METHODS IN MOLECULAR MEDICINE™

Septic Shock

Methods and Protocols

Edited by

Thomas J. Evans

Humana Press

Septic Shock Methods And Protocols Methods In Molecular Medicine 36

Yvonne A. Barnett,Christopher R. Barnett

Septic Shock Methods And Protocols Methods In Molecular Medicine 36:

Septic Shock Methods and Protocols Thomas J. Evans,2008-02-01 Septic shock remains a serious medical condition with high mortality Despite many advances in intensive care medicine and antibiotic devel ment this has not changed appreciably in the last 20 years Frustratingly over the same period of time enormous advances have been made in understa ing the underlying pathogenic mechanisms of this condition This has resulted in the development of several novel therapies for septic shock which despite excellent theoretical grounds for their efficacy have failed in altering mort ity attributable to sepsis The reasons for these failures are multiple but it is clear that further research is required aimed at increasing our understanding of the basic pat physiological processes that occur following infection Research into septic shock draws upon a number of different disciplines ranging from molecular and cellular biology to physiological measurements on whole animals Septic Shock Methods and Protocols is an attempt to draw together into one volume a number of protocols that are of use in the investigation of the mechanisms of septic shock I have divided the book into five sections The first deals with endotoxin the lipopolysaccharide component of the Gram negative cell m brane that can mimic many of the features of septic shock Gram positive organisms are found increasingly as causes of septic shock and several pects of toxins produced from these bacteria are considered in the second s tion **Antiviral Methods and Protocols** Derek Kinchington,Raymond F. Schinazi,2008-02-01 This latest addition to the Methods in Molecular Medicine series Anti ral Methods and Protocols is opportune because there is an increasing int est in discovering compounds that are effective against both chronic and acute viral infections A number of the methods described in the volume are unp lished and their inclusion indicates the speed at which this field is moving This volume is not a review but each chapter contains methods validated by the experts who have spent time in developing the protocols The hallmark of this series is the comprehensive way in which the me ods are described which includes a list of all the reagents needed for each protocol Of importance is the section on tips and pitfalls that the authors have discovered while developing their protocols The manual itself is designed to be used by researchers in universities and industry who are familiar with a range of biological techniques but who want to set up quickly a novel assay system We encourage a dialog between readers and authors which may also result in useful collaborations **Antibiotic Resistance Methods and Protocols** Stephen H. Gillespie,2008-02-01 At a time of rising concern about drug resistance and falling output of new antibacterial compounds antibiotic research has once again returned to the forefront of medical science In Antibiotic Resistance Methods and Protocols Stephen Gillespie and a panel of leading clinical and diagnostic microbiologists describe a series of detailed molecular and physical methods designed to study the growing problem of antibiotic resistance as well as facilitate new antibiotic research programs for its effective redress The techniques range widely from those that provide rapid diagnosis via DNA amplification and phage display to those for plotting the transmission of resistant organisms and investigating their epidemiology The methods are readily adaptable to a wide range of resistant

bacterial organisms In order to ensure successful results each method is described in minute detail and includes tips on avoiding pitfalls Practical and wide ranging Antibiotic Resistance Methods and Protocols provides a collection of indispensable techniques not only for illuminating the basic biology of antimicrobial resistance but also for developing and implementing new diagnostic and epidemiological tools **Aging Methods and Protocols** Yvonne A. Barnett,Christopher R. Barnett,2008-02-01 Aging is an almost universal process within biological systems one which leads to a decline in functional capacity disease onset and eventually death There has been much interest in recent years to elucidate the molecular mec nisms that underlie the aging process Many theories have been proposed since the last century that aim to explain the causes of aging There is no one theory that completely satisfies the phenotype of aging but genetics and environm tal factors play an important role in the etiology of age related pathologies and the aging process However there is still much to be learned about the aging process which has been termed one of the last great frontiers in biology De graphic changes worldwide are leading to increased average life expectancies within our populations These changes in population characteristics will impact upon the economies of the supporting society with increasing healthcare and infrastructural costs arising from the prevalence of age related pathologies and other physical disabilities associated with advancing years Many researchers worldwide are working in the attempt to identify key cellular processes through which it might one day be possible to slow down the aging process and thus increase the health span of humans Numerous research projects from the cellular through to tissue organ and whole organism studies are currently underway to investigate the mul factorial aging process **Angiotensin Protocols** Donna H. Wang,2008-02-01 A qualitative leap in the understanding of cardiovascular and n ral regulation by the renin angiotensin system and of the role of this s tem in tissue damage has occurred as a result of the many recent advances in molecular genetic techniques The cloning of the genes for the components of the renin angiotensin system the design of specific angiotensin receptor ligands and the use of embryonic gene targeting te niques for the creation of mutant strains have established that the renin angiotensin system is important in blood pressure regulation ion and fluid homeostasis and tissue growth and remodeling Further investigation of the mechanisms by which this system p ticipates in cardiovascular regulation may shed some light on the pat genesis of several cardiovascular diseases e g hypertension congestive heart failure and chronic renal failure Despite the promise of this system as a target for therapeutic interventions for these diseases there are great challenges in the integration of the attempts to close the gap between the traditional literature of medicine and the explosion of information from the new technologies This book s title Angiotensin Protocols reflects the authors strong efforts to translate expert knowledge into easy to follow practice The book opens with introductory chapters and each specialty section provides detailed methods covering a wide variety of techniques ranging from genetic manipulation of targeted genes to functional studies of the renin angiotensin system **Mycobacterium Tuberculosis Protocols** Tanya Parish,Neil G. Stoker,2008-02-01 The aim of this book is to provide detailed protocols for

studying the molecular biology of the pathogen Mycobacterium tuberculosis and its int actions with host cells As established mycobacterial laboratories move wards exploiting the genome and laboratories with expertise in other fields apply them to mycobacteria both traditional and novel methodologies need to be reviewed Thus the chapters in Mycobacterium tuberculosis Protocols range from perspectives on storage of strains and safety issues to the application of the latest functional genomics technologies The last few years have been remarkable ones for research into M tuber losis The most important landmark by far has been the completion of the genome sequence of the widely studied H37Rv strain 1 We can now predict every protein and RNA molecule made by the pathogen This information is or will soon be enriched by the addition of genome sequences of other strains from the M tuberculosis complex a second strain of M tuberculosis My bacterium bovis and the vaccine strain M bovis BCG Valuable comparative data will also be provided by the genome sequences of Mycobacterium leprae Mycobacterium avium and Streptomyces coelicolor Another recent milestone for M tuberculosis has been the development of efficient mutagenesis me odologies the lack of which has been a major handicap in functional studies **Molecular Pathology Protocols** Anthony A. Killeen,2008-02-02 The era of molecular pathology has arrived From its promising beg nings in research laboratories the field has grown and continues to grow to become a vital part of the care of an ever increasing number of patients Because of its recent emergence from the research taboratory many molecular pathology protocols we still to be found in the primary litcramre and have not appeared in a text MO PCU Q Padhoiogy Protocob contains la ratory protocoIs that have been developed by many of the authors for use in clinical molecular pathology laboratories and describe in detail Row to perform these assays This book is therefore intended for clinical laboratory use by medical technologists and pathologists It will dso be of interest to research workers who are performing these assays In its broadest meaning pathology is the study of disease and therefore it follows that any disease for which the molecular basis is understood would be suitable as a topic for inclusion in this work When seiecting protocols it was necessary to place limits on the number of chapters that could be feasibly presented in a single work Those protoculs that were selected are performed more frequently or have achieved recognition as having important diagnostic utility in contemporary practice A decision was made to exclude inherited genetic diseases with certain exceptions such as those diseases that are associated with thrombotic states and are part of the traditional dumain of pathology *Gene Therapy Protocols* Jeffrey R. Morgan,2007-10-26 Efforts in gene therapy have grown dramatically in recent years Basic research as well as clinical activity have made exciting progress and are beg ning to offer renewed hope that gene therapy may be able to deliver novel approaches for the treatment of inherited as well as such acquired diseases as cardiovascular disease and cancer With the sequencing of the human genome complete we now have a comprehensive catalog of genes that further expands the potential role of gene therapy into such new fields as tissue engineering Central to gene therapy is the process of gene transfer thus advances in the technology of gene transfer are at the heart of this field s progress Numerous

technologies based on a variety of methods e g viral mediated physical chemical have been developed to achieve gene transfer Some of the earliest methods such as recombinant retroviruses are still widely used have undergone significant improvements and have given rise to new vectors based on lentiviruses *DNA Vaccines* Douglas B. Lowrie,Robert Whalen,2008-02-01 The field of DNA vaccines has undergone explosive growth in the last few years As usual some historical precursors of this approach can be d cerned in the scientific literature of the last decades However the present state of affairs appears to date from observations made discreetly in 1988 by Wolff Malone Felgner and colleagues which were described in a 1989 patent and published in 1990 Quite surprisingly they showed that genes carried by pure plasmid DNA and injected in a saline solution hence the epithet naked DNA could be taken up and expressed by skeletal muscle cells with a low but reproducible frequency Such a simple methodology was sure to spawn many applications In a separate and important line of experimentation Tang De Vit and Johnston announced in 1992 that it was indeed possible to obtain humoral immune responses against proteins encoded by DNA delivered to the skin by a biolistic device which has colloquially become known as the gene gun The year 1993 saw the publication of further improvements in the me ods of naked DNA delivery and above all the first demonstrations by several groups of the induction of humoral and cytotoxic immune responses to viral antigens expressed from injected plasmid DNA In some cases protection against challenge with the pathogen was obtained The latter result was questionably the touchstone of a method of vaccination worthy of the name Metastasis Research Protocols Susan A. Brooks,Udo Schumacher,2008-02-02 The process of metastasis formation is hugely complex as described in the introductory chapter of this book and this complexity has led us to compile two volumes of methods from a vastly divergent background that attempts to encompass the whole spectrum of cancer biology This first volume Metastasis Research Protocols Analysis of Cells and Tissues concentrates on analysis and mapping of molecules produced by cells and tissues and analysis of the molecular biology underlying their expression whereas the second volume Metastasis Research Protocols Cell Behavior In Vitro and In Vivo focuses sharply on the determination of cell behavior in vitro and in vivo We have deliberately included chapters describing well established and familiar te niques for example SDS PAGE and Western blotting Chapter 11 and immunocytochemistry Chapter 2 in addition to the newer and more speci ized approaches and specific examples of their application because although the methodology is readily available in the published literature and established in many laboratories we wished these volumes to stand alone and to make accessible here the standard techniques that underpin much metastasis research for both the newcomer to the field and the seasoned researcher Undoubtedly owing to the complexity of the metastatic cascade and the wealth of research techniques involved in scientific approaches to its unraveling and despite our best efforts to make these volumes as comprehensive as seems feasible this is a tall order and there will inevitably be omissions For these we apologize *Angiogenesis Protocols* J. Clifford Murray,2008-02-02 In the last few years we have been deluged with information on ang genesis Scientists and the public at

large are exposed daily to this new science not just in specialist journals and texts but in the tabloid press where popular articles refer to angiogenic therapies as magic bullets and miracle cures for cancer arthritis retinopathies heart disease and circulatory problems Is there no ill this approach will not cure The fact that so much time effort and resource have been and continue to be dedicated to this new science is clear testament to its importance Yet many fundamental aspects of angiogenesis remain poorly und stood in particular cues that activate the process This fact has to some extent been masked behind a surfeit of fine detail we can t see the wood for the trees Most studies of angiogenesis identify single links in a long chain of events Furthermore each study is itself hampered by the limitations of the biological end point chosen For instance though endothelial proliferation may well be necessary for angiogenesis it is not sufficient Therefore measuring endothelial proliferation in response to a novel growth factor and on the basis of this obs vation stating that the factor is angiogenic is unsound logic It is important that researchers in this field and perhaps more importantly those experimenting at its periphery recognize the limitations of their chosen biological end points **Rotaviruses** James Gray,Ulrich Desselberger,2008-02-01 James Gray and Ulrich Desselberger have assembled a comprehensive collection of established and cutting edge methods for studying and illuminating the structure molecular biology pathogenesis epidemiology and prevention in animal models of infection with rotaviruses an important cause of infant morbidity and mortality Presented by experts in the fields of animal and human rotavirus infections and rotavirus vaccine research these readily reproducible methods detail molecular and other modern techniques and include relevant background information and various notes to ensure reproducible and robust results Authoritative and up to date Rotaviruses Methods and Protocols offers researchers today s benchmark compendium of experimental methods for the investigation of this medically significant virus **Vaccine Adjuvants** Derek T. O'Hagan,2008-02-02 Derek T O Hagan and a team of expert vaccinologists and pharmacologists thoroughly describe the preparation characterization and evaluation of a wide range of alternative vaccine adjuvants for use in preclinical studies Each chapter carefully reviews a single adjuvant and suggests why a specific adjuvant might be preferred for a given antigen depending on what type of immune response is desired Alternate adjuvant choices are also presented so that researchers can choose those most efficacious for their specific purpose Comprehensive and highly practical Vaccine Adjuvants Preparation Methods and Research Protocols provides an effective guide to making and using vaccine adjuvants By closely following directions from the book today s researchers will be able optimally to induce specific immune responses against different types of antigens and to selectively manipulate the immune response in a favorable way **Molecular Pathology of the Prions** Harry F. Baker,2008-02-02 Internationally recognized investigators review the latest developments in and novel approaches to understanding the prion protein and prion diseases at the molecular level Utilizing a variety of cutting edge techniques these distinguished scientists seek to define the normal function of a prion protein to detect and measure the early immune response to prion disease and to discover possible therapeutic targets They also use transgenic mice and new

electrophysiological investigations to elucidate the pathogenetic mechanisms involved in prion diseases State of the art and richly insightful Molecular Pathology of the Prions captures for basic and clinical neuropathologists the latest developments and approaches to understanding the pathogenesis of prion diseases and by analogy suggests possible research techniques for the more common proteinopthies such as Alzheimer s and Parkinson s diseases *Alzheimer's Disease* Nigel M. Hooper,2008-02-02 Alzheimer s disease is the most common cause of senile dementia Since the discovery in 1984 of the amyloid peptide A as the core protein of the senile plaques present in the brains of Alzheimer s disease sufferers an immense amount of research has gone into mapping out the molecular basis of this debilitating disease The aim of Alzheimer s Disease Methods and Protocols is to bring together the main biochemical cell biological and molecular biological techniques and approaches that are being used to investigate the molecular basis of Alzheimer s disease This volume begins with chapters of an introductory review nature Chapter 1 provides a historical introduction to Alzheimer s d ease with particular emphasis on the central role played by A and its re tion to tau Chapter 2 examines the genetics underlying this neurodegenerative disease covering the amyloid precursor protein apolipoprotein E and the presenilins Chapter 3 presents an overview of currently available therapeutic agents and prospects for drugs of the future Vision Research Protocols P. Elizabeth Rakoczy,2008-02-02 Elizabeth Rakcozy and a team of leading clinical and experimental scientists describe in step by step detail the key techniques essential to effective molecular biological research in ophthalmology and optometry These readily reproducible methods are adapted to the special requirements of vision research with coverage that ranges from the most basic to the most sophisticated technologies Included are methods for the down regulation of gene expression new gene therapy techniques and for the development of transgenic and knockout animal models for testing novel therapies Eminently accessible and clinically relevant Vision Research Protocols provides experimental and biomedical investigators in ophthalmology and optometry with a rich panoply of most powerful tools with which to ask and answer all the important questions emerging from the dramatically advancing work in vision research today Interleukin Protocols Luke A. J. O'Neill,Andrew Bowie,2008-02-02 Interleukins are a family of proteins that regulate the maturation diff entiation or activation of cells involved in immunity and inflammation and belong to a broader family termed cytokines Collectively these proteins are the key orchestrators of host defense and the response to tissue injury There are currently 23 different interleukins numbered from IL 1 to IL 23 although the full extent of the interleukin family will only become clear upon analysis of the human genome sequence Most important interleukins are central to the pathogenesis of a wide range of diseases that involve an immune com nent including such conditions as rheumatoid arthritis multiple sclerosis ulcerative colitis psoriasis and asthma Interleukins have also been imp cated in other conditions including cancer migraine myocardial infarction and depression In essence when cells are activated by interleukins a program of gene expression is initiated in the target cell that alters the cell s phenotype leading to enhanced immune reactivity inflammation and or proliferation

Interleukins are therefore at the core of the cellular basis for many diseases They are the subject of intense investigation by biomedical researchers and the targeting or use of interleukins in the clinic is proceeding apace Approaches such as t geting IL 4 in asthma or IL 1 in joint disease are being pursued and it is likely that in the next 5 10 years a number of new therapies based on either inhib ing or administering interleukins will be available Gene Therapy of Cancer Wolfgang Walther,Ulrike Stein,2008-02-01 Since the discovery of the molecular structure of genes and the unveiling of the molecular basis of numerous human diseases scientists have been fas nated with the possibility of treating certain diseases by transducing foreign DNA into the affected cells Initially it was proposed that the foreign DNA could either replace defective nonfunctional genes or code for therapeutic proteins This concept has evolved into the rapidly growing field of gene therapy Even though surgery radiotherapy and chemotherapy are widely ava able and routinely used for cancer treatment these therapies fail to cure approximately 50 percent of cancer patients Therefore since it is a disease characterized by aberrant gene expression cancer has been a target of gene therapy research since the inception of this treatment modality Numerous cancer gene therapy strategies are currently being investigated including gene replacement therapy the regulation of gene expression to modulate immu logical responses to tumors the direct killing of tumor cells and direct int ference with tumor growth In this context gene transfer systems tumor specific expression vectors and novel therapeutic genes have been extensively st ied All these strategies aim for the selective destruction of human malignant disease while circumventing the destruction of nonmalignant cells and tissues thereby minimizing toxicity to the patient *Vascular Disease* Andrew H. Baker,2008-02-01 Molecular biology has revolutionized research into vascular disease Over the past 20 years molecular techniques have enabled us to both elucidate lecular mechanisms in vascular disease and identify appropriate therapies The vast explosion in technical knowledge and the array of protocols that become more advanced and intricate by the day lead us into new and exciting areas of research that were previously unobtainable Vascular Disease Molecular Biology and Gene Transfer Protocols scribes today s most powerful molecular methods for the investigation of the pathogenesis of vascular disease The protocols are highly detailed allowing beginners who have little experience in either vascular biology or molecular biology to embark on new molecular projects This book is also suited to more experienced molecular biologists who wish to grasp new methods for stu ing the involvement of genes in normal vascular physiology and in diseased states It is well established that cardiovascular disease progression has a s stantial genetic influence Part I describes three methods that have been used successfully to identify specific mutations in candidate genes involved in c diovascular disorders These mutations include both single stranded conf mational polymorphism analysis and heteroduplex detection methods In addition technology to map new genes to specific regions of chromosomes by high resolution mapping is described Hepatocellular Carcinoma Nagy A. Habib,2008-02-01 Advances in molecular characterization and novel gene isolation techniques have vigorously expanded our understanding of hepatocellular carcinoma HCC a form of liver cancer that affects one million people annually and

generated many new therapeutic possibilities In Hepatocellular Carcinoma Methods and Protocols Nagy Habib and a team of basic and clinical researchers describe the wide variety of powerful new laboratory based molecular methods currently being used for investigating and treating this disease The book focuses on gene therapy approaches including the use of such vectors as lipids adenovirus and baculovirus and virus detection assessment using electron microscopy It also provides preclinical and clinical data on the killing of cancer cells using tumor suppressor genes antisense compounds to growth factors immunotherapy remove gene and virus directed enzyme prodrug therapy A perspective on future treatment of the failing liver is given along with a clinical protocol for p53 gene therapy Hepatocellular Carcinoma Methods and Protocols offers experimental and clinical investigators a rich source of both basic science and clinical information on today s optimal use of gene therapy to treat and manage patients suffering from hepatocellular carcinoma

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Septic Shock Methods And Protocols Methods In Molecular Medicine 36 Introduction

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