METHODS IN MOLECULAR BIOLOGY

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Innate Immune Activation

Methods and Protocols

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Dedication

Dedicated to our daughter, Francesca Mariam Hope De Nardo (19 months)—the strongest, funniest, and most inspiring little girl we know. You will never read this book, or care about it, but you will enjoy looking at the Owl below—hoot hoot! You are our sunshine and life, "Frankie the fighter."

You are ours and we are yours.

Love mum and dad xxx

Preface

The major function of the innate immune system is to induce a rapid and controlled acute inflammatory response to "danger signals" aimed at eliminating invading microorganisms and/or restoring tissue homeostasis. "Danger signals" come in many forms including highly conserved components of microbes, termed pathogen-associated molecular patterns (PAMPs); host-derived molecules that accumulate or become modified following tissue injury, metabolic dysfunction, and uncontrolled cell death to mediate sterile inflammatory responses, which constitute danger-associated molecular patterns (DAMPs); as well as certain environmental irritants. Families of highly conserved pattern recognition receptors (PRRs) expressed primarily by specialized immune cells, such as macrophages and dendritic cells, have evolved to recognize these danger signals to activate an innate immune response. The innate immune receptors can be broadly classified according to their localization either on plasma/endolysosomal membranes or within the cytosolic compartment. Toll-like receptors (TLRs) and C-type lectin receptors (CLRs) represent the membrane bound receptors, while retinoic-acid-inducible gene I (RIG-I)-like receptors (RLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), and several DNA receptors (e.g., AIM2, cGAS) are expressed in the cytosol. Activation of innate immune receptors on tissue resident cells initiates specific signal transduction pathways leading to transcription and secretion of inflammatory mediators such as cytokines, chemokines, and interferons that mobilize recruitment of additional immune cells and facilitate the inflammatory process. Certain PRRs are able to form large oligomeric protein structures, termed inflammasomes, that instigate proteolytic maturation of members of the IL-1 family of cytokines and drive pyroptosis, a form of programmed cell death. Innate immune activation underpins both pathological and physiological inflammatory responses and is thus critical for the host.

Although innate immune activation was initially observed by Elie Metchnikoff in the early 1900s, the field only gained impetus some 100 years later following the discovery of a critical role for the Toll protein in *Drosophila melanogaster* immunity. Subsequently, a Toll homologue (TLR4) was uncovered in mammals and the field of innate immunity exploded in active pursuit of other TLRs, additional families of PRRs, as well as adaptor and effector proteins. From its humble beginnings, innate immunity has become a highly vibrant and prominent field and innate immune activation is recognized as the underlying mechanism driving acute inflammatory responses in a wide variety of biological processes.

Given the importance of the innate immune system, studies into its activation remain a major focus of research, and along with rapid technological developments in molecular, biochemical, and computational approaches, the field continues to reveal new and exciting discoveries. We have the unique opportunity to work with experts in the field to present some gold standard approaches used to study innate immune activation as well as some of the more recent technical advances in one volume, which we hope will help broaden the field by allowing others to more successfully perform these assays. We begin with an overview chapter discussing the emerging concepts in the field, which also acts to elegantly place the method chapters into context. The chapters presented include a variety of methodologies ranging from general approaches, such as the assessment of macrophage activation (bioinformatics); measuring innate immune responses to bacterial viability; quantification of secreted proteins (proteomics); generation of model (differentiation of BLaER1

cells, CRISPR/Cas9 knock out in myeloid cells) and reporter systems (retroviral transduction); to protocols examining specific innate immune activation by TLRs (Myddosome formation by immunoprecipitation and LUMIER, NFκB activation), RLRs (confocal imaging-based quantification of MAVs), cGAS (detecting activation by cytosolic dsDNA using confocal imaging), and inflammasomes (activation and cleavage of Gasdermin-D, ASC speck formation, cell swelling).

We would like to thank all the authors for their excellent contributions and enthusiasm throughout this project. Our hope is that these protocols and the personal tips and notes shared by the authors will form an invaluable tool for labs interested in studies of the innate immune system. This project would not have been possible without excellent advice and support from Dr. John Walker and David Casey. Finally, we would like to thank Thijs van Vlijmen for the initial invitation to contribute a new volume to Springer.

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