Various TCA cycle metabolites were shown to accumulates in inflammatory macrophages (in orange) or in IL-4-stimulated macrophages (in pink). Citrate is metabolized into itaconate, which induces NRF2-dependent and NRF2-independent regulation of inflammatory responses. Together with fumarate, itaconate exhibits antibacterial properties. Fumarate was shown to downregulates GADPH through 'succination', the1reby dampening inflammatory pathways associated to innate immune receptor-induced glycolytic switch. Furthermore, fumarate regulate cytokine expression through the activation of the histone demethylase KDM5 upon stimulation with β-glucans. Succinate, which accumulates in inflammatory macrophages, and, α-ketoglutarate, which accumulates in IL-4-stimulated macrophages, exert opposing roles on histone demethylase such as JMJD3 thereby regulating cytokine expression through epigenetics. Red arrows indicate activating/promoting effects while blue arrows indicate inhibiting/repressing events. NRF2, nuclear factor erythroid-derived 2-like 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; JMJD3, Jumonji C-domain-containing histone demethylases 3.

Another important feature by which TCA cycle-derived metabolites may critically control innate immunity is through epigenetics means. Succinate, fumarate or α-ketoglutarate were all shown to contribute to histone and DNA demethylation in innate immune cells. For instance, glutaminolysis-dependent α-ketoglutarate production sustains Jumonji C-domain-containing histone demethylases (JMJDs) and Ten–eleven translocation

(TET) family of 5mC hydroxylases activities in IL-4-stimulated BMDMs by acting as a co-factor, while succinate, which is induced by LPS stimulation, would repress JMJDs by competing with α-ketoglutarate [32]. Similarly, β-glucans stimulation of monocytes induces glutaminolysis-dependent fumarate accumulation, which was linked to the inhibition of the KDM5 histone demethylase [33]. Such metabolic control of epigenetics has revealed to be critical to establish trained immunity, a form of innate immune memory that potentiate gene transcription after a second stimulation, which does not have to be related to the first one.

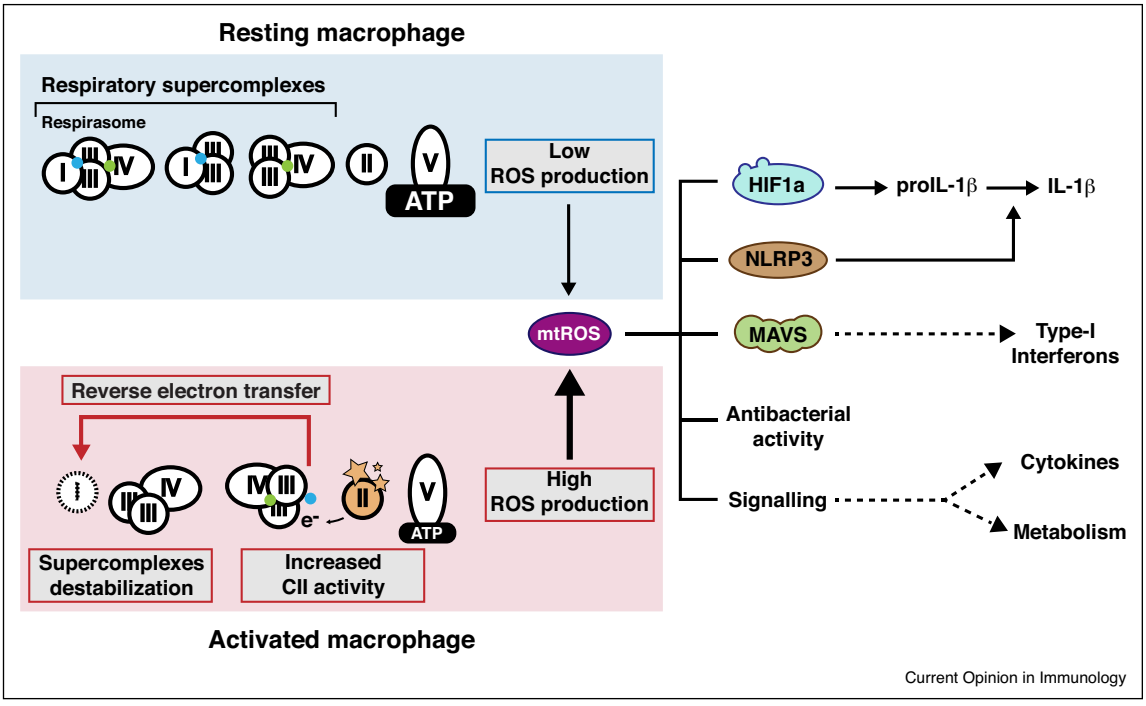
The mitochondrial respiratory chain adapts to innate immune signals

The mitochondrial electron transport chain (ETC) — also known as mitochondrial respiratory chain — comprises two electron carriers (coenzyme Q [CoQ]/ubiquinone and cytochrome c) and four respiratory complexes (complex I-IV [CI-CIV]). Many metabolic processes in the mitochondrion converge on the ETC by supplying electrons through the reductive equivalents of nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$), the ratio of which is postulated to vary according to the nature of the fuel feeding the mitochondrion [34]. NADH and $FADH_2$ are respectively oxidized by CI and CII and the subsequent transfer of electrons through the respiratory complexes generates a H^+ gradient that is used by the H^+-ATP synthase (CV) to generate ATP. Interestingly, respiratory complexes I, III, and IV can assemble as large molecular supercomplexes (SCs) in the mitochondrial inner membrane where they accumulate in the cristae [35]. This includes SC I + III_2 (composed of one unit of CI and two units of CIII), SC III_2+IV and the so-called respirasome (SC I + III_2+IV). Whether this supramolecular organization of the ETC serves physiological purposes and how SCs assembly is regulated is a matter of intense investigation and stimulating debates. Briefly, SCs were proposed to 1) decrease ROS production, 2) stabilize or assist the assembly of individual complexes, 3) regulate the respiratory chain activity, 4) prevent protein aggregation in the mitochondrial inner membrane, and 5) organize the substrate channeling within the ETC by compartmentalizing CoQ and cytochrome c pools. Pros and cons for each of these hypothesis exist and are well summarized elsewhere [36].

Several of these SCs are found in mitochondria from murine macrophages and human monocytes (Figure 3). However, the abundance of SCs in which CI is engaged is significantly decreased upon recognition of viable Gram-negative bacteria in a TLR-dependent and NLRP3-dependent manner [28••]. This likely reflects a decreased assembly of CI but the molecular mechanisms involved are still poorly defined [37]. It is however tempting to speculate that because innate immune receptors engagement induces a switch in the nature of the fuel that feed mitochondria and reprograms mitochondrial metabolism, intramitochondrial $FADH_2$ /NADH ratio should vary thereby driving the respective mobilization of CI and CII. In line with this, such a shift in nutrient-depending control of CI-containing and CI-containing SCs abundance was recently shown in fibroblasts, probably because of local superoxide production that oxidizes specific residues within CI [38]. An alternative explanation relies on the enhanced production of mitochondrial reactive oxygen species (mROS) mediated by PRRs. Engagement of TLRs at the plasma membrane leads to the recruitment of mitochondria in the proximity of bacteria-containing phagosomes through the E3 ligase TRAF6-mediated ubiquitination of the mitochondrial assembly factor ECSIT [39]. This process is regulated by a TLR-Mst1/2-rac axis and contributes to engulfed-bacterial clearance by inducing mROS [39,40]. Furthermore, ECSIT is critical for the assembly of CI in macrophages and its absence surprisingly leads constitutive mROS production but prevent further mROS increase upon TLR stimulation [41]. In line with this, recent studies demonstrated that the activity of macrophage CII (which is also the SDH in the TCA cycle) is increased upon bacterial infection [15,28••] or TLR activation [28••,29]. Because succinate level is high in activated innate immune cells [17], this may create the conditions where electron flux is going backward from CoQ to CI in a process known as reverse electron transfer leading to mROS production by CI [42]. Taken together these studies strongly underline the fundamental role of mROS for innate immunity. Finally, because respiratory SCs abundance is tightly associated to the mitochondrial cristae architecture [43], it is possible that changes in mitochondrial dynamics occurring during microbial infection contributes to the modulation of the ETC architecture in phagocytes. Therefore, more work is needed to determine how SCs adapts to innate immune signals and what are the physiological consequences of such adaptations for innate immunity. New structural models of the mammalian respirasome obtained by cryo-electron microscopy [44,45] together with new mechanistic details on the super assembly of CIII and CIV and the development of new proteomics tools to assess SC compositions [46] will certainly help understanding the physiological roles those SCs. In this context, innate immune receptor-induced adaptations of SCs may represent a model of choice.

In all the above scenarios, post-translational modifications controlled by PRR signaling are likely to contribute to ETC structural and functional regulation but the mechanism involved remains elusive. For example, TLR-mediated increases in CII activity requires the Src-family tyrosine kinase FGR [28••], which phosphorylates the tyrosine 604 in the SDHA subunit of CII [47], while PTPMT1 dephosphorylates this tyrosine in other systems [48]. Thus, it is possible that a molecular switch on/off system based on phosphorylation/dephosphorylation

Figure 3



Mitochondrial electron transport chain (ETC) reprogramming upon innate immune cell activation. In resting macrophages, the respiratory complexes I (CI) to IV (CIV), except for CII, can assembled into supercomplexes (SC), the compositions of which are: CI/$CIII_2$/CIV (the respirasome), CI/$CIII_2$ or $CIII_2$/CIV. Another fraction of the respiratory complexes may move freely in inner mitochondrial membrane. Electrons are sequentially shuttled from CI or CII to CoQ (blue point), CIII, cytochrome c (green point), and CIV. This pumps protons (H+) into the intermembrane space to create am mitochondrial membrane potential and a pH gradient that are used by the ATP synthase (CIV) to generate ATP. In macrophages activated through TLR engagement or live bacteria engulfment, CII activity is increased. This overloads CoQ with electrons that are pumped back towards CI by a process called reverse electron transfer. This induces mitochondrial reactive oxygen species (mtROS) production. CI is then oxidized, leading to its destabilization and the disassembly of CI-containing SCs. ETC is thus reprogramed towards the production of ROS and fumarate rather than ATP production, thereby contributing to macrophage mediated- immunity. In turn, mtROS were shown to stabilized the hypoxia-induced transcription factor 1α (HIF-1α) to induce the expression of pro-inflammatory cytokines such as pro-IL-1β. They also promote NLRP3-inflammasome, which is required for the proteolytic activation of IL-1β. MtROS may favor MAVS activation leading to Type-I interferon responses. Finally, mtROS may further regulate different signaling pathways to drive cytokine expression and metabolism reprogramming.

is controlling respiratory complexes in phagocytes. The transitory increase in CII activity induced by PRRs is accompanied by a diminished CI activity and is necessary to maintain the respiratory reserve capacity in activated macrophages suggesting that CII may ensure sufficient electron flux within the ETC while allowing for mROS production at CI and proper cytokine production upon microbial sensing through the stabilization of hypoxia inducible factor-1α (HIF-1α) [29,30]. Consistently, CI inhibition in LPS-treated macrophages diminishes mROS production and IL-1β production [49]. In addition, the inhibition of CII in PRR-activated macrophages diminishes microbicidal capacity and pro-inflammatory cytokine production by macrophages, while it sustains production of anti-inflammatory cytokines such as IL-10 [28••,29,30]. Furthermore, mouse macrophages deficient for the CI subunit NUDFS4 show an exacerbated inflammatory phenotype, which is attenuated by the absence of TLR2/4, emphasizing the interconnection between PRR signaling and the ETC [50]. Therefore, mitochondrial CII and CI emerged as critical elements downstream innate immune receptors and their pharmaceutical manipulation may offer significant advantages to control inflammation.

Conclusion

Despite tremendous progress made in our understanding of the intertwining of mitochondrial metabolism and innate immunity, several critical aspects remain to be determined. What PRRs control mitochondrial functions? What is the immune functions of mitochondrial supercomplexes and individual respiratory complexes? How Mitochondrial superoxides achieve their critical mediator function in innate immunity? Do they act in the proximity of their production sites by altering component of the ETC or at distant sites when converted to H_2O_2? Why the two TCA cycle derived-metabolites itaconate and fumarate exert both host-defense and immune regulatory

functions? How mitochondrial dynamics govern metabolism-dependent innate immune signaling? Our understanding of the PRR-mediated orchestration of the mitochondrial respiratory chain plasticity and organelle dynamics will certainly provide valuable information on these matters and will be useful to clinically manipulate inflammation and design new vaccines.

Conflict of interest statement

Nothing declared.

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