(S6) Mitochondria in sepsis symposium lecture abstracts

S6/1 Cellular energetic metabolism in sepsis

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Sepsis is a complex pathophysiological disorder arising from a systemic inflammatory response to infection. Patients are clinically classified according to the presence of signs of inflammation alone, multiple organ failure (MOF), or organ failure plus hypotension (septic shock). The organ damage that occurs in MOF is not a direct effect of the pathogen itself, but rather of the dysregulated inflammatory response of the patient. Although mechanisms underlying MOF are not completely understood, a disruption in cellular energetic metabolism is increasingly implicated. Our aim is to understand the relative importance of factors affecting cellular ATP supply and demand in contributing to the onset of organ failure, and also organ recovery, in sepsis. We will emphasise the need for an integrated systems approach and preliminary data will be presented that demonstrate how a modular framework can be used to assess the integration of cellular energetics in this complex pathophysiological condition.

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S6/2 Functional import of exogenous mature cytochrome *c* into mitochondria of deficient cells

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Cytochrome *c* (cyt *c*), the electron carrier between Complexes III and IV, is required for oxidative phosphorylation. It is believed that only the immature form of the protein is imported into mitochondria. Here we test the hypothesis that exogenous mature cyt *c* can be functionally imported. Cyt *c* null cells (cyt *c*−/−) and control fibroblasts (NIH/3T3) were exposed to cyt *c*, acetylated cyt *c*, or buffer for 2 h. Immunoblot analysis demonstrated markedly increased cyt *c* levels in mitochondria of cyt *c* exposed null and 3T3 cells. Following exposure to FITC-conjugated cyt *c*, green punctate fluorescence was readily visualized in both cell types and co-localized with MitoTracker Red. Using electron microscopy, gold particles were clearly seen within mitochondria and endosomes of null and 3T3 cells following exposure to gold-conjugated cyt *c*. Cyt *c*-dependent respiration was significantly decreased in null cells exposed to buffer or acetylated cyt *c* and increased following exposure to exogenous cyt *c* approaching 3T3 rates. The percentage of TUNEL positive nuclei was similar between groups. Exogenous cyt *c* readily entered the intact cell via endocytosis and was imported into mitochondria without initiating apoptosis. This import increased null cell cyt *c*-dependent respiration. This may have important implications for the treatment of acquired and inherited mitochondrial disorders.

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S6/3 Mitochondrial UCP 1 and thymus function

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We have recently discovered through empirical investigations and confocal imagery, that thymus from rats and mice have UCP 1 in their mitochondria. As part of our investigations into the role of UCP 1 in thymus function, we are endeavouring to establish, using wild-type and UCP 1 knock-out mice, (a) whether there is a regional expression bias for UCP 1 in thymus tissue, (b) whether UCP 1 is thymocyte-type specific, (c) whether bioenergetic and (d) immunological parameter are effected by the absence/presence of UCP 1 and (d) and whether susceptibility to apoptosis is effected by UCP 1. A summary of our findings will be presented and include data clearly showing that UCP 1 knock-out mice are more sensitive to LPS treatment than wild-type controls.

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(S6) Mitochondria in sepsis symposium abstracts (poster and raised abstracts)

S6.4 Mechanisms of increased mitochondrial ROS generation in sepsis: The impact of AIF and delta Ψ

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The aim of this study was to clarify the mechanisms underlying increased mitochondrial ROS production in-vitro (primary hepatocytes) and in-vivo (endotoxic shock model). We found that elevated ROS generation in both models was not accompanied by an increase in delta Ψ, which is known to stimulate ROS production. Additionally, the comparison of the rates of respiration induced either by ADP or by CCCP suggested that the increase in ATP synthesis in endotoxic shock was possibly due to an upregulation of ATP-synthase. The latter was confirmed by the analysis of mitochondrial proteome. Surprisingly, ROS generation was sensitive to PJ34, a PARP inhibitor. PAR, a product of PARP is known to induce AIF-release from complex I. A pro-apoptotic phenotype was confirmed by an increase in ratio Bax/Bcl. Although it has been suggested that the AIF release stimulates ROS production at complex I we did not observe increased ROS production in AIF knockouts. Also we have found that AIF has not been transferred to the nucleus, yet suggesting that it remains in mitochondria. All data together suggest that AIF itself is the source of ROS in endotoxic shock as was recently suggested (Susin et al., 2007).

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