SEPSIS: NEW INSIGHTS, NEW THERAPIES
The Novartis Foundation is an international scientific and educational charity (UK Registered Charity No. 313574). Known until September 1997 as the Ciba Foundation, it was established in 1947 by the CIBA company of Basle, which merged with Sandoz in 1996, to form Novartis. The Foundation operates independently in London under English trust law. It was formally opened on 22 June 1949.

The Foundation promotes the study and general knowledge of science and in particular encourages international co-operation in scientific research. To this end, it organizes internationally acclaimed meetings (typically eight symposia and allied open meetings and 15–20 discussion meetings each year) and publishes eight books per year featuring the presented papers and discussions from the symposia. Although primarily an operational rather than a grant-making foundation, it awards bursaries to young scientists to attend the symposia and afterwards work with one of the other participants.

The Foundation’s headquarters at 41 Portland Place, London W1B 1BN, provide library facilities, open to graduates in science and allied disciplines. Media relations are fostered by regular press conferences and by articles prepared by the Foundation’s Science Writer in Residence. The Foundation offers accommodation and meeting facilities to visiting scientists and their societies.

Information on all Foundation activities can be found at http://www.novartisfound.org.uk
SEPSIS: NEW INSIGHTS, NEW THERAPIES
Contents

Symposium on Sepsis: new insights, new therapies, held at the Novartis Foundation, London, 14–16 February 2006

Editors: Derek J. Chadwick (Organizer) and Jamie Goode

This symposium was based on a proposal made by Mitchell Fink and Mervyn Singer

Mitchell P. Fink  Chair’s introduction  1
Discussion  4

Alessandro Protti and Mervyn Singer  Strategies to modulate cellular energetic metabolism during sepsis  7
Discussion  16

Jérôme Pugin  Immunostimulation is a rational therapeutic strategy in sepsis  21
Discussion  27

Alfred Ayala, Doreen E. Wesche-Soldato, Mario Perl, Joanne L. Lomas-Neira, Ryan Swan and Chun-Shiang Chung
Blockade of apoptosis as a rational therapeutic strategy for the treatment of sepsis  37
Discussion  49

John C. Marshall, Zeenat Malam and Songhui Jia  Modulating neutrophil apoptosis  53
Discussion  67

Haichao Wang, Wei Li, Richard Goldstein, Kevin J. Tracey and Andrew E. Sama  HMGB1 as a potential therapeutic target  73
Discussion  85
Csaba Szabó  Poly (ADP-ribose) polymerase activation and circulatory shock  92
Discussion  103

Xavier Leverve, Cécile Batandier and Eric Fontaine  Choosing the right substrate  108
Discussion  121

Laura Dugo, Marika Collin, David A. Allen, Nimesh S.A. Patel, Inge Bauer, Eero M. A. Mervaala, Marjut Louhelainen, Simon J. Foster, Mohammad M. Yaqoob and Christoph Thiemerman Inhibiting glycogen synthase kinase 3β in sepsis  128
Discussion  142

Mitchell P. Fink  Ethyl pyruvate: a novel treatment for sepsis  147
Discussion  156

General discussion I  160

Stefan W. Ryter and Augustine M. K. Choi  Cytoprotective and anti-inflammatory actions of carbon monoxide in organ injury and sepsis models  165
Discussion  175

Andrea Polito, Jérôme Aboab and Djillali Annane  The hypothalamic pituitary adrenal axis in sepsis  182
Discussion  199

Ilse Vanhorebeek, Lies Langouche and Greet Van den Berghe  Modulating the endocrine response in sepsis: insulin and blood glucose control  204
Discussion  215

Luis Ulloa and Ping Wang  The neuronal strategy for inflammation  223
Discussion  233

C. T. Pereira, M. G. Jeschke and D. N. Herndon  Beta-blockade in burns  238
Discussion  248

Mervyn Singer  System interactions  252
Discussion  262
Participants

Djillali Annane Address Service de Reanimation, Hôpital Raymond Poincaré (AP-HP), Faculté de Médecine Paris Ile de France Ouest (UVSQ), 104 Boulevard Raymond Poincaré, 92380 Garches, France

Alfred Ayala Aldrich 227, Division of Surgical Research/Shock-Trauma Research Laboratories, Rhode Island Hospital / Brown University School of Medicine, 593 Eddy Street, Providence, RI 02903, USA

Jean-Marc Cavaillon UP Cytokines and Inflammation, Institut Pasteur, 28 rue Dr Roux, 75015 Paris, France

Augustine M. K. Choi Division of Pulmonary, Allergy and Critical Care Medicine, University of Pittsburgh School of Medicine, MUH628, 3459 5th Avenue, Pittsburgh, PA 15213, USA

Jon Cohen Brighton and Sussex Medical School, University of Sussex, Falmer, Brighton BN1 9PX, UK

Tom J. Evans Level 4, Glasgow, Biomedical Research Centre, University of Glasgow, 120 University Place, Glasgow G12 8TA, UK

Mitchell P. Fink (Chair) Department of Critical Care Medicine, University of Pittsburgh, 616 Scaife Hall, 3550 Terrace Street, Pittsburgh, PA 15261, USA

Derek Gilroy Division of Medicine, BHF Laboratories, University College London, 5 University Street, London WC1E 6JJ, UK

Richard D. Griffiths Intensive Care Research Group, Division of Metabolic & Cellular Medicine, School of Clinical Science, Faculty of Medicine, University of Liverpool, Liverpool L69 3GA, UK
Paul G. Hellewell Division of Clinical Sciences North, Northern General Hospital, University of Sheffield, Sheffield S5 7AU, UK

David N. Herndon Chief of Staff, Shriners Hospitals for Children, Director of the Blocker Burn Unit, University of Texas Medical Branch, Galveston, TX 77550, USA

Xavier Leverve LBFA-INSERM 221, Nutrition Humaine et Sécurité des Aliments, Université Joseph Fourier, Institut National de la Recherche Agronomique (INRA), 147, Rue de l’Université, Paris 75007, France

John C. Marshall Department of Surgery and the Interdepartmental Division of Critical Care Medicine, St Michael’s Hospital, University of Toronto and Room 4–007, 30 Bond Wing, Toronto, Ontario M5B 1W8, Canada

Claude A. Piantadosi Division of Pulmonary and Critical Care Medicine, Duke University Medical Center, Durham, NC 27710, USA

Jérôme Pugin Division of Medical Intensive Care, University Hospital, 24, r. Michelli-du-Crest, Geneva 14, CH-1211, Switzerland

Peter Radermacher Sektion Anästhesiologische Pathophysiologie und Verfahrensentwicklung, Universitätsklinik für Anästhesiologie, Universität Ulm, Parkstrasse 11, 89073 Ulm, Germany

Anne Marie Schmidt Division of Surgical Science, Columbia University Department of Surgery, New York, NY 10032, USA

Mervyn Singer Department of Medicine and Wolfson Institute of Biomedical Research, University College London, Cruciform Building, Gower Street, London WC1E 6BT, UK

Csaba Szabó Department of Surgery, University of Medicine and Dentistry of New Jersey, 185 South Orange Avenue, University Heights, Newark, NJ 07103-2714, USA

Chris Thiemermann The Department of Experimental Medicine, Nephrology and Critical Care, William Harvey Research Institute, St Bartholomew’s and The Royal London School of Medicine and Dentistry, London EC1M 6BQ, UK
Jack Tinker Royal Society of Medicine, 1 Wimpole Street, London W1G 0AE, UK

Pierre Tissières (Novartis Foundation Bursar) Laboratory of Intensive Care, Department of Microbiology and Molecular Medicine, University of Geneva Medical Center, CMU-1, Rue Michel Servet, 1211 Geneva 4, Switzerland

Luis Ulloa North Shore University Hospital, 350 Community Drive, Manhasset, NY 11030, USA

Greet Van den Berghe Department of Intensive Care Medicine, Catholic University of Leuven, B-3000 Leuven, Belgium

Haichao Wang Laboratory of Emergency Medicine, Feinstein Institute for Medical Research, North Shore-Long Island Jewish Health System, 350 Community Drive, New York, NY 11030, USA
Chair’s introduction

Mitchell P. Fink

Department of Critical Care Medicine, University of Pittsburgh, 616 Scaife Hall, 3550 Terrace Street, Pittsburgh, PA 15261, USA

Let me try to set the stage for this symposium. We will be discussing new treatments for sepsis. In 1992 a landmark publication appeared, which attempted to create some definitions that would bring order to the field of sepsis research (American College of Chest Physicians/Society of Critical Care Medicine 1992). At this time the concept of a tiered approach to diagnosing systemic inflammation, sepsis and severe sepsis was developed. The notion was that the combination of the cardinal signs of inflammation and infection constituted sepsis. If organ dysfunction is layered on this mix, the condition is now severe sepsis, and if arterial hypotension is included in the mix, this condition is septic shock.

In 2001 another consensus conference was held that slightly modified this concept (Levy et al 2003). This meeting recognized that there are many findings that tip clinicians off to the possibility that a patient has a deleterious response to infection. The list is long and complete. To keep peace, the notion was adopted that everything should be listed and the new definition does not specify how many features are needed for a diagnosis of sepsis. This is one of the current weaknesses in our field: unlike our brethren in oncology and cardiology, we don’t have a tissue diagnosis for the disease we are interested in, and we don’t have a biochemical marker. So we are not all studying the same thing, and when we enrol patients in clinical trials we tend to enrol a very heterogeneous group of people, poorly characterized with respect to genotype and phenotype.

This being said, no matter how you diagnose sepsis, it is an important public health problem. Angus et al (2001) published a paper that has been cited more than 900 times. The findings in the paper were the result of a thorough epidemiological analysis of the incidence of sepsis in the USA, using data from seven states and then generalizing it to the rest of the population. This study showed that sepsis is a disease of the very young and very old, and there are about three cases per 1000 in the population. This incidence translates to about 750,000 cases annually, with a mortality rate of about 30%. To put this into context, colon cancer (a common form of cancer in the USA) has an incidence of about 50 cases per 100,000 people, and breast cancer has an incidence of about 110 cases per 100,000 women. AIDS gets an enormous amount of research funding, and has an incidence of
17 cases per 100,000 people, and the incidence of congestive heart failure is about 130 cases per 100,000. Sepsis is truly a major public health problem.

In the developed world, the most costly health problems are the neurodegenerative diseases of ageing, such as Alzheimer’s disease. Congestive heart failure and type 2 diabetes mellitus are also epidemics. In this same category of huge public health problems is sepsis. In the developing world the list looks very different; malnutrition heads the list. Malaria remains a huge public health problem internationally, and cerebral malaria is a cytokine-driven illness that is very reminiscent of sepsis. Diarrhoeal diseases, AIDS and schistosomiasis are also enormous worldwide public health problems. Even in the developing world, because of the importance of cerebral malaria, I think sepsis is an important public health problem.

About six years ago a number of us in the Department of Critical Care Medicine at the University of Pittsburgh wrote a proposal to the NIH. Professor Derek Angus was the principal investigator on this project. The goal was to do the largest and best large-scale observational study of human sepsis, focusing in particular on the genetic and biochemical markers associated with development and progression of the syndrome. We chose to study patients with community-acquired pneumonia, because of the diagnostic problems mentioned at the outset. Accordingly, we wanted to study as homogeneous a population as possible. We enrolled patients from 38 hospitals from four states, and the enrolment was concluded in November 2003.

We enrolled 2300 patients, and of these about 2100 were shown to have community-acquired pneumonia. Of these, the vast majority were admitted to the hospital. About 70% of these admitted patients did not have a diagnosis of severe sepsis (no evidence of organ dysfunction). Of these, only 4% died over a 60 day observation period. On the other hand, about 30% did have a diagnosis of severe sepsis, and in this group about 30% died. The difference in the shape of the mortality curves was not just apparent during the acute period, when the patients were in the hospital, but it persisted for months afterwards.

The cytokine profiles for different groups of patients are interesting. At admission, almost all the patients had high circulating concentrations of interleukin (IL)6. In general, the patients who were discharged alive normalized their circulating IL6 level. In contrast, the patients who succumbed to their illness tended to have biochemical evidence of persistent systemic inflammation. This can be shown by plotting the log circulating concentrations for IL6, IL10 and tumour necrosis factor (TNF). There are a couple of messages here: first of all, everyone with sepsis is admitted to the hospital with biochemical evidence of systemic inflammation. The peak inflammatory and counter-inflammatory response is at the time of admission. The notion that there is a delayed anti-inflammatory response that comes up 3, 5 or 7 days after admission is probably wrong. Circulating cytokine levels tend to normalize relatively quickly in patients who are des-
tined to survive. In contrast, both pro-inflammatory and anti-inflammatory cytokine levels tend to remain elevated in patients who are destined for a bad outcome. Additionally, it is worth noting that circulating levels of TNF are not very valuable as a prognostic marker, and it is not too surprising that TNF has not proven to be a useful drug target for the development of therapeutic agents for sepsis.

The history of research in our field can be defined in three epochs. The first is the period prior to the publication of the Beutler et al (1985) paper in *Science*, which identified for the first time that TNF is a mediator of lipopolysaccharide (LPS)-induced lethality in rodents. The premodern era of sepsis research is the period before this paper. The modern era comes after this publication. Another landmark was the report of the results of the PROWESS study in 2001 (Bernard et al 2001). This report led to the approval in many countries of Xigris® as an adjuvant therapy for the management of severe sepsis. The approval of Xigris® doesn’t mean that research in our field is over (Carlet 2006).

In the era before the PROWESS study was published, several clinical trials were carried out using drugs directed at a number of targets. TNF was the favourite target for a long time. Another alarm-phase cytokine, IL1β, was a target of several studies. There were numerous studies of drugs that targeted platelet activating factor (PAF), thromboxane A₂, cyclooxygenase isoforms, bradykinin, endotoxin, nitric oxide (NO) and reactive oxygen species. The results of all of these studies were negative. In February 2006, the current list of drug targets includes TLR4 (including components of a proximal TLR4 signalosome), GSK3β, Fas/FasL, caspases, histone deacetylase, HMGB1, RAGE, the α7 nicotinic receptor and reactive oxygen species. There are also some therapeutics under development that seem to work in sepsis models but no one knows why. These include ethyl pyruvate, carbon monoxide and inosine. Then there is Xigris®, which also has demonstrable efficacy in humans without a clearly delineated mechanism of action.

I think this will have teed up the subject. Sepsis is an enormous public health problem. There has been much progress over the last 20 years, but I think the real progress will be made in the next few years because our understanding of the biology has improved so much.

References


DISCUSSION

Marshall: As someone who has contributed to the 940 citations of Derek Angus’ paper, I want to challenge the notion that sepsis is a common disease. Sepsis in the abstract is common in the same way that cancer is common. Cancer is very common if you include diseases such as carcinoma of the prostate, basal cell cancer and metastatic small cell cancer. The reality is that when we have actually studied it, the disease we think we are modelling in our animals is in fact quite rare. One of the challenges we have is deconstructing a lot of the assumptions we have made in the past and refocusing them on the reality. High profile victims of sepsis have included Jim Henson and that is the disease that we would like to treat. They have also included Pope John Paul II who had a urinary tract infection that the doctors decided not to treat. He died of sepsis as well. We need to recognize that this complex polyglot of diseases is not a single common process that we want to treat in every patient.

Fink: I beg to differ. In the context of doing the GeNIMS study, where our goal was to study patients with community-acquired pneumonia, we had no difficulty enrolling. Busy emergency rooms, such as the one at the University of Pittsburgh, were enrolling at the rate of three or four cases a week. This makes it a common disease. We admit patients to the hospital with community-acquired pneumonia during the winter at the rate of at least one per day. We admit congestive heart failure patients at about one per day. These are diseases of comparable epidemiological significance.

Marshall: Only a third of the patients you recruited had severe sepsis.

Fink: Among the patients who were admitted, about one third had severe sepsis. This means that we admit two to three patients per week with severe sepsis. This number is not a trivial number.

Marshall: Look at the people who come in with community-acquired pneumonia. My father died of sepsis but he was 86 years old and had Alzheimer’s disease; it was the appropriate end for his life. I guess the point I am trying to make is that we have to be a bit more critical at looking at the population of patients we want to target with aggressive, expensive, biologically complex treatments.
Probably for the majority of people this is not such a bad way to leave this planet.

Fink: My dad died of sepsis as well; he was 91 years old. To a certain extent I agree with you; some of these deaths are appropriate. How many of these in the fifth decade and beyond are inappropriate?

Cohen: One of the points John Marshall was making is this question of definition. We probably don’t want to spend the whole meeting rehearsing the definition of sepsis, but we should flag this up because it is a real issue. Because what we are talking about is so vague and nebulous, we will inevitably be dealing with a heterogeneous population and the polar examples you give are bound to occur. We are coming to the end of another era, that of thinking of sepsis as a disease or diagnosis. I don’t think it will be helpful to continue to do this. Should we not perhaps be reverting to a situation where we talk about severe infection complicated by organ failure. This might be pneumonia or it might be peritonitis. This is different from the syndromic approach to sepsis, which is that a slight fevered brow is suddenly sepsis.

Fink: I agree with you. As we improve our level of precision in diagnosis, the whole field will be able to move forward in leaps and bounds. Now we are setting up a disease instead of a loose collection of clinical features or a syndrome. The haematologists and oncologists used to define cancer on the basis of histology. Now they do it on the basis of genetics. They are way ahead of us. Cardiologists have a blood test that establishes the diagnosis of myocardial infarction in the emergency department. We need to move to that biochemical level, phenotyping patients on the basis of biochemistry rather than syndrome description.

Griffiths: One of the things that has always dogged me from a clinical perspective has been the timeline of severe sepsis. Mortality is still progressing, even a year after the event. This has troubled a lot of our understanding of sepsis. We need a pathological explanation for how and why people die at different time points. It may well be that we’re not dealing with a single phenomenon but rather a cascade of events. This could explain why there has been disappointment with Xigris®.

Fink: This gets at John Marshall’s point. Part of the reasons for those excess deaths out at three months, six months and a year is because the people who get sepsis are not healthy people to begin with. They are dying after their sepsis, but what they are really dying from is their pancreatic carcinoma, for example.

Griffiths: That is an assumption I’m not sure of. You can match people to different ages and illness and there outcomes can be quite different. We must not forget the legacy of the septic illness itself.

Fink: This is just some of it. For any of you who have followed your patients out months afterwards, there is clearly a nutritional hit that is associated with two or three weeks in an intensive care unit. It is non-trivial. Patients lose enormous
amounts of lean body mass as a result of this acute illness, and they don’t recover that for months. There is also a neurocognitive hit that is enormous. I don’t think we understand the implications of this and its effect on survival at all: it is currently a black box.

_Herndon:_ The prolonged response to sepsis may be one of the most poignant observations you have made. Individuals do have a cascade of events. There is a prolonged hypermetabolic response, and a prolonged catabolic response. It is not just the loss of weight that occurs during the intensive care unit stay. There is another aspect to this: people who develop sepsis may well be genetically different. There are two aspects of this shift in curve that you mentioned: first, the response or the genetic make-up of the individual as they come in, and second, the prolonged response, which we have studied little if at all. Perhaps we should focus on this prolonged response more to separate out those who may benefit from therapy.

_Singer:_ It is interesting to hear this discussion develop. Certainly, my experience of community sepsis is that it is now relatively rare. These are ones we are waiting to recruit into studies, but we don’t see many. The ones we do get are those who develop sepsis as a complication of another problem. This could be a different population, or there could be syndromes within a syndrome. Could we therefore argue that sepsis is a human-induced disease? We are admitting fewer and fewer people from the community into our intensive care units with sepsis. The sepsis patients we do see come predominantly from the ward, or they are on the unit already recovering from surgery or trauma. Perhaps we have created the monster?

_Fink:_ This may be one difference between the way that healthcare is done in the USA and the UK. At the University of Pittsburgh Medical Center we have more than 150 ICU beds. This is roughly one-quarter of the beds in the adult Medical Surgical hospital. In the medical ICU, I suspect that the vast majority of patients with sepsis or severe sepsis have acquired this in the community.

_Marshall:_ Mervyn Singer’s point is an important one. This is one of the ways that we need to parse this entity. The reality is, if we look at severe sepsis as a construct, it is entirely iatrogenic. We are dealing with organ dysfunction that is lethal if we don’t intervene. We know that many of the interventions we use can become a second hit, or something that modifies the response, such as mechanical ventilation and the cytokine response. This is one of the groups that we systematically exclude from studies. It is probably the group that we should be most keen on studying, although it is also the most difficult to describe.
Strategies to modulate cellular energetic metabolism during sepsis

Alessandro Protti and Mervyn Singer

Bloomsbury Institute of Intensive Care Medicine, University College London, Gower Street, London WC1E 6BT, UK

Abstract. Growing evidence suggests that mitochondrial inhibition plays a major role in the development of multiple organ failure during sepsis. Early correction of tissue hypoxia, strict control of glycaemia and modulation of oxidative and nitrosative stress may protect mitochondria during the acute inflammatory response. Once mitochondrial dysfunction has developed, the regulated induction of a hypometabolic state, analogous to hibernation, may protect the cells from severe bioenergetic failure and a critical fall in ATP. Though this is clinically manifest as organ dysfunction, it may actually represent an adaptive response to a prolonged, severe inflammatory stress. Repair of damaged organelles, stimulation of mitochondrial biogenesis and re-activation of cellular metabolism may accelerate the recovery phase and thus improve clinical outcomes. The aim of this review is to discuss putative interventions aimed at preventing or reversing mitochondrial dysfunction that may have possible clinical relevance, and to stress the importance of the correct timing of intervention.

Sepsis is the systemic inflammatory response to infection and represents a major cause of morbidity and mortality in patients admitted to intensive care units (Padkin et al 2003). Despite recent advances, many aspects of the pathophysiology of sepsis remain to be elucidated. Findings of reduced oxygen consumption, elevated tissue oxygen tension and the absence of significant histological changes in most of the affected organs suggest that multiple organ failure (MOF) during sepsis may be due to an acquired inability of the cells to use oxygen (Fink 2002).

Mitochondria utilize >90% of total body oxygen consumption to produce energy as adenosine triphosphate (ATP). The electron transport chain consists of enzyme complexes and carrier molecules associated to the inner membrane. The reduced nicotinamide (NADH) and flavin (FADH₂) adenine dinucleotides produced by the oxidation of nutrients donate electrons to complex I and complex II, respectively. The electrons then flow through complex III and complex IV.

1This paper was presented at the symposium by Mervyn Singer, to whom correspondence should be addressed.
(cytochrome oxidase) transported by coenzyme Q and cytochrome C, and finally reduce oxygen to water. Electron transfer through complexes I, III and IV generates a proton gradient across the inner mitochondrial membrane that is used by a specific ATP synthase to generate ATP from ADP.

Studies on the early phase of sepsis have produced conflicting results with increases, decreases or no change in mitochondrial function all being reported (Singer & Brealey 1999). Nonetheless, mitochondrial structure and activity were consistently shown to be damaged in studies lasting more than 12–16 hours, in both a time- and a severity-dependent manner. ATP levels were variably affected, depending on the balance between energy production and consumption. A reduction in the latter, eventually leading to MOF, may represent a cellular adaptive strategy to preserve ATP levels above a threshold compatible with survival (Singer et al 2004). Restoration of mitochondrial activity may drive the recovery of these ‘failed’ organs, a hallmark of survival from MOF. Accordingly, the need for long-term support (e.g. renal dialysis, mechanical ventilation) in septic survivors is very uncommon in those whose organs were healthy before the septic insult. This finding suggests that organ failure in sepsis differs from organ-specific diseases (e.g. glomerulonephritis) as it predominantly represents a potentially reversible, functional problem.

Early tissue hypoxia, excess production of inflammatory mediators, and hormonal changes are all implicated in the pathogenesis of mitochondrial abnormalities during sepsis. Nitric oxide (NO) production rises significantly, mainly as a consequence of increased expression of a specific inducible synthase (iNOS) and, possibly, of a mitochondrial isoform of the enzyme. Activation of phagocytic cells accounts for most of the increased production of reactive oxygen species. However, NO-mediated inhibition of cytochrome oxidase increases electron leak from the respiratory chain and subsequent generation of superoxide (O₂⁻·). NO and O₂⁻· accumulating within the mitochondria will react together to generate peroxynitrite and other nitrogen species. These are able to alter the structure and function of several mitochondrial proteins. Changes occurring at the electron transport chain are likely to impair cellular energy production (Liaudet et al 2000).

Only a few specific therapeutic approaches are currently available for treating sepsis. The aim of this chapter is to discuss some putative interventions that could prevent or reverse mitochondrial dysfunction, or that could modulate cellular metabolic activity both during the development of sepsis and in the recovery phase.

**Prevention and early reversal of mitochondrial dysfunction**

If the development of MOF following sepsis or any other major insult (e.g. major trauma) is related to a cellular energetic failure, then strategies aimed at prevent-
ing the impairment of mitochondrial energy production may be potentially beneficial.

Septic mitochondrial dysfunction can occur despite adequate tissue perfusion. Nonetheless, cellular hypoxia resulting from an unresuscitated shock state will limit aerobic ATP generation and will thus contribute to the development of progressive mitochondrial damage. We have recently demonstrated that activated macrophages experience an earlier impairment of mitochondrial oxygen consumption when incubated in 1% oxygen compared to room air (Frost et al 2005). The competitive NO-mediated inhibition of cytochrome oxidase is more likely to occur at low oxygen tension; this, in turn, may accelerate the production of highly reactive species that are able to persistently impair mitochondrial respiration.

At a cellular level, optimization of oxygen delivery can ameliorate energy failure as long as mitochondria retain their ability to produce energy. Patients with severe sepsis or septic shock clinically managed to achieve an adequate balance between global oxygen supply and demand (defined as a central venous oxygen saturation, \( \text{SvO}_2, \geq 70\% \)) early after admission to an Emergency Department experienced significantly better survival than control patients; the faster normalization of lactate levels, arterial base deficit and pH was consistent with an improvement in aerobic cellular metabolism (Rivers et al 2001). When critically ill patients with established MOF were treated in a similar way, often after several days in intensive care, there was either no benefit (Gattinoni et al 1995) or harm (Hayes et al 1994). Thus the same intervention, performed at different time points, had a different clinical impact: in the early phase, when the cellular energetic machinery is still likely to be functional and oxygen delivery may represent a limiting factor (as suggested by a baseline \( \text{SvO}_2 \) around 49% in the Rivers’ study), it may correct the impending cellular energetic failure and reduce the incidence of organ dysfunction. In a late phase, when mitochondrial inhibition and damage have eventually occurred, and the cell has become ‘intrinsically’ unable to produce ATP, such an approach may not provide any benefit. Lack of improvement of oxygen consumption despite a re-established oxygen supply has been associated with unfavourable outcomes in septic patients (Hayes et al 1997).

Hyperglycaemia and insulin resistance are common among critically ill patients and represent an additional potential threat to mitochondrial integrity. Even though most of the evidence comes from studies on diabetes, recently published work suggests that acute hyperglycaemia can dramatically increase the mitochondrial production of reactive oxygen species in normal bovine aortic endothelial cells (Nishikawa et al 2000). Moreover, insulin may contribute to the regulation of mitochondrial protein synthesis and oxidative phosphorylation (Stump et al 2003). Maintenance of normoglycaemia with intensive insulin therapy has been demonstrated to improve outcomes in a surgical intensive care unit population.
The greatest reduction in mortality was seen in deaths due to sepsis-related MOF in patients requiring intensive care for more than five days. A study performed on a subgroup of non-survivors found a protective effect of intensive insulin therapy on hepatocyte mitochondrial ultrastructure and respiratory enzyme activities (Vanhorebeek et al 2005).

Oxidative and nitrosative stress occur within the mitochondria during sepsis. Reactive oxygen and nitrogen species are overproduced whereas mitochondrial antioxidants (reduced glutathione [GSH] and manganese superoxide [MnSOD]) are depleted because of increased oxidation and altered metabolism. In the presence of persistently high levels of NO and other free radicals, mitochondrial proteins may undergo (semi-)permanent modifications. Damage to the iron-sulfur centres, nitrosylation of thiol groups and nitration of tyrosine residues of complex I may occur in a stepwise process, leading to a prolonged inhibition of mitochondrial respiration (Brown & Borutaite 2004).

Studies performed on cells in culture demonstrated that the membrane permeable glutathione ethyl ester can protect the functioning of complex I in an early phase, by either preventing or reversing its oxidation and nitrosylation (Clementi et al 1998). Reduced concentrations of GSH and increased levels of nitrite and nitrate (products of NO metabolism) were associated with greater inhibition of complex I in skeletal muscle biopsies taken within 24 hours of admission to intensive care from patients in septic shock (Brealey et al 2002). Incubation of the tissue samples with exogenous glutathione did not ameliorate the activity of the mitochondrial enzyme, suggesting that GSH-irreversible changes, such as nitration, may have occurred by this point. Provision of glutamine, N-acetylcysteine and other precursors may stimulate glutathione synthesis and potentially enhance the mitochondrial antioxidant state.

Manganese superoxide dismutase (MnSOD) scavenges superoxide anions, preventing them from further reacting with NO to generate peroxynitrite within the mitochondria. Nitration and inactivation of the enzyme may occur in the presence of high levels of reactive nitrogen species (MacMillan-Crow et al 1996). MnSOD mimetics may exert a protective effect towards oxidative and nitrosative damage, possibly reducing mitochondrial superoxide accumulation and peroxynitrite generation (Salvemini et al 2002).

**Prevention of cellular energetic failure in the presence of mitochondrial dysfunction**

Once permanent mitochondrial dysfunction has developed, optimization of the residual cellular ability to produce energy, and/or a reduction in metabolic requirements, may prevent the ATP level from dropping below the threshold that stimulates cell death pathways.