Preface

Cytokines are pleiotropic regulatory proteins involved in essentially all biological processes and associated with a wide range of diseases, including immune and inflammatory disorders, as well as many types of cancer and leukemia. Examples of cytokines include interleukins, chemokines, growth factors, interferons, and the tumor necrosis factor family. Information about the qualitative and quantitative nature of cytokine expression and release is essential for the understanding of physiological and pathological processes. However, the cytokine detection in biological and clinical samples faces many challenges that include the low abundance, the need to distinguish between active and latent cytokine forms, and the need to measure multiple cytokines in a single assay. This volume provides a comprehensive collection of classic and cutting-edge methodologies that are used to analyze and quantify cytokines and their biological activities in complex biological and clinical samples.

The chapters are divided into three main categories. The *first category* focuses on the *immunodetection of released cytokines* in tissue culture supernatants, plasma, serum, and whole blood samples by immunoassays. These immunoassays measure the total concentrations of released cytokines regardless of their biological activities, and include ELISPOT, immunoblotting, and ELISA. In addition, the complexity of cytokine responses has led to the development of multiplex technologies that can simultaneously detect large numbers of cytokines in small sample volumes. Thus, the first part also includes the recently developed multiplex arrays that allow the simultaneous measurement of multiple cytokines in small sample volumes with time-saving advantages and less cost compared to other immunoassays.

Since immunoassays cannot distinguish between biologically active and inactive cytokines, *bioassays* are used to measure different biological activities of cytokines, such as cytokine-induced cytokine release, chemotaxis, phagocytosis, proteasome activity, and immunoglobulin class switching. The *second part* focuses on the analysis of biologically active cytokines by bioassays using neutralizing antibodies, chemotaxis assay, cytokine-induced phagocytosis assay, proteasome activity assay, and analysis of cytokine-induced immunoglobulin class switching. In addition, since cytokines exert their functions through binding to cytokine receptors, this category also includes methods focused on the analysis of expression and function of cytokine receptors.

Often, there is a need to identify the cytokine-producing cells, or to analyze the intracellular cytokine protein levels or the mechanisms regulating cytokine expression. The *third part* focuses on the *analysis of intracellular cytokines* by flow cytometry, immunohistochemistry, immunofluorescence confocal microscopy, and western blotting. In addition, this category includes protocols for quantitative *analysis of cytokine gene expression* by real-time RT-PCR, luciferase assay, analysis of the cytokine promoter occupancy by chromatin immunoprecipitation, and analysis of alternative splicing of cytokine genes.

There is no one best method, since each has its own merits and limitations. Choosing the right method depends on the sample, purpose of the assay, and the available instrumentation. Often, using combined information from several different approaches can yield the most accurate information about the quantitative and qualitative nature of cytokine expression. The reliability of all the protocols has been tested in laboratories around the world.

Each chapter is appended by notes that navigate through the protocol and serve as a troubleshooting guide. By covering a broad variety of methods used in cytokine research and analysis, we hope that this book will be useful not only to biochemists, molecular biologists, and immunologists, but also to physician-scientists working in the field of cytokine research.

I would like to thank all the authors for their enthusiastic help and support in assembling this volume; I fully realize that in the highly competitive environment of academic research, many scientists are reluctant to commit their time to writing book chapters and method articles. I also would like to express my gratitude to the series editor, Dr. John Walker, and the outstanding staff of Humana Press for their support, help, and encouragement.

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