Sepsis represents a whole-body inflammatory response to infection that will often progress to multiple organ failure. In this state, organ function is altered in an acutely ill patient such that homeostasis cannot be maintained without interventions such as mechanical ventilation to support gas exchange, renal dialysis to act as a surrogate kidney removing nitrogenous waste products and excess fluid, and/or catecholamines to elevate critically low blood pressures in the condition of septic shock. Multiple organ failure is both common and potentially deadly. A quarter of patients admitted to British intensive care units have sepsis and it is the predominant cause of mortality in the critically ill. Indeed, patients with the failure of three or more organs have a >50% chance of dying.

Despite three decades of intensive research and the billions of pharmaceutical companies’ dollars invested in the search for immunotherapeutic magic bullets, mortality rates due to sepsis have not changed considerably. Its incidence is predicted to increase still further as elderly and immunosuppressed populations grow.

There remain many unanswered questions. Why are some people more susceptible than others? How does excessive inflammation actually cause the organs to fail? Why do these failed organs look remarkably normal, with minimal evidence of cell death? Unlike many other conditions that cause single organ failure, why is there, in general, (near) total recovery of organ function should the patient survive? Why is this recovery seen even in organs with poor regenerative capacity?

Genetic, immune and exogenous factors (e.g. more pathogenic bacteria) are responsible for triggering this excessive degree of inflammation, yet precise mechanisms and interactions remain poorly understood. There is an increasing appreciation of the importance of other systems such as hormonal, immune, metabolic and bioenergetic. The degree of perturbation of each of these systems has been independently associated with increased mortality. Furthermore, many of the conventional paradigms that have attempted to explain the underlying pathophysiology have been successfully challenged in recent years. For example, the traditional belief held that organ failure was directly related to inflammatory mediator-induced release of vasoactive agents, activation and aggregation of neutrophils and platelets, and a disseminated intravascular coagulation that would result in an abnormal microvasculature, with consequent tissue hypoxia, cell death and organ dysfunction. However, this dogma has been undermined by the findings of a normal histology and a paradoxical rise in tissue oxygen tension. This suggests that oxygen is actually freely available but is not being utilized, and that a large part of the problem may lie at a cellular level. As cytochrome oxidase is by far the predominant consumer of molecular oxygen, a mitochondrial pathology is therefore implicated in the pathophysiology of sepsis-induced multiple organ failure.

Mitochondrial abnormalities — both biochemical and ultrastructural — have been recognized in in vivo and in vitro models of sepsis for more than 30 years. A systematic review of these models showed variable findings in short-term models of varying severity, lasting several hours with either increased, decreased or unaltered mitochondrial function being reported. However, when the study duration exceeded 16 hours, dysfunction and/or injury were consistent features. Corresponding functional changes were noted, which supported the concept of mitochondrial dysfunction. For example, Rosser et al. found that maximal oxygen consumption was markedly increased in a hepatocyte cell line exposed to endotoxin after six hours, but was significantly depressed by 24 hours. In a study on patients, Kreymann et al. noted that increasing sepsis severity was associated with progressive falls in O2 consumption.

Data from humans are still relatively scanty; small case series reported decreases in ATP or decreased activity of various respiratory chain complexes. A larger study that we published in 2002 consisted of a group of 28 patients in septic shock, and a control group of patients undergoing elective hip surgery. A significant association was seen...
between sepsis severity and Complex I inhibition in muscle biopsies taken within 24 hours of admission to intensive care. Interestingly, there was a clear delineation between subsequent survivors and non-survivors, with ATP levels being preserved in the former (compared to the orthopaedic controls) and significantly reduced in the latter. This was found notwithstanding the lack of any clinical differentiation between the two septic groups at the time of biopsy. This human study prompted us to develop a long-term (3 day) rat model of faecal peritonitis that remained adequately fluid resuscitated throughout to ensure an adequate circulating blood volume. This model mimics many of the physiological, biochemical, histological and outcome characteristics of the human patient and enables comparison of muscle data with other ‘vital’, deeper organs such as liver and kidney. Mitochondrial results were comparable to the human muscle data in both liver and kidney with the more severely septic animals also demonstrating greater degrees of Complex I inhibition and a decrease in ATP levels. Importantly, recovery in mitochondrial function paralleled clinical and biochemical recovery. A crucial question that I will return to is whether this mitochondrial recovery is fundamental to the restoration of organ function and, if so, how this could arise.

An important step in unravelling the mechanism of mitochondrial inhibition in sepsis arose from the discovery of nitric oxide. This reactive species is produced in greater quantities in sepsis than in any other clinical condition and is largely responsible for the characteristic hypotension and vascular hyporeactivity (i.e. decreased responsiveness to vasoconstrictor catecholamines) of septic shock. The subsequent recognition that nitric oxide and, more particularly, peroxynitrite (formed from the reaction between nitric oxide and superoxide) were potent inhibitors of the electron transport chain suggested its likely relevance in sepsis. In both our human and animal model studies, elevated nitric oxide production (measured as tissue nitrite/nitrate) correlated with the degree of sepsis severity and Complex I dysfunction. Glutathione, an endogenous mitochondrial antioxidant that protects Complex I from nitrosative damage, was correspondingly reduced and the inability to reverse this inhibition with exogenous glutathione suggested nitration of the enzyme leading to a longer-lasting, if not irreversible, inhibition. In a macrophage cell line incubated with endotoxin, we found a progressive decrease in oxygen consumption and Complex I inhibition. In conjunction with these findings, an early nitrosylation was followed by a progressive increase in nitrination; this was accentuated in the presence of concurrent hypoxia. If these findings can be extrapolated to patients, co-existing tissue hypoxia (for example, due to delayed fluid resuscitation) would have a synergistic effect on systemic inflammation and would reduce the competition between nitric oxide and oxygen for the same binding site on cytochrome oxidase. Boulos et al. incubated an endothelial cell line with plasma taken from septic patients and found a decrease in mitochondrial respiration and ATP levels compared to that seen following incubation with plasma from healthy controls. This depression could be reversed by inhibition of either nitric oxide synthase or poly(ADP)-ribose synthase (PARS), a nuclear repair enzyme that depletes NAD stores and yet has anti-inflammatory actions. Excessive nitric oxide has also been implicated in the skeletal and cardiac muscle contractile failure seen in sepsis, for which a mitochondrial aetiology has been suggested.

An important breakthrough in the clinical management of patients with sepsis was made by van den Berghe’s group in Leuven, Belgium, who randomized critically ill surgical patients to receive either conventional management of blood glucose levels (i.e. maintained between 4 and 10 mmol/l) or a regimen of tight glycaemic control (4.5–6.1 mmol/l) and additional insulin and glucose. They reported an impressive decrease in mortality and morbidity in the protocol group, stimulating much discussion as to possible mechanism(s) of action. In an important follow-up paper examining liver and muscle biopsies taken soon after death, they demonstrated minimal cell death, yet considerable hepatic mitochondrial injury in the conventionally managed patients, but almost complete protection in those treated by tight glycaemic control. These changes mirror those we have seen in our long-term septic animal model in terms of both lack of cellular damage, yet significant mitochondrial injury within the liver (Figure 1). This suggests that better glycaemic control (leading to reduced glycation of mitochondrial protein) and/or additional insulin and glucose (enhancing glycolysis and having direct effects on mitochondrial function) represent potential protective mechanisms.

Despite the aforementioned decrease in Complex I activity in both septic patients and the long-term animal model, Complex IV activity tended to increase. This may be misleading, as rapid reversibility of competitive NO inhibition of this enzyme in the room air environment...
in which the in vitro assay was prepared and performed may belie any in vivo inhibition present in an environment where the oxygen tension is more than 20-fold lower. On the other hand, it may possibly represent a true result and be due to an increase in activity of the enzyme per unit mass due, for instance, to a conformational change. It is more likely, however, that total enzyme protein has increased. Although two recent studies have reported a decrease in Complex IV protein and mitochondrial content, these were performed in severe, high-mortality rodent models. On the other hand, Suliman et al. found that bacterial lipopolysaccharide (endotoxin) injected into rats produced a dual oxidant effect of early nuclear damage followed by stimulation of mitochondrial biogenesis. This is consistent with the finding of Elfering et al. that nitration induced a much accelerated turnover of new mitochondrial protein.

In conclusion, prolonged sepsis will induce mitochondrial dysfunc-
tion/failure. Recovery would then be contingent upon restoration of mitochondrial function, through either repair of existing damaged mitochondria or production of new organelles. Excess production of nitrite and other reactive species appears likely to be the main ‘culprit’ of the initial injury and altered bioenergetics, yet, paradoxically, would appear to provide the stimulus for eventual recovery of function.

References


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