PREFACE

The last 25 years have featured many significant discoveries related to the role of chemokines and their receptors as the maestros of cell migration. They are involved in numerous biological processes starting with immune responses that allow successful conception and fetal development. They subsequently provide cues that regulate development of many organ systems such as the cardiovascular system and the central nervous system. And they are involved in the development and function of both the innate and adaptive arms of our immune system that guard us against physiological insults as we navigate through life. Unfortunately, these otherwise developmentally important and protective molecules can contribute to many diseases as we age. Unbalanced or unresolved chemokine/receptor activities are hallmarks of many inflammatory and autoimmune diseases such as rheumatoid arthritis, inflammatory bowel disease, and multiple sclerosis. In 1996, two chemokine receptors were discovered to be the coreceptors that facilitate the entry of HIV into cells and thereby contribute to AIDS. In 2001, the connection between chemokines and cancer metastasis was established, and we now know that chemokines contribute to cancer via numerous additional mechanisms: by promoting angiogenesis, by providing survival and proliferation signals, and by establishing an immunosuppressive microenvironment that facilitates tumor growth and thwarts chemotherapy and immunotherapy. They are involved in cardiovascular disease, diabetes, pain, and the list goes on.

Because of their unusually broad clinical significance, they have been aggressively pursued as therapeutic targets, and the scientific community has realized the importance of understanding the complexities of how these proteins function in order to bring more chemokine-targeted drugs to the clinic. This understanding has been accompanied by increasingly sophisticated methods for studying the molecular and cellular mechanisms of chemokines in normal physiology and disease. It is instructive to look back at the protocols documented in the first *Methods of Enzymology* volumes from 1997 compared to this volume. While many of these early methods remain as standard laboratory practice, others have been replaced by more efficient methods, or methods that were never feasible before. As documented herein, there are improved ways to carry out assays of binding and signaling that in many cases utilize biosensor or imaging technologies. For more

sophisticated assays of cell migration than classic Boyden chambers, microfluidic devices have been developed that allow real-time monitoring of cell migration with control of gradient steepness and flow. Moreover, imaging methods for monitoring migration of specific cell types in *in vivo* models of disease have been developed. It has become possible not only to make chemokines routinely but also to rapidly generate novel modified chemokines with defined pharmacological properties that hold promise as biologics. Whereas the concept of determining structures of chemokine receptors in complex with their natural ligands and with small molecule antagonists was a dream in 1997, it is now possible, and in fact several such structures have now been solved because of the ability to express and purify receptors. This volume covers these and a wide variety of other methods that I believe will be useful to the chemokine community at large. It represents the efforts of many researchers whom I am deeply grateful to for taking the time to share their expertise. I would also like to thank the many other researchers whose work laid the foundation for the evolution of the methods described herein.

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