Preface

Cells of the immune system, such as macrophages and dendritic cells, continuously survey host tissue integrity. Immune cells can quickly respond to invading pathogens or tissue damage by mounting an immune response geared to the reconstitution of tissue homeostasis. Immune cells have the ability to distinguish foreign molecules from self-molecules, detect altered self-molecules, or respond to metabolic derangements via the expression of families of signaling receptors that can recognize such danger signals. Most of these immune signaling receptor families, including Toll-like receptors, Rig-I like receptors or C-type lectin receptors, as well as a number of Nod-like receptors (NLRs), induce signaling cascades that eventually culminate in a transcriptional response of the cell and the production of inflammatory mediators. The activated factors act in concert with many cell types that help in the defense against the invading pathogen or lead to the repair of damaged tissues.

Inflammasomes, which are the main topic of this volume, have a seemingly simple architecture. A sensor molecule of the NLR or PYHIN protein family recruits the common adapter molecule apoptosis-associated speck-like protein containing a CARD (ASC), which then leads to the approximation of pro-caspase-1 molecules inducing their autoproteolytic activation. Intriguingly, following activation, ASC forms a large protein aggregate, termed "ASC speck," which is thought to provide a platform for the activation of caspase-1. Active caspase-1, in turn, cleaves the inactive precursor molecules of the IL-β cytokine family (IL-1β and IL-18) into their biologically active forms. In addition, inflammasomes control the release of mature IL-1β cytokine family members, demonstrating that inflammasomes are key gatekeepers for these highly pro-inflammatory cytokines. Indeed, uncontrolled production and activation of IL-1β cytokines can lead to significant inflammatory reactions that contribute to a range of diseases. For example, the NLRP3 inflammasome, which recognizes a range of microbes as well as many sterile danger signals, can contribute to common inflammatory pathologies, including gout, atherosclerosis, type 2 diabetes, and Alzheimer's disease.

While we have learned a great deal about the mechanisms leading to the production of IL-1β family cytokines, more precise details of how inflammasomes are activated, remain to be elucidated. Elaborate mechanisms have evolved that control the activity of inflammasomes and we are just beginning to understand the upstream mechanisms that lead to the formation of inflammasomes by the many reported triggers. Future work in this area will reveal novel targets for pharmacological interference and could lead to more specific anti-inflammatory interventions that are urgently needed.

Protocols used in the study of inflammasomes can be difficult and intricate to master in the beginning and thus, detailed protocols with tips from the experts can be of great value. A number of expert labs have developed specific techniques to study many aspects of inflammasome function in human or mouse immune cells. Furthermore, some benchmark protocols, such as the analysis of caspase-1 cleavage by immunoblotting, have evolved individually in different labs around the world. We have the unique opportunity to present these protocols in one volume, which will help broaden the inflammasome field by allowing others to more easily and hopefully more successfully perform these assays.

Protocols are provided that detail the generation of inflammasome stimuli, such as the NLRP3-stimulating amyloid beta and islet amyloid amylin peptides. Other protocols describe how perturbance of cellular homeostasis by crystalline materials, infectious agents, or certain adjuvants can be used to activate inflammasomes. Inflammasome activation can be studied at several levels, hence, detailed protocols including the assessment of ASC speck formation, the monitoring of caspase-1 activity and processing, and the activation of IL-1β cytokines are presented. In addition, protocols for the analysis of inflammasome assembly and ATP binding or ATPase activity of inflammasomes are described. Another hallmark of inflammasome activation is the induction of a specialized form of cell death called "pyroptosis." Methods to quantify this process as well as the assessment of consequences of inflammasome activation for the cell and host are also detailed in this volume. These chapters will be a useful resource for investigators who seek a better understanding of inflammasome activation pathways and would like to master techniques optimized by experts in the field. We hope this collection will form the definite lab protocol source for inflammasome research.

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