

MANUAL OF  
MACROPHAGE  
METHODOLOGY

Collection, Characterization, and Function

Edited by

Herbert B. Herscovitz

Howard T. Holden

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## FOREWORD

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Drugs can only repress symptoms: they cannot eradicate disease. The true remedy for all diseases is Nature's remedy. Nature and Science are at one, Sir Patrick, believe me; though you were taught differently. Nature has provided, in the white corpuscles as you call them—in the phagocytes as we call them—a natural means of devouring and destroying all disease germs. There is at bottom only one genuinely scientific treatment for all diseases, and that is to stimulate the phagocytes. Stimulate the phagocytes. Drugs are a delusion. Find the germ of the disease; prepare from it a suitable anti-toxin; inject it three times a day quarter of an hour before meals; and what is the result? The phagocytes are stimulated; they devour the disease; and the patient recovers—unless, of course, he's too far gone.

George Bernard Shaw  
Sir Bloomfield Bonnington in *The Doctor's Dilemma*  
Royal Court Theatre, London  
20 November 1906

The macrophage has not until recently enjoyed its past limelight of general interest that it did in the beginning of the twentieth century when George

#### iv FOREWORD

Bernard Shaw's *The Doctor's Dilemma* was presented at the Royal Court Theater in London. While for Shaw this production was an opportunity for social commentary on contemporary medical practices, the play nonetheless makes clear that the public at the turn of the century at least was as aware of developments in medicine and science and their redeeming social value as the public in the 1980s. Indeed, the role of Metchnikoff's phagocytes were the equivalent of our modern concerns as well as of our hopes for application of genetic engineering.

It is ironic that unlike Shaw and his fictional characters, the medical establishment for nearly half a century persisted in viewing the macrophage as little more than a "big eater." Today, mononuclear phagocytes as well as close relatives such as dendritic cells and Langerhans cells of the skin are now viewed as important contributors to cellular and humoral immunocompetence.

The phagocyte serves at least four separate but yet interrelated functions in host defense. First, the macrophage plays a vital role in the induction of T-lymphocyte proliferation in that the physical association of antigen-bearing macrophages and lymphocytes is required to clonally expand determinant specific subsets of such lymphocytes. Second, the macrophage is the principle site for the immune response gene control. Third, the macrophage possesses a number of plasma membrane receptors such as those for the Fc portion of immunoglobulin which augment the phagocytosis and killing of infectious agents during secondary immune responses. Fourth, the macrophage secretes a wide range of biologically active molecules which influence the development of the afferent limb of the immune response as well as being directly responsible for many of the major clinical and pathological aspects of chronic allergic and inflammatory disease.

As we begin to appreciate the sophisticated and specialized functions which the cells of mononuclear phagocyte lineages can express, it is of absolute importance that those scientists asking similar related questions share a common terminology and technology.

Doctors Herscovitz, Holden, Bellanti, and Ghaffar have achieved a significant synthesis of methodologies in this collection which allows the new investigator to characterize and study the function of the macrophage in a systematic manner. While it was the editors' intent to prepare a working laboratory manual, they have also in effect provided all of us who have a long-standing commitment to this area with a powerful reference by which we can both judge our own peculiar approaches and provide a point of departure for new and innovative methodologies. Knowledge of the biology, physiology,

v FOREWORD

and pathology of the macrophage should provide the basic and clinical scientist with a perspective on disease such as was unavailable to the physicians of George Bernard Shaw's time.

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## PREFACE

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The macrophage has ascended from its historical role as a scavenger cell, which was assumed to provide a poorly defined but essential function in host defense, to its present role, which is considered by many to be central in the immune response. In addition to the participation of these ubiquitous cells in the afferent and efferent limbs of both humoral- and cell-mediated immune responses, macrophages have emerged as important cells in natural host defense capable of being activated to protect the host against a wide variety of invading microorganisms and developing tumors. These cells have also been shown to have extensive biosynthetic capabilities resulting in the secretion of complement components, a wide array of enzymes, interferon, prostaglandins, and numerous other biologically active factors which modulate the activities of other lymphoreticular cells.

Not only does the unraveling of the complex functions of these cells contribute to the satisfaction of the curiosity of immunologists, the macrophage serves as a stimulus for the intellectual pursuits of individuals in a wide variety of disciplines of contemporary biomedical research. In view of the

fact that current interest in the macrophage spans many disciplines, both basic and clinical, and, as in all fields of science, progress is dependent upon the availability of innovative methodology, it was felt that a book describing methods for the study of macrophages would be both useful and timely. In the present text, the contributions have come from both basic and clinical scientists and reflect the breadth and scope of interest in the macrophage.

The *Manual of Macrophage Methodology* is composed of four parts which present tried and tested procedures of fundamental importance in carrying out studies involving macrophages. Part I deals with the collection of macrophages from various anatomic locations. Part II covers the characterization of macrophages based on morphology, cytochemistry, surface receptors, and enzyme content. Parts III and IV are concerned with functional activities of macrophages in vitro and in vivo, respectively.

The methods presented in this collection represent the vast experience and expertise of the contributors and, naturally, their prejudice in carrying out a given experimental design. As a consequence of this the reader may, at first, feel uncomfortable when inconsistencies are perceived. For example, one chapter may recommend that peritoneal exudate cells be harvested after injection of heparin-containing RPMI-1640 which is collected in the same syringe without removing it from the injection site, while another chapter may suggest that this same cell population should be harvested using  $\text{Ca}^{2+}$ - $\text{Mg}^{2+}$ -free balanced salt solution with a Pasteur pipette through a small incision in the peritoneal wall. Where the end result will not be affected by deviation from the given procedure, it is suggested that the reader use the method found to be most comfortable after experimenting with the various procedures.

The chapters in this book are arranged in a consistent format, each beginning with a brief introduction which provides background information on the utility and application of the method. This is followed by a comprehensive list of equipment and supplies, indicating the source where special items are required. The procedure is then presented in a step-by-step manner followed by a discussion of information relevant to the method which includes analysis of the data, special considerations, and alternate methods available. Each chapter also contains a list of pertinent references.

On occasion, the reader will have to extrapolate the information provided herein to meet the needs of a given experimental design or animal system under study; this should be accomplished with facility. An understanding of the basic procedures described in this manual is not only required for designing successful laboratory experiments but also for critically evaluating the literature which is ever increasing in volume with macrophage-related studies.



The concept for this book had its genesis in the numerous discussions with individuals involved both in the presentation of and participation in Workshops on Macrophage Function held in conjunction with the Annual Meetings of the Reticuloendothelial Society over the past four years. A deep sense of gratitude is felt toward Dr. Herman Friedman who, in his term as President of the Reticuloendothelial Society, initiated the concept of the workshops and suggested that their proceedings be published in some useful format. We would also like to acknowledge the help provided by Dr. Walter Ceglowski who was instrumental in getting us "off the ground" during the early planning stages for this book.

We have attempted to compile a series of methods currently used to collect, characterize, and study the function of macrophages. It was our intent to prepare a practical "working" laboratory manual to be used at the bench rather than to be a detailed theoretical treatise. The manual is directed toward the student, research assistant, and investigator about to initiate studies involving macrophages as well as toward experienced investigators desiring to increase their methodological repertoire in this area.

We thank all our colleagues who have shared the methods used in their laboratories in a format which can be reproduced in any laboratory. We also acknowledge the help of Dr. Christine Copple and the staff of Marcel Dekker who waited patiently and worked with us in bringing this concept to fruition.

Herbert B. Herscowitz, Howard T. Holden,  
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