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# The Role of Mitochondria in the Immune Response in Critical Illness

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## Abstract

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## Introduction

Immune dysregulation, characterized by an imbalance between a systemic inflammatory response syndrome and a compensatory anti-inflammatory response syndrome, is often observed in critically ill patients [1, 2]. This imbalance between the pro- and anti-inflammatory responses frequently leads to immunoparalysis in critically ill patients, rendering them more susceptible to further infections, and is associated with increased mortality [3]. Currently, no effective treatments are available to restore immune homeostasis and reduce mortality in these patients, largely due to the heterogeneity in patients' immune status and more importantly the lack of understanding of the underlying cause of such immune dysfunction [2, 4]. Immune response is not a standalone process but is interconnected with other cellular activities, a very important one of which is cellular metabolism. Metabolic pathways and immune response are tightly intertwined both in health and in disease [5]. The link between immune cell function and mitochondrial function is now well recognized and a field known as "immunometabolism" is dedicated to understanding the relationship between immune and metabolic pathways [6–8]. Mitochondria play a crucial role in regulating not

only the growth, but also the function, of immune cells. In addition to providing energy to support the synthesis of the macromolecules essential for immune cell proliferation, mitochondria also act as signaling organelles, driving activation of immune cells via metabolic intermediates, mitochondrial DNA (mtDNA), and reactive oxygen species (ROS). In addition, mitochondrial dynamics (fusion and fission), biogenesis (synthesis of new mitochondria), and mitophagy (degradation of damaged mitochondria) also play important roles in regulating immune cell functions. Knowledge in immunometabolism in critical illness, in particularly sepsis, opens up a new paradigm in patient care. Potential therapies targeting metabolic pathways, instead of solely immune-related pathways, might be the way to repair cellular function and restore immune homeostasis [4]. The other aspect of immunometabolism—looking at how immune responses influence metabolic pathways—is equally important, but beyond the scope of this review. Interaction between metabolism and immune response at the organ level has been reviewed elsewhere [6].

## Mitochondrial Machinery That Mediates and Regulates Immune Responses in Critical Illness

Apart from being the powerhouse of the cell, the mitochondrion has emerged as a signaling hub that shapes and modulates how the immune system responds to infection or trauma. Mitochondrial dysfunction is evident in leukocytes from critically ill patients, and is

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believed to be the underlying cause of immunoparalysis and may account for the development of organ dysfunction [7–9]. Early recovery of mitochondrial function correlates with improved recovery in critically ill patients [10].

### Metabolic Reprogramming

The immune-regulating mitochondrial machinery is a complex network involving many pathways and mechanisms that diverge and converge at various levels. Metabolic reprogramming is one mechanism that has been well studied in both innate and adaptive immune cells. Immune cells at different activation states (quiescent vs. activated), or with different functions (pro-inflammatory vs. anti-inflammatory), and different cell types (granulocytes, macrophages, dendritic cells, T- and B-lymphocytes), make use of different metabolic pathways (e.g., glycolysis, oxidative phosphorylation, fatty acid metabolism) to produce ATP [11]. The choice of different metabolic pathways, supports the energy demand of cells at different activation state. For example, upon infection or stimulation, immune cells become activated and produce cytokines and hence tend to favor glycolysis over oxidative phosphorylation for fast turnaround of ATP. Although the same amount of starting material, such as glucose, is used, oxidative phosphorylation generates 18 times more ATP than glycolysis, although is a lot slower. On the other hand, the choice of metabolic pathway determines the fate of the immune cells, i.e., naïve or memory, effector or regulatory, etc. However, the environment that the cells are in the first place, triggers the changes in the metabolic pathways. The overall trend is that neutrophils, inflammatory macrophages (M1 macrophages), activated effector T cells, and dendritic cells rely more on aerobic glycolysis, whereas alternatively polarized macrophages (M2 macrophages), regulatory T cells (Tregs), and memory T cells prefer oxidative phosphorylation and fatty acid oxidation for energy production [8, 11, 12]. Metabolic reprogramming serves an important role in catering for the immune cells' energy demand at different phases of their activation and proliferation. However, imbalance across the metabolic pathways could have serious pathological impact. One example may be the hyperlactatemia often seen in critically ill patients. Increased aerobic glycolysis in the activated immune cells during the initial hyper-inflammatory response is believed to contribute to the increase in blood lactate levels in sepsis [13, 14].

### Mitochondrial ROS and mtDNA

Metabolic reprogramming sets the scene for the immune response, which is then subjected to many more modifications and regulations by factors that are directly or

indirectly related to mitochondrial metabolism. Two important mitochondria-related immune regulators that have been well studied are mitochondrial ROS and mtDNA. Mitochondrial ROS are produced in healthy mitochondria, as a by-product of oxidative phosphorylation. At low dose, mitochondrial ROS serve important signaling functions, especially in the innate immune response. They are known to mediate NLRP3 inflammasome activation, leading to production of the pro-inflammatory cytokines, interleukin (IL)-1 $\beta$  and IL-18 [8, 15]. Mitochondrial ROS also induce a type-I interferon (IFN) response via mitochondrial antiviral-signaling (MAVS) and the IFN regulatory factor 3 (IRF3) pathway [16]. However, the level of mitochondrial ROS needs to be tightly regulated by the antioxidant system. Excessive mitochondrial ROS can cause oxidative damage to proteins/enzymes involved in oxidative phosphorylation and create mutations in mtDNA, contributing to the immune dysregulations as seen in critical illness [17]. Like mitochondrial ROS, mtDNA also plays an important role in innate immunity [12]. In healthy cells, mtDNA is located in the matrix of mitochondria, encoding 13 proteins, all of which are components of oxidative phosphorylation. mtDNA is released to the cytosol upon mitochondrial dysfunction which involves changes to the integrity or permeability of the mitochondrial membrane. mtDNA, released into the cytosol, can activate the NLRP3 inflammasome with release of IL-1 $\beta$  and IL-18. Due to its bacterial origin, cytosolic mtDNA also serves as a damage-associated molecular pattern (DAMP), which can be recognized by intracellular pattern recognition receptors (PRRs), such as Toll-like receptor 9 (TLR9), and initiate the nuclear factor-kappa B (NF- $\kappa$ B)-dependent pro-inflammatory signaling pathway. In addition, cytosolic mtDNA can also be sensed by cyclic GMP-AMP synthase (cGAS) and activate the cGAS/stimulator of IFN genes (cGAS/STING) pathway and its downstream IFN response [18]. mtDNA can also be released into the circulation and cause systemic inflammation. Circulating mtDNA has been associated with mortality in critically ill patients [19].

### Succinate and Itaconate

In addition to mitochondrial ROS and mtDNA, metabolites such as succinate and itaconate have also emerged as part of immune-regulating mitochondrial machinery [4, 20]. Both succinate and itaconate are intermediates from the tricarboxylic acid (TCA) cycle with opposite effects on the immune response. The TCA cycle generates nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH<sub>2</sub>), providing electrons to fuel oxidative phosphorylation. Succinate accumulation occurs under conditions such as hypoxia or inflammation. It can

be released from mitochondria into the cytosol and functions as a signal transducer promoting pro-inflammatory gene expression via hypoxia-inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ) activation. Accumulation and oxidation of succinate by succinate dehydrogenase (SDH) in the mitochondria also leads to increased production of mitochondrial ROS via a process called reverse electron transport. This further enhances the pro-inflammatory effect of succinate. Like ROS, the level of succinate needs to be carefully regulated due to its inflammation aggravating effect. Plasma succinate has been proposed as a predictor of mortality for critically ill patients who are severely injured [21].

Itaconate, which is derived from cis-aconitate of the TCA cycle, is a succinate-regulating factor. It is shown to counteract the pro-inflammatory effect of succinate by inhibiting SDH. Itaconate can also be released into the cytosol and activate transcription factor NF-E2 p45-related factor 2 (Nrf2), a master regulator of antioxidant and anti-inflammatory responses [22]. Recently, itaconate has also been shown to inhibit the inflammatory response in macrophages through activating transcription factor 3 (ATF3).

### Mitochondrial Dynamics

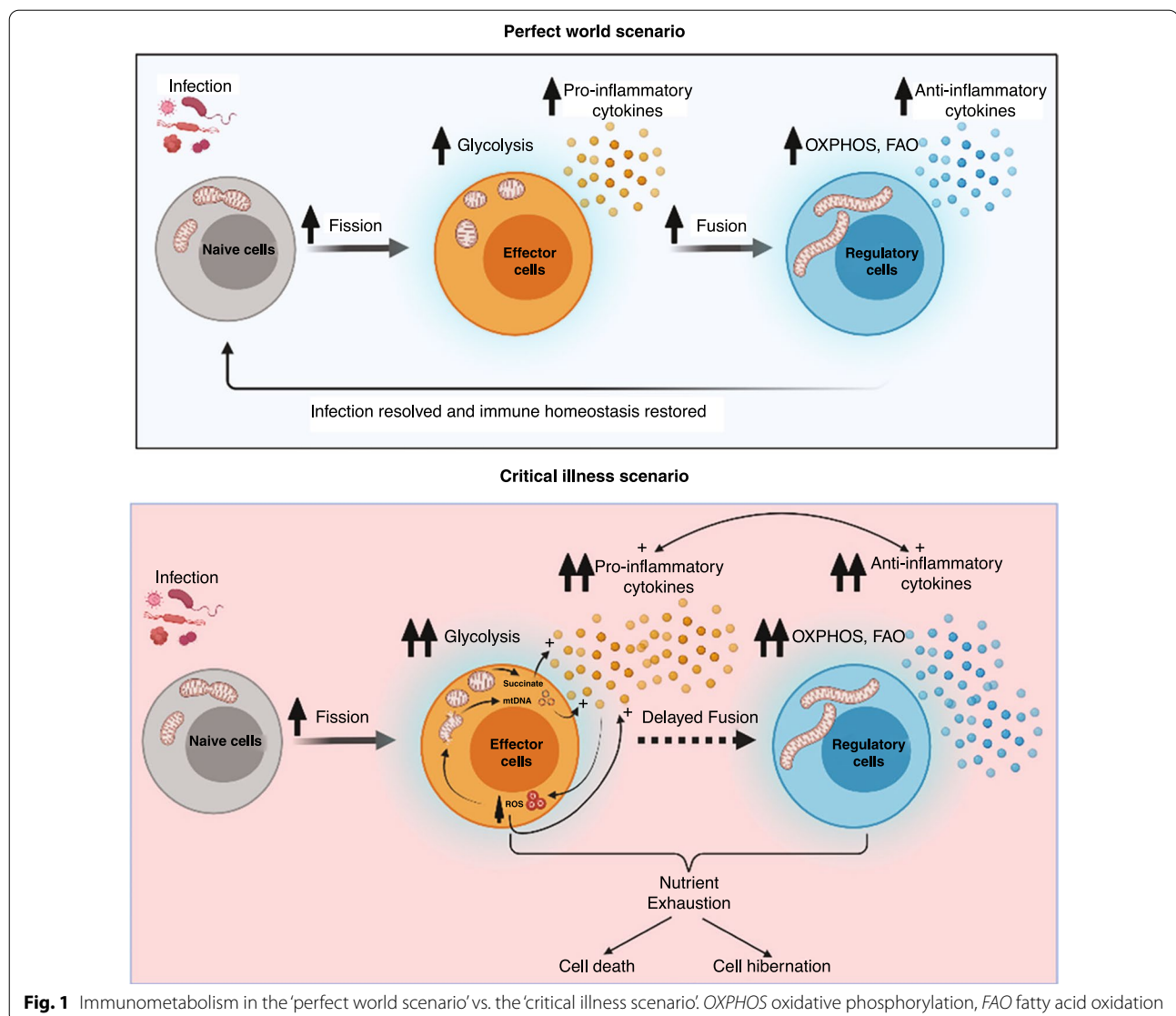
The above mentioned immune-regulating mitochondrial factors are centered around the biochemical aspect of mitochondrial biology. Another important aspect of immune-regulating mitochondrial machinery is mitochondrial dynamics, which is to maintain and provide infrastructural support for the immune response. The size and shape of mitochondria undergo constant change through fusion and fission, which is important for maintaining the health and function of mitochondria. First, fusion incorporates newly synthesized mitochondria (from mitochondrial biogenesis) into the current mitochondrial network. Second, fusion also allows for mixing of proteins and/or mtDNA between the existing mitochondria, which on one hand enhances the metabolic capacity of the mitochondria, and on the other enables the damaged proteins and/or mutated mtDNA to be segregated from the healthy ones. Finally, segregation is achieved via fission and the damaged mitochondria can be destroyed through a process known as mitophagy. The proportion of mitochondria with damaged proteins or mutated mtDNA is kept below a critical threshold level through this process to maintain mitochondrial function [23, 24]. In addition to quality control, mitochondrial fusion and fission also participate in immune regulation. In activated T cells, there is an increase in fission, which creates round and fragmented mitochondria with loose cristae, favoring aerobic glycolysis. And in 146 memory T cells, increased fusion generates elongated mitochondria

which favors oxidative phosphorylation and fatty acid oxidation [8, 25].

### Immunometabolism: The Perfect World Scenario vs. the Critical Illness Scenario

So far, we have presented a list of mitochondrial components that are thought to play important roles in regulating the immune response. Our list is far from complete, but does highlight a few mechanisms that could relate to the development of immune dysregulation in critical illness. Figure 1 illustrates what we think would happen to the immune response when metabolism was in perfect control (the perfect world scenario) and when it became inconsistent and changeable (the critical illness scenario). In the perfect world scenario, the presence of an insult (e.g., infection or a trauma-related stress signal), would trigger metabolic reprogramming, switching from oxidative phosphorylation to glycolysis. This would enable activation of immune cells and production of pro-inflammatory cytokines and other mediators. At the same time, mitochondrial fission would increase to keep up with the metabolic reprogramming. The slightly elevated mitochondrial ROS and succinate in response to initial insult or cytokines would promote the pro-inflammatory response. Once the insult was eliminated, mitochondrial fusion would increase to create fused elongated mitochondria that favor oxidative phosphorylation and fatty acid oxidation. This would allow activation of regulatory immune cells and production of anti-inflammatory cytokines and other mediators. And itaconate would counteract the effect of succinate, activate the Nrf2-mediated antioxidant pathway to dampen down mitochondrial ROS, and activate ATF3 to inhibit the inflammatory response in macrophages. Immune homeostasis would be achieved as a result.

In the critical illness scenario, initial metabolic reprogramming from oxidative phosphorylation to glycolysis would go on for longer than necessary, generating excessive lactate (hyperlactatemia) and pro-inflammatory cytokines and mediators. A disrupted mitochondrial fusion/fission cycle could be to blame, one which could not support the timely switch to oxidative phosphorylation and fatty acid oxidation. The anti-inflammatory response would eventually kick in but by then damage would already have occurred to mitochondria and mtDNA because of excessive production of ROS in response to stress or cytokines. Excessive ROS and released mtDNA would aggravate the pro-inflammatory response, which in turn would trigger a more aggressive anti-inflammatory response to try and salvage the situation. The competition between pro- and anti-inflammatory responses would exhaust the nutrients and lead to



**Fig. 1** Immunometabolism in the 'perfect world scenario' vs. the 'critical illness scenario'. OXPHOS oxidative phosphorylation, FAO fatty acid oxidation

shutdown of the whole metabolic system. Cells would either die or go into hibernation to preserve energy [26]. This scenario is an over-simplified version of what might happen in the actual disease setting, without considering the crosstalk between cells and organs and many other factors that are not included here. It is designed to shed light on the interaction between the immune response and metabolism.

### Potential of Mitochondria-Targeting Therapy in Critical Care

Our understanding thus far leads us to think that targeting mitochondria could perhaps correct the underlying cause of immune dysfunction in critical illness and lead to better recovery of the patients. The central role of mitochondrial dynamics in supporting and initiating

metabolic reprogramming would make it the perfect therapeutic target. To get the fusion/fission cycle going, the mitochondrial network needs to be replenished by newly synthesized mitochondria via biogenesis. Therapies that could potentially boost mitochondrial biogenesis are mitochondrial transplantation, metformin, nitric oxide (NO), and carbon monoxide. Mitochondrial transplantation has been used successfully in pediatric patients with myocardial ischemia–reperfusion injury [27]. Metformin can activate peroxisome proliferator-activated receptor (PPAR)-gamma coactivator-1 $\alpha$  (PGC-1 $\alpha$ ), and Nrf2, the master regulator of mitochondrial biogenesis and antioxidant systems [28]. Premorbid use of metformin is associated with lower mortality in sepsis [29]. NO and carbon monoxide can also enhance mitochondrial biogenesis [30–32]. Dietary nitrite has been trialed

in patients with coronary artery disease (ClinicalTrials.gov Identifier: NCT00069654). Other therapies, such as mitochondria-targeted antioxidant (MitoQ) [33], could also be beneficial in protecting mtDNA and oxidative phosphorylation from oxidative damage. MitoQ has been trialed in people with Parkinson's disease (ClinicalTrials.gov Identifier: NCT00329056).

### Challenges of Applying Mitochondria-Targeting Therapy in Critical Care

There are challenges to overcome before mitochondria-targeting therapy would be possible. First, how do we assess mitochondrial dysfunction in the clinic and identify patients who would benefit from such therapy? A few possible ways could be considered. Non-invasive assessment of mitochondrial oxygen metabolism using a novel device called the COMET monitor was tested on 40 patients during the acute phase of sepsis. This device is based on the protoporphyrin IX-triplet state lifetime technique (PpIX-TSLT) and has been shown to be feasible [33]. This technology is still in its early phase of clinical application but does offer some hope. Another possible biomarker that could potentially be used for assessing mitochondrial dysfunction is plasma mtDNA, but its sensitivity and specificity need further investigation [19, 34, 35]. Furthermore, we could consider using immune response markers as a surrogate markers, one such example could be IFN $\alpha$  inducible protein 27 (IFI27) [36]. If we could overcome the first challenge, the second would be how to deliver mitochondria-targeting therapies to the right organ at the right time.

### Conclusion

In this chapter, we have demonstrated the important role of mitochondria in regulating the immune response and proposed a scenario that explains immune–metabolism crosstalk in the context of critical illness. We have highlighted the role of mitochondrial dynamics in overseeing and supporting metabolic reprogramming during immune cell activation. Mitochondrial ROS can be friend or foe when it comes to immune regulation. Two TCA intermediates—succinate and itaconate—with opposite effects have emerged as important players of the immune-regulating mitochondrial machinery. Our understanding in immunometabolism could take us to the next era of critical care: mitochondria-targeting therapy.

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#### Authors' contributions

YW was responsible for manuscript writing. AM was responsible for the concept and manuscript revision. Final approval for submission was given by all authors.

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#### Competing interests

The authors declare that they have no competing interests.

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