

Methods in Immunology

A Laboratory Text for Instruction and Research

THIRD EDITION

Justine S. Garvey

Natalie E. Cremer

Dieter H. Sussdorf

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Completely revised, enlarged, reset

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In dedicating the third edition of *Methods in Immunology* to Dan H. Campbell, it seems appropriate to provide a brief history of our association with Dan. In so doing, a more exact concept of this text may emerge for you who use it. For the three of us, the rap session with Dan at morning coffee was an important event in the course of a day, if not its highlight. Sharing our feelings about the importance of this daily encounter are a large number of scientists who spent a part of their scientific career with the Immunochemistry Group that Dan developed and headed at Caltech for 32 years. Research was discussed and sometimes lengthily at these sessions. We all remember the freshly brewed coffee, the large table around which everyone assembled, the paper of the white meat-market type covering the table and used for penciled exchanges of ideas, and the large blackboard close by, used to review data or to obtain input from the group. It was there, in Room 315 of the Church Building of Chemical Biology, known as the coffee room, that Dan "threw out" the idea of the four of us writing a laboratory manual. The aim was to describe procedures that demonstrate immunological principles, together with necessary general methods. With the decision made to write the manual, we established a rigid format of style, and each of us assumed a share of the writing, along with the responsibility for the bench work. The exchange of written material and discussions continued in the coffee room until the manuscript for the first edition was completed. The intimate and relaxed atmosphere of these meetings resulted in high-spirited defense of varying viewpoints, often of a decibel level long to be remembered by associates working in adjacent laboratories. This initial cohesiveness of the group probably has propelled us through the preparations of the second and third editions when distance made communication increasingly difficult.

Dan was unique in his lifelong association with the laboratory bench. When he was no longer a doer himself, he continued to inspire by his presence, keen insight, and willingness to help with laboratory problems. In the first edition of *Methods in Immunology*, the use of do-it-yourself materials and equipment was much encouraged. This was a natural consequence of Dan's close association with shop personnel at Caltech and of his general philosophy of using what was at hand. Knowing this, it is with considerable regret that in this edition we are deleting some of the materials from the earlier editions, such as the use of food cartons to construct chambers for gel electrophoresis. Also some of the early techniques have been omitted, such as Schultz-Dale, a method taught to us by Dan, who modified this particular procedure for his anaphylaxis research begun at the University of Chicago.

The numerous scientific citations and awards which Dan received in recognition of his accomplishments appear meager compared with the imprint left by his highly effective scientific style. Everyone who spent even a brief time in his laboratory realized that there was a unique quality about this man and the environment he created at Caltech. At the center of this uniqueness was the manner in which he inspired bench work. He expected his postdoctoral students to learn the very basics of biological and immunological methodology—how to inject and bleed rabbits, clarify serums, and perform precipitation analyses; this was his way of building the confidence and independence of an investigator. No one became a cog in the wheel of a research project. Rather than having his laboratory generate a series of research papers by a unidirectional, inflexible approach, Dan encouraged individual input with complete objectivity. A viewpoint could differ from current thinking on a subject if supported by sound bench work.

Dan was always known for his informality at Caltech. In addition, the days of deep-sea fishing in the Pacific with his postdoctoral fellows and frequent backyard barbecues at his home presented other circumstances to know him and his wife Margaret. Dan's favorite pastime may have been the rap sessions at Allergy and FASEB meetings, at the Midwinter Conferences of Immunologists, and at the Summer Course on Immunology sponsored by AAI. He could be irascible with a peer scientist who needed a reminder of an overlooked fact. At the same time, he humored the newcomer who made mistakes in his learning experience. Regardless of the circumstances, his comments always were witty and meant both to be helpful and to relieve meeting doldrums. In combining good humor with a great love for science and for the doers of good science, few have equaled Dan H. Campbell.

JSG
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PREFACE

Although biological findings are not as precise and unchangeable as laws of physics or mathematics, certain basic principles do emerge and withstand the test of time. The new and ingenious techniques to demonstrate these basic principles are usually variations of the old, often with another dimension added. Although new methods are developed and come into general use because of greater ease of operation, better quantitation, better resolution, or greater applicability, underlying reactions remain the same. From the precipitin reactions have come the many forms of immunodiffusion-in-gel techniques that were in turn the forerunners of the various and innovative immunoelectrophoretic procedures. Other methods such as radioimmunoassay and fluorescent antibody techniques depend also upon fundamentals of the precipitin reaction. Some of the technical variations are popular for a time and then disappear, while others, proven reliable after extensive usage, become routine in teaching, research, and clinical laboratories.

In the present edition as in the previous editions, the format of the book is built around immunological principles. Techniques, both new and old, are selected to demonstrate these principles and are embodied in Part V, Immunological Reactions. The first four parts of the text present basic biological and physical methods and the preparation of immunological reagents (antigens and antibodies) that are used in Part V. The major changes in the third edition from that of the second are the deletion of some techniques little used today, their replacement by newer ones and by expansion of studies on cellular immunology.

The text introduces to the advanced students in biology, chemistry, and medicine, either by way of a formal classroom or in an investigator's laboratory, the fundamental concepts of immunological reactions and their effect under *in vitro* and *in vivo* conditions. More techniques and their application are presented than can be performed in a course, thus allowing selection of those most suited to particular needs and interests.

We wish to thank Betty Aalseth, Aftab Ahmed, Nicola Green, Donald Hoffman, Norman Weliky, and Leo Yuan for their contributions, reviews, and helpful comments. Thanks are also extended to the immunology

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Justine S. Garvey
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INTRODUCTION TO THE STUDENT

As you become increasingly familiar with fundamental concepts of immunology and with the laboratory methods that demonstrate them, you will realize that, with all its ramifications, immunology utilizes most of the disciplines of the basic sciences. Evidence of this is shown by the use of such designations as immunochemistry, immunobiology, immunopathology, immunogenetics, and immunopharmacology. Since all these disciplines must contribute to a laboratory text introducing basic immunological principles, it will be an advantage to have a background that has as much breadth and depth as possible in the basic sciences.

Regardless of your particular interests, it is important that you make an effort to visualize immunological reactions at both the molecular and the cellular levels. Thus, the terms antigen and antibody should suggest discrete three-dimensional entities that have shape and size and consist of specific numbers and arrangements of amino acids and carbohydrates. You also should have some knowledge of the structure, origin, and function of cells. In recent years genetic principles have become increasingly significant in many areas of immunology. For example, genetic factors are of importance in tumor and transplantation immunology, as well as in studies of *in vitro* cellular interactions.

The selection of animals and antigens for a given problem should receive careful consideration, since species, even strains, vary in their immune responsiveness to given antigens. In fact, the genetic basis of responsiveness has emerged as a major field in immunological research. Many of the antigens used in immunological studies commonly occur in our everyday environment. For example, antigens associated with the normal bacterial flora of the intestine and the upper respiratory tract, or even food products, may induce mild but significant sensitizations in so-called normal animals.

When selecting analytical procedures, it must be kept in mind that biological systems often are several orders of magnitude more sensitive than physical methods. Thus, specific skin reactions can be induced by picogram amounts of antigen. This means that preparations considered pure by physical methods may actually contain contaminants that will give cross-reactions when tested biologically.

You, and certainly your instructor, will realize that the field of immunology is progressing at such a rate that certain currently acceptable definitions may change in the future. You often must accept some confusion in terminology, focusing on a general concept rather than a definition. For example, sensitized lymphocytes release, upon contact with antigen, a variety of soluble, biologically active substances (lymphokines) whose present nomenclature relating structure and function is far from resolved.

Trends in immunology are changing rapidly, owing to a new methodology and the resulting accumulation of new information. As you carry out your laboratory studies, fundamental questions will occur to you, and you will discover that many of them have not been answered. Extra laboratory effort on your part, help from your instructor, and reading may suggest answers and provide routes for further investigation. Such exploratory work not only has contributed in the past to our knowledge of immune mechanisms, but also will help in training you to design meaningful experiments.

Because *Methods in Immunology* essentially is a guide for introductory laboratory studies, it will be advisable to consult some of the following texts, reviews, and journals for more information. You can keep abreast of ongoing investigations and recent findings by referring to current journals and publications of conferences and symposia devoted to immunology.

General References

The following reference list is divided into: A. Textbooks, B. Methods Books, C. Monographs, D. Proceedings of International Colloquia, Symposia, and Workshops, E. Reviews, and F. Journals.

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11. *International Archives of Allergy and Applied Immunology*.
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