Monoclonal Antibodies in Clinical Medicine

edited by

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Preface

It is only 7 years since G. Kohler and Cesar Milstein published their work on the production of mouse monoclonal antibodies to sheep erythrocytes, but in that short time monoclonal antibodies have had an astonishing impact on almost every facet of biology. They are now beginning to be used in clinical practice and there can be little doubt that in the next few years they will have as profound and wide-ranging an influence on clinicians as they have had in the past few years on laboratory scientists. As is illustrated in innumerable examples in this volume, every aspect of clinical medicine – diagnosis, prognosis, understanding of pathophysiology, therapy and prophylaxis – will be enormously enhanced by the advent of monoclonal antibodies.

A wealth of benefits in clinical application lay before us. Quite clearly, however, the promise of monoclonal antibodies will be most fully and rapidly realised only if clinicians are aware of their potential benefits, and if laboratory scientists with clinical interests apply themselves to the relevant problems. At this early stage it is difficult for those not directly involved with monoclonal antibodies to assimilate disconnected papers, or to understand the background and look to the future. It is particularly with this in mind that this volume was produced, and we hope that it will serve as a milestone in the dissemination of awareness of the clinical benefits that lie ahead, and thereby hasten their becoming a reality.

The production of monoclonal antibodies represented the culmination of much basic biological research on cell fusion. Their clinical application will be an excellent example of how even the most basic research can have quite unexpected and profound clinical value and it is salutory to stress that point in a volume such as this. All of us who work with monoclonal antibodies owe a large debt of gratitude to Dr. Cesar Milstein, not only because it was largely due to his pioneering work with Dr. G. Kohler that monoclonal antibodies are a reality today, but also because of the generosity with which he distributed, from the earliest days, his myeloma lines to all of those interested in the field. We

are therefore delighted and much honoured that he agreed to write the introductory chapter of this book, as it forms a most appropriate and informative background for the succeeding chapters.

We could not hope, even in a volume of this size, to be fully comprehensive, but we have tried to give an idea of the scope that exists for clinical application, and have chosen subjects where monoclonal antibodies are already or will very likely soon be of clinical benefit. Within each subject we have sought illustrative chapters; for example the chapters on influenza and Epstein-Barr viruses represent virology, although monoclonal antibodies have been made against a large number of different viruses. The last section of the book consists of 4 chapters giving detailed methodology on the production and use of monoclonal antibodies. We felt that a detailed exposition of the practical aspects of monoclonal antibodies would be invaluable to many readers, as this is rarely given in the literature and quite obviously, however valuable monoclonal antibodies might be, their value is diminished if one has difficulty producing them or cannot put them to optimal use.

It is our hope that the book will have a broad educative function and reach a diverse readership. With this in view, the authors were asked to make their chapters accessible to non-specialists, and they have done this admirably well. We are grateful to all the authors for their contributions, which were submitted within a 3 month period and include much unpublished data. The book should therefore be as current as any major journal issue at the time of publication. We are grateful to Peter Brown of Academic Press who initiated and encouraged the project, to Jean Broadis, Eunice Berry and Rosemary Bryan for expert secretarial assistance, and to Phyllis Hildreth for help with the index and appendices.

June 1982

A. J. McMichael
J. W. Fabre

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1982

ACADEMIC PRESS
A Subsidiary of Harcourt Brace Jovanovich, Publishers
LONDON NEW YORK PARIS SAN DIEGO SAN FRANCISCO SAO PAULO
SYDNEY TOKYO TORONTO

ACADEMIC PRESS INC. (LONDON) LTD.
24/28 Oval Road
London NW1

United States Edition published by
ACADEMIC PRESS INC.
111 Fifth Avenue
New York, New York 10003

British Library Cataloguing in Publication Data
Monoclonal antibodies in clinical medicine.
1. Immunoglobulins 2. Gammopathies, Monoclonal
I. McMichael, A. II. Fabre, J.
616.079'3 QR186.7

Library of Congress Catalog Card Number: 81–71580

ISBN: 0-12-485580-6

Phototypeset by Oxford Verbatim Limited
Printed in Great Britain by St. Edmundsbury Press, Bury St. Edmunds.

A INTRODUCTION

1 Monoclonal Antibodies from Hybrid Myelomas: theoretical aspects and some general comments

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I INTRODUCTION

Antibody producing lymphoid cells from immunized animals have a very short life when cultured under *in vitro* conditions. Individual myeloma cell lines can be grown permanently in culture, but the antibody they produce does not express a pre-defined specificity. When both types of cells are fused, hybrids can be derived which retain the essential properties of (a) permanent growth, and (b) production and secretion of antibody with a pre-defined specificity. Since the hybrid cells can be cloned, it is possible to dissect the heterogeneous response of an animal (Fig. 1·1). The procedure therefore permits the derivation of

clean reagents directed against very well defined antigenic determinants, regardless of the complexity of the immunogen: this allows a new strategic approach to a wide variety of problems, and its impact in the field of clinical medicine is the object of this book.

Although the experimental set up looks simple and straightforward, the development of the technique was the result of many years of fundamental development in unconnected areas of cell biology and immunology. The discovery of cell hybrids formed by spontaneous fusions of two different cells in culture was discovered in 1960, but this was a very rare event with little practical importance. The exploitation

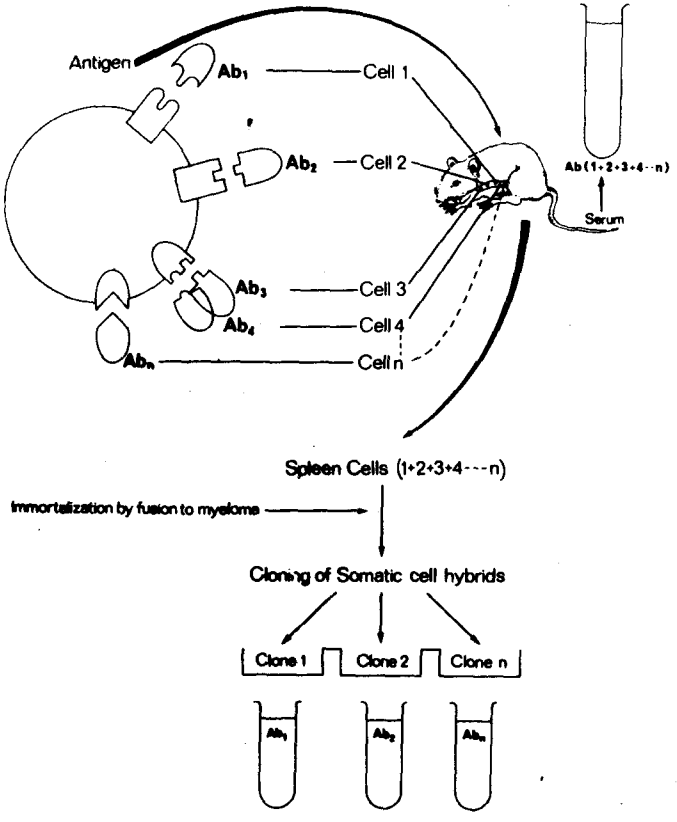

FIG. 1·1 Hybrid myelomas allow dissection of the immune response. The response of an animal to an antigen is very complex. But each antibody is synthesized by individual cells. These can be immortalized by cell fusion to provide an inexhaustible supply of monoclonal antibody (from Milstein and Lennox, 1980).

of the phenomenon for research purposes was made practical by further developments permitting the increase of fusion efficiency and the selection of hybrid cells. (For a comprehensive review of the historical developments, see Ringertz and Savage, 1976). In the following 10 years, somatic cell hybrids were extensively used for two purposes; namely for gene mapping and for studies of gene expression and differentiation. The immortalization of a given differentiated function and the use of hybrid cells as a permanent source of specific products, in this case monoclonal antibodies, added a new application to somatic cell hybrids.

II DERIVATION AND SELECTION OF HYBRID MYELOMAS SECRETING SPECIFIC ANTIBODY

The procedure first used for the derivation of anti sheep red cells (Kohler and Milstein, 1975) and schematically described in Fig. 1·2 requires myeloma cell lines, well adapted to permanent growth in cell culture conditions, but including genetic deficiencies which do not allow them to grow under certain conditions. The most commonly used are mutants lacking the enzymes hypoxanthine guanine ribosyl transferase or thymidine kinase. The mutants are usually selected among those able to grow in the presence of azaguanine or bromodeoxyuridine. Such mutants are resistant to these DNA analogues, because they lack enzymes of the salvage pathway. For the same reason, they are unable to incorporate externally supplied hypoxanthine or thymidine. When endogenous synthesis of DNA precursors is blocked with aminopterin, the cells die, even when hypoxanthine or thymidine are also included (HAT medium) (Szybalski *et al.*, 1962). Hybrids between them and spleen cells, which contain the wild type salvage pathway enzymes can then be selected from the parental components as the only cells that actively multiply in HAT medium.

The growing hybrids coexpress certain genotypic and phenotypic characteristics of both parental cells, but there are restrictions, which will be discussed later. When selected parental myelomas are used, the majority of the hybrid cells express antibody molecules derived from the lymphoid cells (e.g. spleen) of the immunized animal. Among those hybrids, some may express and secrete desired antibodies. Such hybrid cells can be individually isolated, and grown as clones secreting a specifically selected antibody (Fig. 1·3).

Practical problems and strategic approaches have been extensively reviewed (Galfre and Milstein, 1981; and see Chapter 21). The

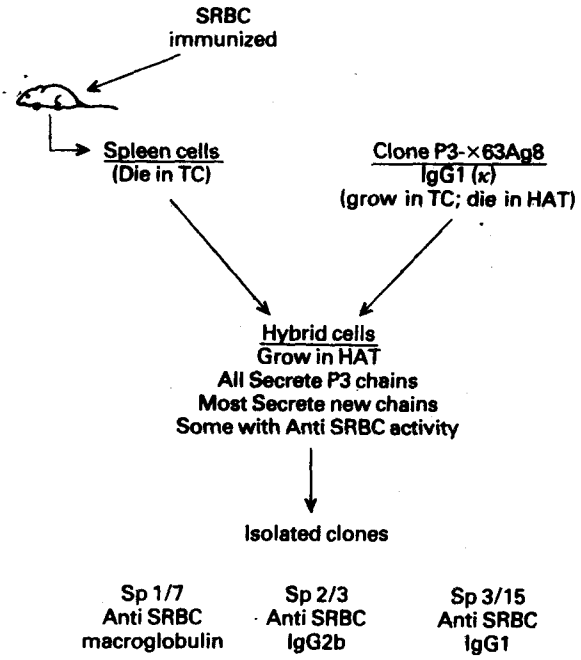

FIG. 1·2 Fixation of the specific antibody production of a transient spleen cell in a permanent tissue culture line (from Milstein and Kohler, 1977).

response of an animal to a given immunogen usually results in the stimulation of a highly heterogeneous population of cells, each secreting different antibody molecules. The hybrid myelomas represent a cross-section of the heterogeneous population (Fig. 1·1). Among those, some will secrete antibody molecules exhibiting desired properties. It follows that the successful derivation of desired lines will largely depend on the appropriate immunization of the animal, and on the ability of the experimenter to select the desired clones from among the large number of hybrid clones randomly produced.

Immunogens are often complex structures, or impure substances, but these factors are of little importance, provided that the desired responses can be induced, and not less importantly, that the relevant antibody can be specifically recognized in the presence of the contaminating components (Williams *et al.*, 1977). For instance, the antibodies induced to impurities of an interferon crude preparation are totally ignored by testing the ability of the antibody to neutralize or remove interferon activity (Secher and Burke, 1980). A monoclonal antibody specifically recognizing thymocytes can be recognized, be-

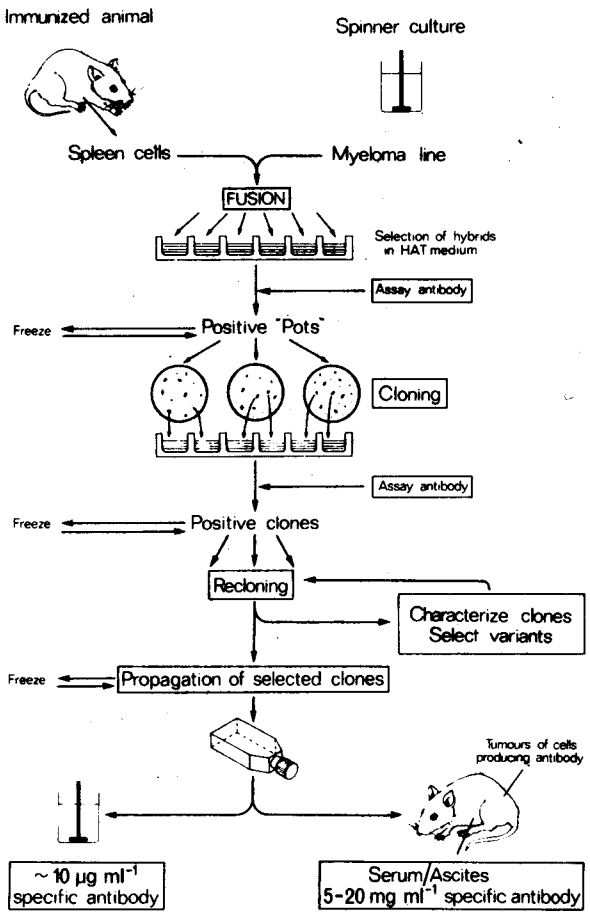

FIG. 1·3 Usual steps involved in the derivation and cloning of antibody producing hybrid myelomas (from Galfre and Milstein, 1981).

cause it will bind to thymocytes, but not to peripheral lymphocytes (McMichael *et al.*, 1979). Here, unlike the case of interferon, antibodies to thymocytes which also recognize peripheral lymphocytes are not ignored in a simple binding assay. This introduces certain complications in screening procedures.

On the other hand, complex antigenic mixtures may contain dominant immunogenic components. These may effectively decrease the response to a desired antigen, in which case partial purification of the antigen in question may become essential. One general way to achieve