

Received: 2020.03.16  
Accepted: 2020.03.16  
Available online: 2020.03.23  
Published: 2020.03.30

# Emerging Roles of Blood-Borne Intact and Respiring Mitochondria as Bidirectional Mediators of Pro- and Anti-Inflammatory Processes

Authors' Contribution:  
Study Design A  
Data Collection B  
Statistical Analysis C  
Data Interpretation D  
Manuscript Preparation E  
Literature Search F  
Funds Collection G

**ABCDEF 1 Tobias Esch**  
**ABCDEF 2 George B. Stefano**  
**ABCDEF 2 Radek Ptacek**  
**ABCDEF 2 Richard M. Kream**





1 Faculty of Health, School of Medicine, University Clinic for Integrative Health Care, Institute for Integrative Health Care and Health Promotion, Witten/Herdecke University, Witten, Germany  
2 Department of Psychiatry, First Faculty of Medicine, Center for Cognitive and Molecular Neuroscience, Charles University and General University Hospital in Prague, Prague, Czech Republic

**Corresponding author:** Tobias Esch, e-mail: Tobias.Esch@uni-wh.de  
**Source of support:** Self financing

Over the past two decades, a major goal of our research group has been elucidation of the functional roles of several key regulatory molecules in proinflammatory preconditioning involved in the pathophysiology of seemingly diverse human disease states. By necessity, operational definitions of proinflammation must be intrinsically fluid based on recent advances in our understanding of complex regulation of innate and adaptive immune processes. Similar to systemic acute stress, a physiological proinflammatory state appears to be a key autoregulatory mechanism for maintaining optimal immune surveillance against potentially infective microorganisms, viruses, and toxic xenobiotics. Perturbation of normative biochemical and molecular mosaics of ongoing proinflammatory tone, exemplified by altered expression of pro- and anti-inflammatory cytokines and their respective protein complexes, is hypothesized to be a common modality for initiation and full expression of various autoimmune diseases and comorbid syndromes evolving from metabolic and metastatic diseases. The newly reported presence of “free” (extracellular) mitochondria exponentially adds to our hypothesis that in conditions of acute stress, a new source of potential ATP producers may be recruited and present to deal with such an acute process. Furthermore, given this phenomenon, an early surveillance role and a dysfunctional chronic inflammation-prolonging component may also be surmised.

**MeSH Keywords:** Inflammation • Mitochondria • Morphine • Nitric Oxide • Stress, Physiological

**Full-text PDF:** <https://www.medscimonit.com/abstract/index/idArt/924337>

 836  —  —  9



Over the past two decades, a major goal of our research group has been elucidation of the functional roles of several key regulatory molecules in proinflammatory preconditioning involved in the pathophysiology of seemingly diverse human disease states. By necessity, operational definitions of proinflammation must be intrinsically fluid based on recent advances in our understanding of complex regulation of innate and adaptive immune processes. Similar to systemic acute stress, a physiological proinflammatory state appears to be a key autoregulatory mechanism for maintaining optimal immune surveillance against potentially infective microorganisms, viruses, and toxic xenobiotics. Perturbation of normative biochemical and molecular mosaics of ongoing proinflammatory tone, exemplified by altered expression of pro- and anti-inflammatory cytokines and their respective protein complexes, is hypothesized to be a common modality for initiation and full expression of various autoimmune diseases and comorbid syndromes evolving from metabolic and metastatic diseases. The newly reported presence of “free” (extracellular) mitochondria exponentially adds to our hypothesis that in conditions of acute stress, a new source of potential ATP producers may be recruited and present to deal with such an acute process. Furthermore, given this phenomenon, an early surveillance role and a dysfunctional chronic inflammation-prolonging component may also be surmised.

In effect, as noted above, aberrant proinflammatory preconditioning most likely is a permissive common stimulus for engendering dysregulated immune responses leading to markedly negative clinical outcomes in cellular and organ systems [1]. This contention is clearly corroborated by the large number of clinically-used monoclonal antibody pharmaceutical agents engineered to neutralize unregulated secretion of circulating proinflammatory cytokines for the treatment of diverse autoimmune syndromes.

Furthermore, a potentially novel area of recent empirical investigation has centered on the observed proinflammatory effects on innate and adaptive immune responses engendered by blood-borne mitochondrial degradation products emanating from sites of cellular and tissue damage [2–4]. Arrays of mitochondrial degradation products have been qualitatively termed mitochondrial damage-associated molecular patterns (mtDAMPs) and have been found to contain varying concentrations of N-formylated peptide fragments derived from mitochondrial proteins, intact full-length mitochondrial DNA (mtDNA), and associated mtDNA fragments, as well as small organic molecules such as ATP and cardiolipin [5]. Additionally, several recent studies have demonstrated that intact mitochondria released from apoptotic or necrotic dying cells directly mediate proinflammatory processes with downstream effects

on adaptive immune responses, leading to varying degrees of negative clinical outcomes [2–4]. In sum, in healthy individuals, it is likely that ongoing cellular turnover provides basal levels of blood-borne mtDAMPs and circulating mitochondria to maintain a constitutive proinflammatory tone, which is markedly enhanced after the initiation and persistence of systemic traumatic stimuli.

A recent study published in the popular scientific press has demonstrated the presence of intact cell-free mitochondria displaying normative O<sub>2</sub> consumption in the blood of healthy human subjects [5]. The authors provided an estimate of 200 000 to 3 700 000 respiratory-competent mitochondria per ml of extracted plasma, and boldly asserted that circulating cell-free respiratory-competent mitochondria are a novel class of signaling organelles involved in regulatory activities and intercellular communication. It remains to be empirically determined, however, whether respiratory-competent mitochondria possess distinct functional properties distinct from circulating proinflammatory mtDAMPs and respiratory-incompetent mitochondria due to extruded cytochrome C following apoptotic or necrotic cell death. In this regard, previous work from our laboratory may provide mechanistic insights into the potentially unique functional roles of circulating respiratory-competent mitochondria.

We have provided empirical evidence and critical discussion in support of the regulatory role of opiate alkaloids and endogenously expressed morphine in modulating essential mitochondrial processes, including O<sub>2</sub> consumption, electron transport, and oxidative phosphorylation linked to ATP production [6–8]. Of equivalent importance, we have demonstrated the presence of constitutive nitric oxide (NO) signaling pathways functionally coupled to selective opiate alkaloid regulation of essential mitochondrial processes [8,9]. Accordingly, collective evidence from our group supports the potential role of blood-borne respiratory-competent mitochondria that serve as sentinels for maintaining homeostatic metabolic activities, as well as providing an emergency pool of functional mitochondria for restoration of essential cellular functions via targeted endocytotic activities.

In conclusion, the potential significance of the existence of extracellular mitochondria resides in their presence outside a cell, and thus their putative ability to restore homeostasis induced by stress by accumulating rapidly at sites of energy deficits. This initial process of coupling recruitment and accumulation also represents a “sentinel” function based on microenvironmental perturbation as physiological processes are initiated. Furthermore, we surmise this phenomenon is normally inhibited by blood-borne down-regulation of endogenous chemical messengers, such as endogenous morphine. Once a stressor reaches a critical level of activation,

other chemical messengers, such as proinflammatory cytokines, disinherit mitochondrial downregulation. This process may be prone to malfunction (intrinsic factors, extrinsic factors, or both), leading to disorders. This molecular mechanism, with the ability to downregulate energy metabolism, resides within prokaryotes and evolved in the symbiotic mitochondrion. Finally, the presence of extracellular free-living mitochondria in blood offers the potential to also

create novel pharmaceuticals that could prevent hyperactivation of a proinflammatory response, as well as its initiation, leading to chronic conditions, by diminishing mitochondrial activity.

#### Conflict of interest

None.

#### References:

1. Esch T, Stefano GB: Proinflammation: A common denominator or initiator of different pathophysiological disease processes. *Med Sci Monit*, 2002; 8(5): 1–9
2. Pollara J, Edwards RW, Lin L et al: Circulating mitochondria in deceased organ donors are associated with immune activation and early allograft dysfunction. *JCI Insight*, 2018; 3(15): pii: 121622
3. Zhu M, Barbas AS, Lin L et al: Mitochondria released by apoptotic cell death initiate innate immune responses. *Immunohorizons*, 2018; 2(11): 384–97
4. Nagakawa K, Soyama A, Hidaka M et al: Elevated plasma levels of mitochondria-derived damage-associated molecular patterns during liver transplantation: Predictors for postoperative multi-organ dysfunction syndrome. *Tohoku J Exp Med*, 2020; 250(2): 87–93
5. Al Amir Dache Z, Otandault A, Tanos R et al: Blood contains circulating cell-free respiratory competent mitochondria. *FASEB J*, 2020; 34(3): 3616–30
6. Stefano GB, Kream R: Endogenous opiates, opioids, and immune function: Evolutionary brokerage of defensive behaviors. *Semin Cancer Biol*, 2008; 18(3): 190–98
7. Stefano GB, Kream RM: Alkaloids, nitric oxide, and nitrite reductases: evolutionary coupling as key regulators of cellular bioenergetics with special relevance to the human microbiome. *Med Sci Monit*, 2018; 24: 3153–58
8. Stefano GB, Mantione KJ, Capellan L et al: Morphine stimulates nitric oxide release in human mitochondria. *J Bioenerg Biomembr*, 2015; 47(5): 409–17
9. Stefano GB, Mantione KJ, Casares FM et al: Anaerobically functioning mitochondria: Evolutionary perspective on modulation of energy metabolism in *Mytilus edulis*. *Invertebrate Survival Journal*, 2015; 12: 22–28