Monoclonal Antibodies: A Manual of Techniques

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PREFACE

In the past 10 years since the original description of antibody production by a somatic cell hybrid line, monoclonal antibodies have become standard reagents in many biological applications. There have been few major changes in the techniques used to make and to utilize monoclonal antibodies, and the mechanism of hybridization is still not understood in any detail. However, a few years of experience with the techniques have brought about important refinements, and have established a body of data which allows choices to be made between some of the alternative procedures. The production and use of monoclonal antibodies can, as a result, proceed in a fairly predictable way, provided that the best techniques are chosen for the particular objectives of the project. This book describes, in detail, techniques for the production and use of monoclonal antibodies. Methods are set out in the form of experimental protocols, so that the book can serve as a laboratory manual. The rationale behind each method is discussed, where appropriate. Where several alternative methods are available, a rational basis is provided, where possible, for the choice of methods appropriate to the reader's own needs. The book does not set out to review specific fields of use, however, since it is intended to cover in detail those aspects of interest to all scientists working with monoclonal antibodies.

ACKNOWLEDGMENTS

This book is principally concerned with methodology. The techniques described have been used in projects which I have either directed or participated in. However, many of my colleagues have contributed in important ways, either with ideas or by doing the actual work. Current or former higher-degree students (Doug Brooks, Ian Beckman, and Russell Hogg), research assistants (Peter McNamara, Ian Hunter, Helen Moore, Joe Webster, and Angela Potter), and Hospital Scientists (Miriam Thomas, Pete Macardle, and Virginia Griffith) have done much of the work which serves as a base for this book. Scientific colleagues who have contributed ideas and suggestions, and have taught me some of the methods, include Drs. Anthony Hodgson, Sim Hee Neoh, Keryn Williams, John Bradley, and Art Hohmann. Professor John Bradley, Head of the Department of Clinical Immunology, has provided an environment which made the research possible, and has given active encouragement and support to research in his Department.

I prepared the early draft of this book on an Apple (and an Apple look-alike) and I owe a real debt to Messrs. Jobs and Wozniak. Writing books must have been such hard work in the days before microcomputers for the masses. My drafts, together with a multitude of alterations, were converted to the finished manuscript by Mary Brown, whose diligence and careful work is greatly appreciated. The art-work was produced by Dennis Jones of the Media and Illustration Unit, Flinders Medical Centre, and by Adrian Wright and Angela Potter. The photographs are my own, but my colleagues had to wait patiently with syringe or scalpel poised, while I fiddled with the focus or the flash.

Dr. Anthony Hodgson read a later draft and provided valuable criticism, particularly of the sections dealing with immunohistochemistry, an area in which his expertise is much greater than mine. Dr. Sim Neoh provided details for the immunoglobulin purification methods of Chapter 4. Dr. Keryn Williams read the whole book in draft form, and provided extensive and valuable critical comments on the scientific content, the clarity, and the English. It is all too easy to write text which is quite clear to the writer, and totally unclear to the reader. If this book turns out to be readable, this will be due, in large measure, to Keryn Williams' constructive criticism. Having sought the help and advice of my colleagues, I chose to ignore some of their comments. The responsibility for any residual flaws is therefore squarely mine.

THE AUTHOR

Heddy Zola is Chief Hospital Scientist in the Department of Clinical Immunology, Flinders Medical Centre, and Reader in Immunology in the Flinders University of South Australia. He received a Bachelor of Science degree in Chemistry from the University of Birmingham, in England, and a Doctor of Philosophy degree in Biophysics, from the University of Leeds, England.

Following his Ph.D. studies Dr. Zola worked in the Biophysics Department of Leeds University, before joining the Wellcome Research Laboratories, Beckenham, Kent, where he carried out biophysical and biochemical studies on a variety of biologically active proteins. Involvement in a project aimed at the preparation of antilymphocyte serum, for the use in the treatment and prevention of transplant rejection in man, led to an interest in the immunology of the lymphocyte surface. When Kohler and Milstein described the first hybridoma, the potential of this technique as a tool in studies of cell membrane molecules soon became apparent. Dr. Zola started to make monoclonal antibodies against human lymphocyte antigens, initially at Wellcome and subsequently in Australia.

Dr. Zola was born and grew up in Africa, where his parents had settled as refugees from Europe. After attending schools in Zanzibar and Kenya, he went to England in 1959, initially to study, married and stayed on in England until 1978. The lure of sunshine and opens spaces eventually proved irresistible, and Dr. Zola, his wife Marion, and their two daughters moved to Australia, where they live in the city of Adelaide.

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INTRODUCTION

I. AIM AND SCOPE OF THIS BOOK

The first publication describing the preparation of a monoclonal antibody by somatic cell hybridization appeared in 1975.[1] Less than a decade later, in 1984, the authors of that paper (Kohler and Milstein) have shared a Nobel Prize, and monoclonal antibodies are regularly mentioned in the lay press. The layman really does not need to know much about monoclonal antibodies; they are high technology, the product of genetic engineering, used in making magic bullets, and quite definitely "a good thing."

On the other hand, biological and medical scientists in a variety of disciplines do need to know much more about monoclonal antibodies, and if they are not immunologists (and, perhaps, even if they are) they may need some help. Monoclonal antibodies are available for use, or at least talked about, in a variety of fields, from cancer diagnosis to the food industry; from transplant surgery to plant pathology. The scientist in any biological or medical field may need to answer these questions:

- Do I need monoclonals?
- Do I need to make them myself?
- How do I use them?
- What can I realistically expect from them?

This book is intended to enable biologists and medical scientists to answer such questions for themselves, in the context of their own particular needs. The book provides detailed methodology to enable readers to make their own antibodies and to use monoclonal antibodies to their best advantage.

II. ANTIBODY AS A SPECIFIC PROBE

Antibodies are proteins made by many animal species as part of the specific immune response to foreign substances. Specificity is the hallmark of the immune response; recovery from measles leads to lifelong immunity from measles, but confers no protection against chicken pox. One component of this specific response is the synthesis, by B lymphocytes and plasma cells, of antibody which reacts with a high affinity with the stimulating substance (the antigen), but not with other substances. This natural ability to make proteins with a high order of specificity in distinguishing different molecular structures can be used in a variety of ways. Antibody obtained by injecting a rabbit with human insulin reacts specifically with human insulin and can be used as a probe for the hormone. The antibody can serve as the basis for a quantitative assay for the hormone; it can be used to detect cells secreting the hormone in tissue sections; it can be used to purify the hormone from a complex mixture of proteins. Thus, antibodies have long been used as reagents in the detection, measurement, and purification of biological molecules. Antibodies have also been used for treatment; antibody made in horses against bacterial toxins or snake venoms provides emergency treatment, albeit with considerable risk of an adverse immune reaction to the horse antibody, itself seen as a foreign molecule by the immune system of the patient.

Until recently such antibodies were obtained by immunizing an animal and then bleeding it. The antibodies are secreted into the serum, and the protein fraction con-