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J Clin Invest. 2011;121(5):1683-1685. <https://doi.org/10.1172/JCI57748>.

Commentary

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Mitochondrial Ca²⁺ and ROS take center stage to orchestrate TNF- α –mediated inflammatory responses

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Proinflammatory stimuli induce inflammation that may progress to sepsis or chronic inflammatory disease. The cytokine TNF- α is an important endotoxin-induced inflammatory glycoprotein produced predominantly by macrophages and lymphocytes. TNF- α plays a major role in initiating signaling pathways and pathophysiological responses after engaging TNF receptors. In this issue of *JCI*, Rowlands et al. demonstrate that in lung microvessels, soluble TNF- α (sTNF- α) promotes the shedding of the TNF- α receptor 1 ectodomain via increased mitochondrial Ca²⁺ that leads to release of mitochondrial ROS. Shedding mediated by TNF- α –converting enzyme (TACE) results in an unattached TNF receptor, which participates in the scavenging of sTNF- α , thus limiting the propagation of the inflammatory response. These findings suggest that mitochondrial Ca²⁺, ROS, and TACE might be therapeutically targeted for treating pulmonary endothelial inflammation.

Role of TNF- α in sepsis

During sepsis, activation of proinflammatory pathways leads to dysfunction of mitochondria and cells, which contributes to multiorgan failure and poor outcomes. TNF- α simultaneously activates the JNK and NF- κ B pathways and increases expression of the mitochondrial antioxidant protein SOD2, leading to an increase in H₂O₂ and the inactivation of JNK phosphatases. TNF receptors are differentially expressed in various cells and tissues and play a key role in modulating the cellular inflammatory responses (1). The severity of injury to the pulmonary endothelium during sepsis is determined by a complex interplay among proinflammatory cytokines

(including TNF- α), adhesion molecules expressed on the endothelial cells, and leukocytes recruited to the site of injury. Overwhelming sepsis or perturbation of the adaptive responses may lead to exacerbation of inflammation (2). An important mechanism to limit the TNF-mediated proinflammatory response involves shedding of the TNF- α receptor 1 (TNFR1) ectodomain, which is controlled by TNF- α –converting enzyme (TACE; also known as ADAM17), a disintegrin metalloproteinase (3, 4). Here, Rowlands et al. provide evidence that in the lung microvascular endothelium, administration of soluble TNF- α (sTNF- α) increased cytosolic levels of Ca²⁺ via inositol-1,4,5-triphosphate-mediated (IP₃-mediated) release from the ER (5). The increase in cytosolic Ca²⁺ was followed by a rapid increase in mitochondrial Ca²⁺, leading to a rise in mitochondrial ROS generation from complex III of the electron transport chain. These ROS were required for

TACE-mediated shedding of the TNFR1 ectodomain, which in turn modulated the endothelial inflammatory response.

Mitochondrial function and Ca²⁺ signaling

Ca²⁺ uptake by mitochondria is highly regulated, and the levels of mitochondrial Ca²⁺ play a central role in the metabolic (ATP production) and signaling (ROS production, release of proapoptotic molecules) functions of the organelle (Figure 1A). In patients with acute sepsis, mitochondrial function is impaired (Figure 1B), and in patients with septic shock, the severity of impairment in mitochondrial function is associated with adverse clinical outcomes (6, 7). Normalization of mitochondrial biogenesis is necessary to restore oxidative metabolism during sepsis (Figure 1C) so that adequate O₂ delivery and O₂ tissue consumption can resume, allowing cellular metabolic needs to be met (8, 9).

Rowlands et al. suggest that the TNF-mediated increase of mitochondrial Ca²⁺ is dependent on ER Ca²⁺ release leading to IP₃-mediated depletion of intracellular stores (5). It is known that store depletion triggers the activation of an inward rectifying Ca²⁺ current mediated either by the action of specialized Ca²⁺ release-activated (CRAC) channels or by the opening of voltage, receptor, or second messenger operated channels. TNF- α –induced increases in cytosolic Ca²⁺ lead to increased mitochondrial Ca²⁺ uptake through mitochondrial voltage-dependent anion channels, the mitochondrial

Conflict of interest: J.I. Sznajder is the editor of the *American Journal of Respiratory and Critical Care Medicine* and receives a stipend from the American Thoracic Society for this function.

Citation for this article: *J Clin Invest*. doi:10.1172/JCI57748.

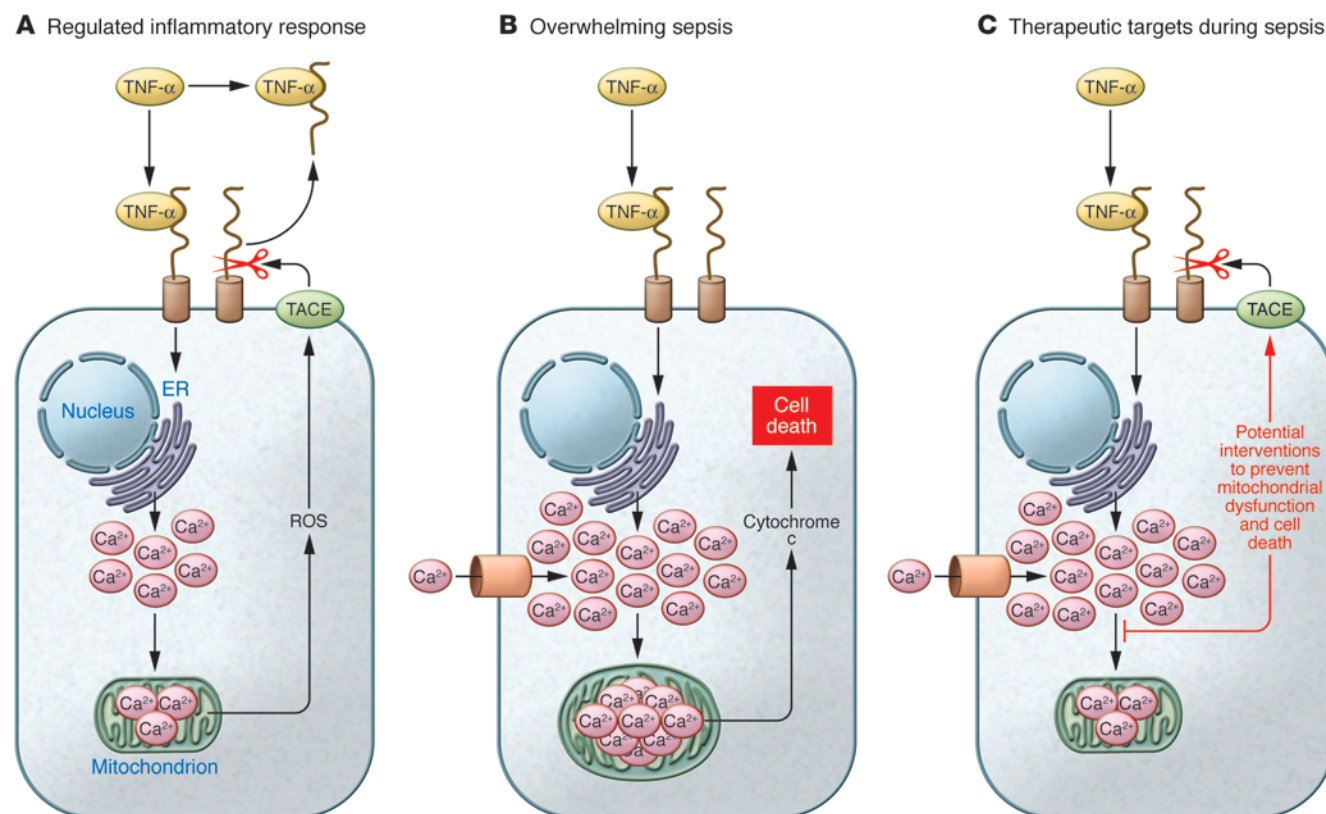


Figure 1

Role of mitochondrial Ca^{2+} and TACE in modulating inflammation of the pulmonary endothelium. **(A)** During regulated inflammatory response, sTNF- α increases mitochondrial Ca^{2+} and ROS, leading to TACE-mediated TNF receptor ectodomain shedding in endothelial cells. **(B)** During overwhelming sepsis, increased Ca^{2+} influx into the mitochondria leads to its dysfunction and to cell death. **(C)** Restoring the mitochondrial function by decreasing Ca^{2+} influx into the mitochondria may be a potential therapeutic target during sepsis.

Ca^{2+} uniporter (MCU) and rapid mode of uptake channels. This process also involves the high-conductance permeability transition pore (PTP), which spans the outer and inner mitochondrial membrane and can allow rapid ion transfer, leading to mitochondrial swelling and dysfunction. The molecular Ca^{2+} signaling machinery is complex, and studies into the role played by the many channels involved has been limited by incomplete identification of the proteins comprising them, limiting the development of genetic or specific pharmacologic tools. Instead, investigators have relied on studies using relatively nonspecific inhibitors, for example, ruthenium red to inhibit MCU and cyclosporine A to inhibit the PTP. Interpretation of these studies is complicated by the off-target effects of these agents. Recently, substantial progress has been made in identifying the critical protein constituents of these channels. For example, Perrochi et al. recently identified the mitochondrial calcium

uptake 1 (MICU1) channel, providing a novel molecular target to prevent mitochondrial Ca^{2+} overload (10).

Mitochondrial Ca^{2+} and ROS signaling in response to TNF

During oxidative phosphorylation, electrons from reduced substrates are transferred to O_2 through a chain of electron transporters, and the energy provided by these electron transfer reactions is used to transfer protons across the mitochondrial inner membrane. The energy stored in the resulting chemiosmotic gradient is used to generate ATP. During electron transfer, some electrons react with molecular O_2 to produce a superoxide anion that is converted to H_2O_2 by mitochondrial or cytosolic SODs. Complexes I and II of the electron transport chain release ROS exclusively in the mitochondrial matrix, whereas complex III generates ROS on both sides of the mitochondrial inner membrane (11). Influx of Ca^{2+} accelerates the generation

of reduced substrates from the TCA cycle and the rate of several reactions in the electron transport chain, including O_2 consumption and ROS generation. Ca^{2+} also increases the activity of NO synthase, elevating NO levels, which in turn inhibit cytochrome oxidase (complex IV) and might also increase the production of ROS. Although classically considered toxic byproducts of cellular respiration, more recent evidence suggests that ROS released at moderate levels may activate cytosolic signaling pathways and transcriptional programs, allowing the cell to adapt to environmental stress (12).

The increase in mitochondrial Ca^{2+} leads to ATP and ROS production, which can trigger additional increases in Ca^{2+} as a result of further extracellular Ca^{2+} influx (13–15). This represents a tipping point in cell survival: low levels of ROS generated at complex III can activate adaptive signaling pathways, but higher levels of ROS result in the release of cytochrome *c* and cell death (12, 16).



In a series of elegant experiments, Rowlands et al. transfected several custom-designed fluorophore probes into mouse lung microvascular endothelium; confocal microscopy then showed that the intracellular Ca^{2+} increase occurs in two steps (5). The initial increase corresponds with sTNF- α activation of the lung microvascular endothelium and is associated with expression of the leukocyte adhesion receptor E-selectin and TACE-mediated TNF receptor shedding. This initial spike is followed by prolonged elevation of Ca^{2+} that persists even when TNF- α infusion is stopped and is mediated by the purinergic receptor P_2Y_2 (5).

TNF receptor shedding as an antiinflammatory adaptation response

Ectodomain TNF receptor shedding is an important mechanism of adaptation and/or protection against inflammation. The unanchored receptor binds the soluble TNF, decreasing its ability to transduce intracellular signals, and thus curtails the cytokine's proinflammatory effects. TNF receptor inhibition is an accepted therapeutic modality in many inflammatory diseases, and the TNF- α inhibitors etanercept (a recombinant soluble fusion protein consisting of TNF receptor coupled to the Fc portion of IgG), adalimumab (a human anti-TNF- α antibody), and infliximab (a chimeric IgG1 anti-TNF- α antibody) are widely used therapeutics (17). An impaired ability to shed or endocytose TNF receptors can lead to inflammatory diseases. For example, a mutation in the gene encoding TNFR1 that impairs receptor shedding causes TNF receptor-associated periodic syndrome (TRAPS), an autosomal-dominant autoinflammatory disease (18). Consistent with the findings of Rowlands et al. (5), a protective role of TNFR1 shedding by TACE was also reported in TNF- α sensitization of hepatocytes to Fas-mediated death (19).

Relevance to sepsis

Sepsis remains a common clinical problem in the United States; therapeutic options to address the resulting multiorgan failure are limited, and the mortality in patients with sepsis remains unacceptably high. Sepsis occurs in patients with overwhelming infections and leads to the inability of cells to use the O_2 delivered to them. In patients with septic shock,

cardiac output is normal or even supranormal, and the delivery of O_2 to tissues after acute resuscitation is normal or increased. However, the response to infection in patients with sepsis can result in impaired mitochondrial function, which may contribute to cell death and multiorgan dysfunction (Figure 1B and refs. 8, 20). We reason that in severe sepsis, there is a massive influx of Ca^{2+} , part of which is taken up by mitochondria via the above-described Ca^{2+} channels. This rapid influx of Ca^{2+} leads to swelling and mitochondrial dysfunction, which, in conjunction with proinflammatory TNF signaling, contributes to cell death.

Rowlands et al. provide evidence that controlled TNF infusion leads to sustained mitochondrial Ca^{2+} elevation and mitochondrial ROS generation in the pulmonary endothelium (5). At the levels they observed, these ROS-activated signaling pathways resulted in TNF receptor shedding via TACE, thereby limiting the inflammatory response (Figure 1A). A limitation of this study — and of most research in this area — is that researchers use a model of moderate sepsis to elucidate mechanisms underlying the response to TNF- α . It is tempting to speculate that higher levels of sTNF- α in more severe sepsis would lead to influx of Ca^{2+} and that these same pathways would cause mitochondrial dysfunction and perhaps death.

Anti-TNF- α therapy has been ineffective in the treatment of sepsis and critical illness, but has been used with success in rheumatoid arthritis, Crohn disease, ankylosing spondylitis, and other chronic inflammatory diseases. This therapy has been associated with potentially severe side effects, including tuberculosis and serious bacterial and opportunistic infections. The pathways Rowlands et al. have identified (5) and the recent discovery of MICU1 protein (10) are encouraging and highlight mechanisms that might be targeted to ameliorate the inflammation and organ dysfunction observed in patients with sepsis (Figure 1C).

Acknowledgments

The authors are supported by NIH grants R37-HL48129, HL-85534, and PO1-HL71643.

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