

BOOK REVIEWS

Methods in Molecular Medicine

Septic Shock – Methods and Protocols

Edited by T. J. EVANS. 1999. ISBN 0-896-03730-4 (hard-back). Humana Press, NJ. Pp. 224. US\$89.50.

Septic shock remains an important cause of morbidity and mortality despite improvements in intensive care medicine and antibiotic therapy. In recent years there has been a significant advance in our understanding of the molecular pathophysiology of this condition, which has led to the development of novel therapeutic approaches. However, the results of clinical trials of most of these therapies have been largely disappointing, emphasising the complex interactions of the mechanisms that lead to septic shock.

Septic shock – methods and protocols provides a useful collection of protocols and techniques for both the researcher and clinician to gain insight into the mechanisms of septic shock. The book is divided into five sections, each presenting a different aspect of septic shock with its own methodology. These include: endotoxin, other (non-endotoxin) bacterial products, cytokines, nitric oxide and cell culture techniques. Each section is complete in itself, divided into chapters which describe pertinent methods and, importantly for a book of protocols, a useful introduction to place the methods into context.

Measurements of endotoxin and endotoxin-binding proteins are important determinants in the evaluation of many anti-endotoxin therapies, although they are frequently ignored when evaluating the outcomes of such trials. The first section of this book is dedicated to endotoxin and provides useful protocols for measuring endotoxin (itself a complex task), preparing purified endotoxin from pathogenic gram-negative bacteria and measuring endotoxin-binding proteins (anti-endotoxin antibodies, bactericidal/permeability-increasing protein and lipopolysaccharide-binding protein). It is increasingly recognised that many products of gram-positive bacteria, such as the exotoxin superantigens, can contribute to the development of septic shock either in their own right or by acting synergically with endotoxin. The second chapter in the book details assay protocols for the purification and assay of streptococcal pyrogenic exotoxin A (SPEA). The assay methods are particularly useful, including the extraction, PCR amplification, cloning and expression of the rSPEA gene to generate antibodies for use in ELISA protocols. The biological activity of the rSPEA is confirmed by a T-cell proliferation assay.

The bioassay theme is developed in chapter three, which describes a bioassay for the key inflammatory cytokines TNF- α and TNF- β and an ELISA protocol for the assay of the soluble TNF receptors. Techniques for the assay of cytokines in whole blood and plasma are included and are a useful addition for the analysis of cytokines in patients with septic shock and many other conditions.

Nitric oxide is recognised as an important contributor to the profound hypotension of septic shock and the inducible nitric oxide synthase (iNOS) is activated by many of the bacterial products and mediators associated with the development of septic shock. Thus, the largest section of the book is devoted to methods concerned with nitric oxide, including the NOS inhibitors, in-situ detection of nitric oxide, immunochemical detection of NOS in human tissue

and two protocols for the detection of nitric oxide products; nitrotyrosine and peroxynitrite. Analysis of the last two products can be particularly challenging and the protocols clearly identify the pitfalls and the appropriate controls to be made.

The final section of the book describes methods for the culture of different primary human cells, which is particularly useful for the evaluation of pathogenic mechanisms at the site of tissue injury *in vivo*. Once the most difficult aspect of primary cell culture is overcome (obtaining the original tissue) the methods detail clearly the way to isolate, grow, manipulate and store the precious primary cells.

Septic shock – methods and protocols attempts to provide a number of protocols relevant to the many different aspects of the study of septic shock. Our knowledge of the pathogenic mechanisms that underly septic shock is rapidly expanding and there are some notable omissions from this book. For example, such a book might be expected to include protocols for the assay of CD14 and sCD14, the detection of Toll-like receptors (TLR4) and the haematological factors such as activated protein C, which hold much promise for the treatment of septic shock. However, as acknowledged by the editor, the book cannot provide an exhaustive account of every protocol that might be used in sepsis research. It provides selected methods that are of intrinsic importance to the study of septic shock, written by experienced investigators in the topics covered. The methods are clear, extensive and complete and will allow the researcher to take them directly to the bench top. Potential problems and ways to overcome them are clearly delineated. *Septic shock – methods and protocols* is an important addition to the series *Methods in molecular medicine* and will be a useful practical companion to the many researchers investigating this enigmatic clinical condition.

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Methods in Molecular Medicine Cytomegalovirus Protocols

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This volume provides detailed laboratory protocols for a range of techniques relevant to human cytomegalovirus research. Molecular, biochemical, immunological and phenotypic approaches are described, in a manner suitable for direct application. As such, it is very much a book for the bench top. The chapter on construction of recombinant viruses, which is increasingly important in order to understand the function of specific genes and/or mutations within the genome, is particularly informative. Unfortunately, a couple of newer techniques are not covered within this book. Firstly, there is no mention of real-time PCR, which provides for much more rapid detection and quantitation of CMV, as well as detection of mutations. Secondly, there is no mention of the use of tetramer technology to quantify CMV CTL responses, which is increasingly used within clinical CMV research. Other than these omissions, the volume is a high quality one, and I recommend it to those working in the field.

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