
Preface

Natural killer (NK) cells are the large granular lymphocytes of innate immune system. NK cells play a central role in defense against viral/microbial infections and immune surveillance against tumors. The phenomenon of “natural” or “spontaneous” killing was first observed against tumor cell lines in the early 1970s, and the lymphocytes responsible for this “natural” killing were later characterized in 1975 and dubbed Natural Killer cells. However, little was known about the phenotype of these cells and how they function, and these lymphocytes were often referred to as “null cells.”

Our understanding of NK cells has come a long way from their simple portrayal as “null cells”; NK cells are now regarded as a complex subset of lymphocytes that function along the boundaries of innate and adaptive immunity. With the identification of a plethora of activating and inhibitory receptors that are expressed on NK cells, we have gained significant understanding of the orchestration of signaling balance that drives the “natural” killing by NK cells. In addition to direct lysis, NK cells regulate immune responses by other lymphocytes through the secretion of cytokines. The complexity of NK cell behavior is epitomized by the discovery of antigen-specific memory response, a feature normally associated with adaptive immunity. Additionally the diversity and adaptability of NK cells is exemplified by the delineation of (tens of) thousands of phenotypically distinct NK cell subsets in the peripheral blood within an individual that emerge through “adaptable expression” of activating receptors in response to prevailing stimulatory environment. NK cells have therefore emerged as one of the most appealing candidates for adoptive immunotherapy for cancer, and there is a significant momentum to take NK cells from “Bench to Bedside.”

Natural Killer Cells: Methods and Protocols provides a collection of research methodologies relevant for both basic and translational research. In this methods book, a compilation of techniques is presented pertaining to new developments in NK cell field such as understanding the influence of NK cell metabolism on its function, identifying complexity of NK cell subsets through mass cytometry, and determining the emergence of memory NK cells in murine model of MCMV infection. This book also includes methods to study - NK cell migration and cytotoxicity through endpoint analysis or live single cell imaging, assessment of calcium mobilization, the role of lipid raft in NK cell signaling and NK cell immunological synapse with target cells or cell-free lipid bilayers using high-resolution microscopy; in order to further our understanding of the intricacies of inhibition and activation, receptor and lytic granule polarization to synapse as well as to study the signaling of individual receptors or class of receptors in isolation. Additionally, detailed protocols are described for genotyping single nucleotide polymorphisms and copy number of Fc receptors expressed on NK cells as well as determining the effect of replication on telomere length. The methods pertaining to translational application of NK cells include ex vivo expansion of NK cells on K562 cell lines genetically modified to express either membrane bound IL-15 or membrane bound IL-21, large-scale NK cell culture, current techniques for engineering NK cells to express chimeric antigen receptors or chemokine receptors

using retroviral vectors, electroporation of mRNA, as well as through the natural phenomenon of trogocytosis. Also included are chapters detailing protocols for siRNA knockdown of gene expression in NK cells and differentiation of gene modified NK cells from hematopoietic stem cells.

Additional chapters include protocols for preclinical evaluation of NK cell adoptive immunotherapies in murine models and methodologies to track adoptively infused NK cells in vivo using fluorescence and MRI imaging. Also included are overview chapters on; bi- and tri-specific killer cell engagers that are emerging as appealing therapeutic molecules for NK cell adoptive immunotherapy, and the regulations and logistics that govern the clinical translation of NK cell adoptive immunotherapies.

Though our understanding of NK cells has dramatically evolved over the past decades, the lytic function against tumors that led to the identification of these lymphocytes remains to be fully exploited for cancer therapy. With the emergence of robust platforms to expand NK cells, and methods to improve tumor recognition and homing, the field is well positioned for propelling our enhanced understanding of these cells towards novel and effective adoptive immunotherapy applications.

I would like to take this opportunity to express my gratitude to all the authors for their time and for sharing their expertise through the contribution of detailed protocols and notes for this book on *Natural Killer Cells: Methods and Protocols* in *Methods in Molecular Biology* series. I hope that the protocols provided here would be valuable resources to researchers not only to understand mechanisms that govern NK cell behavior and diversity but also to systematically evaluate NK cells for adoptive immunotherapy applications.

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