
Preface

Neisseria meningitidis (the meningococcus, family *Neisseriaceae*) is a major causative agent, worldwide, of potentially life-threatening meningitis and septicemia. The organism is a Gram-negative β proteobacterium that resides normally as a commensal on the mucosal epithelium of the human nasopharynx. However, a combination of the expression of key microbial virulence factors, host susceptibility, inadequate innate immune recognition, and changes in niche environmental conditions can favor bacterial invasion of the host.

The pathogenesis of meningococcal infection involves initial penetration of the nasopharyngeal mucosal epithelium, entry into the blood, and the development of a bacteremia, which occurs in the absence of host humoral immunity. The meningococcus is a classical meningeal pathogen, i.e., it is capable of passing across the blood-cerebrospinal fluid (CSF) barrier to enter the CSF-filled subarachnoid space (SAS) to colonize the leptomeninges [1–3]. The consequences to the host are a compartmentalized intravascular inflammatory response, septicemia, and/or a compartmentalized intracranial inflammatory response, leptomeningitis [4]. Meningococcal infection can be sporadic, hyper-sporadic, or epidemic in nature, and global estimates for infection are ~1.2 million cases each year with a toll of ~135,000 deaths [5]. Despite successful antibiotic treatment and advances in intensive care management of patients with meningococcal infection, the mortality rate is still high at ~15% in industrialized countries, and the disease is rightly feared by the public, parents, and physicians for the rapidity of onset of both clinical symptoms and the decline of patients' health. Moreover, survivors are often faced with permanent physical and neurological sequelae, including loss of limbs; auditory and visual impairment; cognitive dysfunction; educational, behavioral, and developmental problems; seizures and persistent headaches; and motor nerve deficits, hydrocephalus, and permanent brain damage [6]. These outcomes impact the quality of life of the affected individuals and their families and on the providers of health, welfare, and social services.

Since Weichselbaum first identified the meningococcus from the CSF of a patient with meningitis in 1887 [7], key discoveries about the nature of the organism, the pathogenesis of infection, and the mechanisms of natural immunity have led, finally, to the development and introduction of safe and effective vaccines. The classical studies by Goldschneider and colleagues in the late 1960s identified the correlation between the development of serum bactericidal antibodies and protection from meningococcal infection [8, 9]. Combined with our increased understanding of meningococcal surface antigen structure and function, capsule polysaccharide-protein conjugate vaccines against meningococci that express the serogroup A, C, Y, and W-135 capsules have been developed and introduced into adolescent and adult immunization schedules [10, 11] and trialed in young children [12]. Conjugate vaccines have already significantly reduced meningococcal disease in industrialized countries, e.g., for serogroup C infection [13], and the long-term expectation is that the introduction of new conjugate vaccines against serogroup A will lead to a decline in epidemic disease reported in the "African Meningitis Belt" [14]. Today, a concerted effort is aimed at developing vaccines to serogroup B meningococci, which present a greater technical challenge, due to the poor immunogenicity of its capsule and the molecular mimicry of foetal NCAM, thereby necessitating a search for subcapsular antigens capable of inducing protective

immune responses. The past decade has seen the publication of many *Neisseria* spp. genomes [15–22], and these have under-pinned advances in molecular methods and techniques with applications to vaccine design. The result is new vaccines for serogroup B that are showing promise in clinical trials [23–25] and a plethora of experimental vaccines that have shown success in preclinical, laboratory studies.

The key discoveries about the meningococcus would not have been possible without significant developments in laboratory methods for studying the pathogen at the molecular and cellular levels. Many of these laboratory methods have been described in the landmark books on meningococcal vaccines and meningococcal disease, edited by Andrew Pollard and Martin Maiden for the series *Methods in Molecular Medicine* [26, 27]. This new book, *Neisseria meningitidis: Advanced Methods and Protocols*, does not simply revisit and update the methods and protocols described in the books from Pollard and Maiden, a task of itself unnecessary due to the comprehensive nature of these previous volumes, but it offers a collection of advanced methods and protocols that in many ways reflect the development and refinement of several new technologies applied to the meningococcus. Several of the chapters in this book describe methods that rely on the collection of complete sets of biological data, for example, using the genome to generate transcriptomes, proteomes, and metabolomes. However, there are many new *-omics* that are being developed both theoretically and practically (e.g., the interactome, molculome, cytome, and regulome, to name but a few), and these are now beginning to be applied to the study of *Neisseria* and many other human pathogens. Laboratory methods and protocols for these new *-omics* could be the subjects of future volumes.

Neisseria meningitidis: Advanced Methods and Protocols begins with a review of the biology, microbiology, and epidemiology of the meningococcus, which is followed by two chapters that provide a clinical context, namely, in the classification and pathogenesis of meningococcal infections and a technique for detecting the pathogen in CSF samples from patients. In cases of undefined meningeal irritation, the latter method provides a means for identifying between the important bacterial and viral causes of meningitis.

A number of chapters then follow that provide methods and protocols for investigating the molecular biology and biochemistry of the meningococcus. These techniques can provide useful tools for vaccine and pathogen–host interaction studies and include methods for generating knock-out and complementation strains of the meningococcus, for identifying and characterizing small RNA molecules and for the expression of purified meningococcal proteins for crystallization. A particular area of *Neisseria* research that, if not exactly neglected, is not always appreciated relates to the metabolism of the meningococcus. Hence, a chapter is provided that explains how genome-scale metabolic networks can be constructed using a constraint-based modeling approach, using available genome sequence databases and high-throughput bioinformatics. This is followed by a complementary method for studying adaptations in meningococcal/microbial proteomes to changing environmental conditions.

The next collection of chapters broadly covers pathogen–host cell interactions and is prefaced with an introductory review of our current knowledge of meningococcal surface ligands and their respective host cell receptors. A protocol for studying meningococcal interactions with an animal model is presented, followed by methods used for culturing and investigating biofilms *in vitro*. Interactions of bacteria with host cells are subject to environmental stress and physical forces applied to bacterial ligand–host cell receptor binding events, and methods are provided for investigating bacterial adhesion under shear stress and the forces exerted by the meningococcal pilus adhesin. The literature is replete with *in vitro* cell culture models used for studying bacterial interactions, from human explant models to

monocultures of primary cells and transformed cells of myeloid and nonmyeloid origins: for this book, protocols are described for isolating human dendritic cells and using them to study host–*Neisseria* interactions, acknowledging the important role that these cells have in sentinel immune recognition during *Neisseria* infection and in driving polarization of naïve T-cell helper responses. Finally, the events that follow bacterial interaction with host cell receptors are considered in two chapters that present methods for investigating ligand–receptor interactions, by using hydrogen/deuterium exchange coupled to mass spectrometry and nanoscale imaging techniques to visualize the interactions between pathogen-associated molecular patterns (PAMP) and host pattern recognition receptors (PRR).

The next chapters consider the consequences of meningococcal interaction with host cells; in-depth protocols are provided for analyzing the transcriptome of the pathogen and host epithelial and endothelial cell models, followed by a detailed technical review on the experimental design that allows the researcher to generate valuable and reliable data from using the pan-*Neisseria* microarray. A major consequence of meningococcal infection is host cell damage, clearly seen in patients with sepsis and meningitis, and in vitro methods are provided for investigating host cellular apoptosis/necrosis induced by the pathogenic *Neisseriae*.

The final part of this book focuses on methods and protocols for vaccine antigen discovery and vaccine design. Methods are provided for two different approaches, one using proteomics to analyze the human immune response to *Neisseria meningitidis* and the other, “reverse vaccinology.” In particular, the latter chapter provides detailed methods for in silico identification and selection of antigens, through production of recombinant proteins for immunization and analyses of the immune response. The final chapter provides a protocol for preparing experimental DNA vaccines to bacterial polypeptides.

Many of the techniques described herein can be readily used to study other pathogens and diseases and should have broad appeal to clinical and nonclinical scientists alike. I do accept that some of these methods can seem daunting or require specialized equipment, but I do hope that they stimulate collaboration between readers and authors. This book could not have been possible without the contributions of many, and I would like to express my gratitude toward all authors, all of whom enthusiastically contributed their articles and showed patience with my editing; to the staff at Humana Press for commissioning this volume and especially to the series editor, John Walker, who has provided support and advice when needed. Finally, although this past decade has seen tremendous advances in the fight against meningococcal infection, there is still much to learn about the meningococcus and not only does it continue to surprise us with its complex nature, but also its relationship with its host reveals a great deal about human biology.

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