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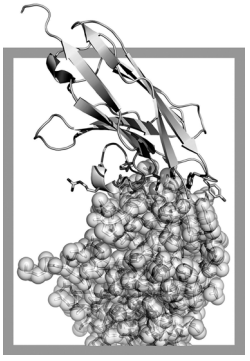

EDITED BY

Edward A. Greenfield

Dana-Farber Cancer Institute

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To my mom, Frances Greenfield,
my sister, Sandie Sternstein,
and my wife, Patricia Bixby,
who have had to share me with
all the hybridomas over the years

Contents

General Safety and Hazardous Material Information

This manual should be used by laboratory personnel with experience in laboratory and chemical safety or students under the supervision of such trained personnel. The procedures, chemicals, and equipment referenced in this manual are hazardous and can cause serious injury unless performed, handled, and used with care and in a manner consistent with safe laboratory practices. Students and researchers using the procedures in this manual do so at their own risk. It is essential for your safety that you consult the appropriate Material Safety Data Sheets, the manufacturers' manuals accompanying equipment, and your institution's Environmental Health and Safety Office, as well as the General Safety and Hazardous Material Information in Appendix V for proper handling of hazardous materials described in this manual. Cold Spring Harbor Laboratory makes no representations or warranties with respect to the material set forth in this manual and has no liability in connection with the use of these materials.

All registered trademarks, trade names, and brand names mentioned in this book are the property of the respective owners. Readers should consult individual manufacturers and other resources for current and specific product information. Appropriate sources for obtaining safety information and general guidelines for laboratory safety are provided in the General Safety and Hazardous Material Information Appendix of this manual.

Preface

I HAVE REALLY ENJOYED UPDATING AND REVISING THIS MANUAL. Everyone I know in the antibody world has a copy of the first edition of this book. Ed Harlow and David Lane must be acknowledged for being the first to demystify hybridoma generation for the nonimmunologist. It is my hope to build on their work, continuing to provide easy-to-follow information and protocols that will allow anyone with a desire to make a monoclonal antibody to succeed.

For many years I have thought about collecting my notes and protocols—all the little tricks of the trade and lessons learned through years of experience—and writing them up as guide for antibody makers. I wanted it to be something of a troubleshooting guide—a manual to go to when things were not going as planned and a place to turn when the usual protocols were not giving the desired results. With the arrival of the internet—antibody-related websites like the Antibody Resource Page and antibody network groups providing information and connections between antibody developers and users—it did not seem to be a pressing need to take the time to gather all the little tidbits of information I had accumulated over the years. Friends and colleagues told me that they still thought it would be worthwhile putting it all together in one place as a reference. Then I was asked if I would be interested in helping to revise the 1988 Harlow and Lane text. Opportunity came knocking; how could I refuse?

When I first got involved making hybridomas, I researched the technology, read the 1975 Köhler and Milstein *PNAS* paper that had been published less than eight years previously, and talked with as many people as I could find who had been making monoclonal antibodies. Then I had my first encounter with *Antibodies: A Laboratory Manual* by Ed Harlow and David Lane. It was during my days as a graduate student in Pathology at Albany Medical College in New York. Immunology was a developing field that was just being applied to pathology (autoimmune disease), which piqued my interests. I was involved with setting up a hybridoma laboratory at the Wadsworth Center, New York State Department of Health, at Empire Plaza, Albany. The *Antibodies* manual provided our laboratory with guidance in the form of protocols and recommendations for setting up a hybridoma facility. Everything we needed to know to make a monoclonal antibody was all there, neatly packaged in an easy-to-understand book.

Those were the days! Preparation for fusion started around 7 a.m. collecting macrophage feeder cells and plating out 200- to 300-microtiter plates for the fusion. The guest of honor would arrive around 9 a.m., its spleen would be harvested and teased apart, and the lymphocytes counted (by hand of course). By midday we would have the lymphocyte-to-myeloma cell ratio calculated and the number of plates ready for the newly fused hybridoma cells at 0.5 cells per well. We did not have sterile plasticware like pipettes or multichannel pipetters. Everything was made from glass that we acid-washed and then autoclaved. ELISA was just catching on as a screening assay that was read on a beam spectrophotometer, one well at a time. Caring for and screening a fusion took a significant amount of time.

After 25 years, monoclonal technology has not changed all that much. Laboratories still immunize mice with an antigen mixed with Freund's adjuvant. They boost the mice every other week or so, collect the spleen, and fuse myeloma cells with murine B lymphocytes to produce hybridomas using PEG and HAT selection. Yes, other methods like electrofusion have been developed, but most laboratories still use the traditional method. What has changed considerably is our understanding of the immune system and the methods we employ to screen fusions. The original edition of this book

refers to many radiological screening assays like RIA (radioimmunoassay). Today, radioisotope methodologies have been replaced by safer, more environmentally friendly protocols using chemical colorimetric and chemiluminescent readouts. The instruments we use are now automated and more accurate. Developing monoclonal antibodies is still a lot of effort, but the work has become more manageable.

Monoclonal antibody generation is most definitely a learning experience. No two antigens induce the same response. Some can be very elusive. Having mastered the fusion technology really well, I find the challenge is in getting the animal to respond and make the antibody necessary for a particular investigator's project. Antibodies can be very odd moieties. Some bind particular epitopes with single-amino-acid specificity, whereas others can be extremely promiscuous, binding similar epitopes in unrelated proteins. Some antibodies work well in some conditions but not at all in others. Different antibodies to the exact same epitope can bind stronger or only weakly, or activate a receptor or block it. Each antibody project is a unique challenge. It is important to select the best form of immunogen for a particular application, choose the right species of animal, present the immunogen to its immune system in the correct way, and then devise a screening assay to identify hybridomas making the antibody. All these things have to line up perfectly to ensure success.

As with the first edition of *Antibodies*, the second edition is intended to provide the necessary information and protocols to assist investigators with their first monoclonal antibody effort, as well as provide guidance for more experienced antibody makers who are having some difficulties with a particular project. With this in mind, the revised edition was expanded to include chapters on antibody characterization, antibody engineering, and flow cytometry. The original chapter on immunizations was split into two chapters: one covering different forms of antigens used for immunizations and a second on various immunization strategies with an expanded protocol section. Other chapters from the first edition have been updated and augmented, with emphasis placed on including protocols and advice for both novice and well-seasoned immunologists.

I would like to thank Jim DeCaprio for getting me involved in this project. I am also very grateful to Vijay Kuchroo for being a patient teacher, great mentor, and good friend over the years I was a postdoc in his laboratory and all the many years we have worked together since then. I must also express my appreciation to the Cold Spring Harbor Laboratory Press team—Judy Cuddihy, our Editor; Michael Zierler, our Developmental Editor; Inez Sialiano, Project Manager; Jan Argentine, Director of Editorial Development; Kathleen Bubbeo, Production Editor; Denise Weiss, Production Manager; and John Inglis, Publisher—for all their help, guidance, and patience coordinating all the authors, chapters, and shifting deadlines that made this revised edition possible.

It is my sincere hope that the revised edition of this book will provide help and insights into the development and use of monoclonal antibodies. I find each new antigen challenging and enjoy seeing all of our "kids" going forth into the world making names for themselves as investigators put them to use, making exciting new discoveries that benefit us all.

ED GREENFIELD, PhD

Director, Monoclonal Antibody Core
Dana-Farber Cancer Institute
Boston, Massachusetts