Monoclonal Antibodies against Bacteria

Volume I

Edited by

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ACADEMIC PRESS, INC.
(Harcourt Brace Jovanovich, Publishers)
Orlando San Diego New York London
Toronto Montreal Sydney Tokyo

ACADEMIC PRESS, INC.
Orlando, Florida 32887

United Kingdom Edition published by
ACADEMIC PRESS INC. (LONDON) LTD.
24–28 Oval Road, London NW1 7DX

Library of Congress Cataloging in Publication Data
Main entry under title:

Monoclonal antibodies against bacteria.

Includes index.
1. Bacterial antigens–Analysis. 2. Antibodies, Monoclonal. I. Macario, Alberto J. L. II. Conway de Macario, Everly. [DNLM: 1. Antibodies, Monoclonal. 2. Bacteria. QW 575 M7472]
QR186.6.B33M66 1985 616.9'20793 84-24455
ISBN 0–12–463001–4 (alk. paper)

PRINTED IN THE UNITED STATES OF AMERICA

85 86 87 88 9 8 7 6 5 4 3 2 1

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Preface

The major aim of this multivolume treatise is to assemble contributions in the field of bacterial immunology focused on monoclonal antibodies. The contributors are pioneers in this field. The contributions are, for the most part, first attempts at reviewing data and publications in specific, restricted areas. As a result, they vary in length and depth depending on how far advanced research in these areas was at the time of writing.

This treatise will provide in a single reference source as much fundamental data as possible on generation, characterization, and utilization of monoclonal antibodies against bacteria and on other monoclonal products (e.g., lymphokines) relevant to antibacterial immune responses. As such, it can be regarded as a milestone, a source in which some of those who were the first to generate and to work with monoclonal antibodies against bacteria paused for a while and reviewed their original observations. Consequently, efforts were made to include extrapolations to the future. The intention was to assemble useful data and to provide a foundation for future research.

Another major goal pursued was to create a common ground for human and veterinary medicines and dentistry, one in which findings and speculations concerning pathogenic bacteria in these disciplines could be dealt with in a complementary fashion.

Studies of bacteria relevant to biotechnology and industry involving antibodies are also included. Many of these endeavors are promising for public health application. They are interconnected and require consideration in relation to one another and to medicine, dentistry, and veterinary sciences. This is particularly true if one deals with genetic engineering and monoclonal antibodies.

Updates on each topic are planned for later volumes so that not only the birth but also the initial growth of the field will be recorded.

We thank all those who taught us, worked with us, supported us financially and/or spiritually, and helped us in so many different ways. They are too many for us to name them all. We also thank the authors for their excellent contribu-

tions to this treatise. The staff of Academic Press deserves our warmest appreciation for their expert and unfailing support and encouragement.

Alberto J. L. Macario
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Introduction: Monoclonal Antibodies against Bacteria for Medicine, Dentistry, Veterinary Sciences, Biotechnology, and Industry—An Overview

I. BACTERIAL SEROLOGY

A. Today

Antisera have been the essential reagents of bacterial serology for many years (11). Today, monoclonal antibodies share the central stage with antisera (Fig. 1).

The use of hybridoma technology a decade ago for the production of monoclonal antibodies (31) started a new era in bacterial serology as well as in other areas of basic and applied sciences. Immunology, immunochemistry, and biochemistry were immediately invigorated by the use of monoclonal antibodies. Some areas of microbiology, i.e., virology, were also influenced by these new reagents rather early (32). Reports on monoclonal antibodies against bacteria began to appear later (9,30,50), and only in the last couple of years has their number been climbing steadily (42). A comprehensive review is no longer feasible by a single author or group. Extensive coverage of the topic requires participation of experts in a treatise. This book was conceived to meet both this requirement and the need for a reference work on the novel aspects of bacterial serology dependent on monoclonal antibodies.

In this introductory overview, we intend to sketch the topic of monoclonal antibodies against bacteria, encompassing its scope, research to date, and future, expected directions as a guide to the chapters in this volume and to subsequent ones in this treatise. We will highlight some aspects that are particularly interest-

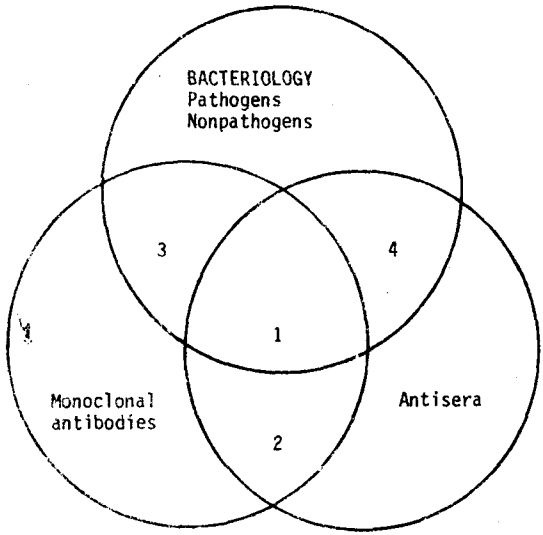

Fig. 1. Contemporary bacterial serology encompasses monoclonal antibodies and antisera. These two types of reagents can be used independently of one another, or coordinately for specific purposes, as shown by the nonoverlapping and overlapping segments of the circles, respectively. (1) Combined, complementary serology; (2) dissection of antisera into their antibody populations; (3) molecular elucidation of antigenic mosaics; chemoimmunotaxonomy; genetic engineering; (4) the classic serology.

ing. For example, it has been realized that the preparation and use of monoclonal antibodies against bacteria are aided in a complementary manner by antisera to the same microbes studied with the antibodies (8) (see Chapters 7 and 11).

Monoclonal antibodies and antisera must be calibrated and thus made into probes for achieving maximal precision and resolution (8,10,40,41) (see Volume II). Poly- and monoclonal antibody probes have specific uses depending on their distinctive properties. If these two types of probes are utilized coordinately, they complement one another multiplicatively rather than additively in terms of amount and quality of data obtained and in terms of the ease and accuracy of their interpretation. Monoclonal antibodies can be considered definitively incorporated into bacteriology, forming a battery of complementary reagents with antisera (Fig. 1).

A comparison of classic serology with that dependent on monoclonal antibodies shows differences between the two, some circumstantial, others more profound. An example of the former is that monoclonal antibodies have not yet been used in all areas of bacteriology in which antisera have. Practically all

pathogens have been studied with antisera (11), whereas only a few have been examined with monoclonal antibodies, and even fewer have been utilized as immunogens to generate these antibodies (42) (a comprehensive tabulation will be included in a subsequent volume).

There are specific applications of monoclonal antibodies in which these antibodies cannot be replaced by antisera. Examples of these applications are dissection of antisera into their antibody populations by competitive inhibition assays (20) (see Chapters 3 and 4) and analyses of specific functions of defined antibody subpopulations *in vitro* and *in vivo* (50) (see Chapter 2).

Another difference between antisera (or polyclonal antibody probes) and monoclonal probes concerns experimental strategies for their utilization. Interpretation of the data is also governed by different rules. A monoclonal antibody is monospecific in the sense that it recognizes a single determinant (and perhaps slight variations of it), whereas an antiserum is of undefinable complexity (8). The range of specificities of an antiserum cannot be ascertained. It may contain a few, several, or many different antibody populations differing in specificity, affinity, and other properties (39,59).

An additional complicating factor that obscures interpretation of results obtained with antisera is that one antibody population may affect the binding of another to its complementary site on a bacterial antigen. These interantibody effects are currently being studied by means of monoclonal antibodies in nonbacterial antigen–antibody systems (24,46,64). The topic is extremely important in bacterial serology since mixtures of monoclonal antibodies would be desirable for screening clinical specimens in search of pathogens, and where polyvalent reagents may enable detection of more than one serotype in a single test (47) (see Chapter 3 and subsequent volumes).

Interactions between antibodies of disparate specificities do not occur if monoclonal probes are used individually. Therefore interpretation of binding data is less subject to error. For example, if two bacterial strains or toxins are assayed with a monoclonal probe and both bind the probe equally, it can be safely assumed that they share a determinant. A similar dual reaction given by an antiserum cannot be interpreted unequivocally. Perhaps the two strains (or toxins) do not share a determinant but each has one or more sites in common with the immunizing strain (or toxin). Both strains (or toxins) then react with the antiserum via different antibody populations involving different determinants. Alternatively, the two strains or toxins may share a determinant and react with the antiserum via a single antibody population. Moreover, several distinct determinant–antibody pairs may contribute to the reaction of the antiserum with both strains or toxins.

How can one discriminate between these various cross-reaction mechanisms? Only indirect methods can be used for this purpose which are cumbersome, such

as cross-adsorptions of the antiserum. However, even with these procedures, one cannot be positively certain about the cross-reaction mechanisms. Practically all this confusion is eliminated with the use of a monoclonal probe.

It has become evident that not only pathogenic bacteria deserve immunologic analysis but also bacteria that are useful in biotechnology and industry (8,40, 41) (see Chapter 11). Antibodies specific for these "useful" bacteria hold considerable potential. Monoclonal probes are needed for identifying them and, perhaps more importantly, for manipulating and utilizing them.

The generalization of genetic engineering for constructing microbes useful in a variety of ways (21,22,49) goes hand in hand with monoclonal antibody technology (31) and some of its recent variations (45). Monoclonal antibodies can aid in the identification of bacterial antigens that induce protective immunity and also in the genetic engineering techniques used to produce vaccines with these antigens (37,54) (see Chapter 2). The same antibodies can be instrumental in the biochemical purification of bacterial antigens by means of affinity chromotography. The quantities purified will be limited only by antigen availability since monoclonal antibodies can be produced in any desired amount for large-scale chromatography. Antigens purified by this means can be used for immunization or for diagnostic purposes such as measuring antibodies in biologic fluids.

B. Yesterday

The search for monoclonal antibodies against bacterial antigens began many years ago. Attempts at obtaining antibodies of restricted heterogeneity against pneumo- and stretococcal antigens *in vivo* (17,34) and against *Escherichia coli* β-D-galactosidase *in vitro* (43) were made in the 1960's. A systematic screening of myelomas was also carried out in search of monoclonal immunoglobulins with specificity for a variety of antigens including bacterial ones (51).

Important information was obtained utilizing these *in vivo* and *in vitro* systems concerning diversity, genetics, structure, and function of antibodies (17,34,51). Progress was also achieved toward lengthening antibody synthesis to several weeks in cultures of limited numbers of lymphoid cells (7). The cyclic character of the antibody response was first observed in these long-term cultures using a bacterial antigen (38), and some early insights into the mechanism responsible for the oscillatory dynamics of antibody titers and affinity were gained (7).

However, the solution to the practical problem of one's inability to produce unlimited quantities of homogenous antibodies for use as chemically "pure" reagents came with the advent of hybridoma technology in 1975 (31).

Bacterial serology can be viewed, historically, as encompassing two eras: pre- and post-hybridoma (42). The latter era has just dawned and will, no doubt, be long-lasting and fruitful. This book will cover the first period of the post-hybridoma era.

II. WHY AND WHAT FOR?

One reason for the importance of monoclonal antibodies against bacteria is their use in a wide variety of disciplines including medicine, veterinary sciences, dentistry, biotechnology, and industry. Despite this diversity of application, the basic strategies and methods for preparation, characterization, and use of monoclonal antibodies against bacteria and the categories of information obtained with their use are common to all areas. Each application in a given area differs from that in another only in detail. Thus, for example, a considerable part of what may have been done and learned concerning a pathogen for man, against which monoclonal antibodies have been generated, may be useful in preparing antibodies against a pathogen for any other animal species. Early knowledge gained in studying pathogens will help in the generation of monoclonal antibodies against useful strains for biotechnology. By the same token, strategies and procedures devised to examine and utilize bacteria for industrial purposes by means of monoclonal antibodies can be adopted to investigate etiologic agents of infectious diseases.

This treatise is intended to be a reference work in which those using, or planning to use, monoclonal antibodies against bacteria will find what is common to all areas as well as what is specific to each of them, or to each microorganism, or to each problem (molecular studies, diagnostics, ecologic surveys, epidemiology, biotechnology, etc.). The common materials will be directly useful, perhaps with slight modifications, to anyone working with monoclonal antibodies or getting ready to prepare them against the strain(s) (or bacterial structures) of his/her interest. Information unique to a given strain or problem should serve as a stimulus to the imagination of those interested in other bacteria, and/or in a different problem, to envision possible developments within their own restricted fields.

Monoclonal antibodies against bacteria are particularly important for the pathologist. Immunologic tests are often used for detecting and monitoring bacterial infections (11,63). Monoclonal antibodies are contributing to the improvement of current methods for measuring bacterial antigens and antibodies. These methods can be used for diagnosis, monitoring of infections and response to treatment, epidemiologic surveys, and for examining foods, water, soil, and other materials in search of environmental contamination with bacteria and their toxins. Monoclonal antibodies are also being tested in experimental systems to treat infection, i.e., in serotherapy by a general route (e.g., intravenously) or locally, and to neutralize bacterial toxins (2,4,15,16,18,20,23,25,26,29,36, 44,47,48,50,52–54,56–58,60–62,65,66).

A list of specific applications of monoclonal antibodies against bacteria follows.

A. Identification

Monoclonal antibodies are becoming prominent alongside antisera in the identification of new isolates (4,6,9,10,15,16,18,23,47,53,54,56,58,61,65) (see Chapters 4–6 and 11).

B. Classification

Immunologic classification of bacteria is of practical value. The coordinated use of antisera and panels of monoclonal antibodies is proving useful for classification as a diagnostic tool with prognostic implications in the case of pathogens, or as a preliminary step in taxonomy (8,18,26,40,42,47,62) (see Chapters 2–7, 11, and Volumes II and III). Definitive phylogenetic assignments require comparative analyses of nucleic acids and other studies, but definition of antigenic relationships helps at the beginning as an orientation. Immunologic tests also aid in sorting strains, i.e., at the infraspecific level, when other tests are no longer useful because they lack the sensitivity required at this level.

The potential of monoclonal antibodies used individually or as mixtures for bacterial identification and rapid classification is considerable. Time will tell to what extent these expectations are fulfilled.

C. Manipulation

Monoclonal antibodies hold great potential as instruments in working with bacteria for industrial or biotechnological purposes, including genetic engineering (see Section II,D below, Chapter 11, and Volume II). An antibody specific for a marker of a strain could be used as a selection tool (40). Positive and negative selections can be envisaged by means of affinity immunoabsorbents made of insolubilized antibody. The desired strain could be first allowed to bind to the immunoabsorbent, then eluted free from the other microbes, and grown as a pure culture. The same procedure could be utilized to eliminate an unwanted microbe from a mixture. Similarly, a monoclonal antibody could be utilized to stop growth of an unwanted strain by adding the antibody to a culture. The antibody and conditions would have to be adjusted so as to obtain a bacteristatic or bactericidal effect.

Industrial production of monoclonal antibodies against bacteria is another important area currently under development which must be distinguished from that involving the use of these antibodies for industrial or biotechnologic purposes. The manufacture of large quantities of antibacterial monoclonal antibodies for routine and widespread use in many areas (medicine, dentistry, veterinary sciences, industry, and biotechnology) is evolving into an industry of its own. The tactics for industrial production with commercial purposes are different from those in basic research. Also research and development endeavors in the

industrial world differ from research efforts in the academic environment (see Volume II).

D. Genetic Engineering

Monoclonal antibodies are instrumental in genetic engineering, first to identify "useful" antigens, for example, those inducing protective immunity, and then to help in the cloning of the genetic codes for these antigens in the preparation of vaccines or diagnostic kits (37,54).

E. Biochemistry and Molecular Studies

Elucidation of the chemical composition and structure of the antigen molecule bearing the determinant recognized by a monoclonal antibody, and eventually of the determinant itself, should become an important part of chemoimmunotaxonomy and other molecular studies (4,6,10,16,18,23,44,53,54,58,61,65) (see Chapters 1, 6–8, and Volumes II and III). For this purpose, panels of monoclonal antibodies show extraordinary resolution power, especially if the fine (molecular) specificity of the antibodies is known. In this case, the antibodies constitute a set of high-precision tools for probing molecular markers and for detecting these markers in a variety of materials. One can foresee the occurrence of monoclonal antibodies specific for a marker of a strain, or species, or higher taxon.

One can also envisage the use of monoclonal antibodies of predefined molecular specificity for tracing molecular "signatures" left by a given strain in other microorganisms, subcellular structures (e.g., mitochondria), and materials from ecologic niches, such as fossils and sediments, and from culture supernatants (see Chapter 11).

F. Physiology and Pathogenecity

Monoclonal antibodies against bacterial molecules are proving useful for the understanding of the physiologic and pathogenetic mechanisms in which these molecules, or the structures carrying them, are involved. Surface-recognition structures, surface receptors, pili, and toxins are particularly interesting for investigations aiming at establishing structure–function relationships (1–3,13,14,19,25,29,48,52,55,57,58,60,65,66) (see Chapters 1, 2, 8, and Volumes II and III).

G. Diagnostics

Although diagnostics is essentially identification and classification, i.e., topics already discussed (see Sections II,A and II,B), it is mentioned again because

of its practical importance and to emphasize its relevance to human and veterinary medicine and dentistry. In this regard, antibacterial monoclonal antibodies do have great potential. Preparation of diagnostic kits useful in the measurement of antibodies against bacterial antigens, or to detect these antigens in biologic fluids, exudates, and pathologic specimens, is becoming a major endeavor in the field of infectious diseases (42) (see Chapters 1, 3–7, and Volumes II and III).

H. Serotherapy

Antibacterial and antitoxin therapies with monoclonal antibodies administered locally or intravenously can be envisaged to replace, or to complement, serotherapy (2,25,29,33,35,48,52,57,58,66). For this purpose, it would be preferable to develop monoclonal antibodies homologous (if not autologous) with the animal species requiring serotherapy. Human monoclonal antibodies would be the best for man (25,33,35). However, this is still a problem under investigation and the difficulties are serious (see Chapters 8, 9, and Volumes II and III).

I. Prophylaxis

Induction of protective immunity by means of vaccines is a classic component of bacterial immunology. Monoclonal antibodies will probably greatly improve vaccine preparation procedures as mentioned in Section II,D. Two novel approaches must be mentioned. One is the use of monoclonal lymphokines and the other is the utilization of antiidiotypic and anticlonotypic antibodies (12,28,58). While lymphokines would stimulate cellular immunity, antiidiotypic and anticlonotypic monoclonal antibodies would mimic antigen (see Chapter 10 and Volume II). Instead of injecting antigen, monoclonal antibodies recognizing idiotopes of the protective antibacterial monoclonal antibodies or recognizing iodiotopes of T-cell clones secreting antibacterial lymphokines, i.e., anticlonotypic antibodies, would be administered. Since these antibodies possess a combining site resembling the bacterial antigen that elicits protective antibodies, or lymphokines, they would also elicit production of these protecting molecules. Such applications must obviously be approached with great caution (see Section IV on Warnings, below).

III. BEYOND B-CELL HYBRIDOMAS

Although the construction of B-cell hybridomas synthesizing monoclonal antibodies (31) is the landmark between the pre- and post-hybridoma eras and antibodies produced by B-cell hybridomas are the most commonly used thus far, it should be noted that there are other kinds of hybridomas. There are also antibodies produced by cell lines which are not hybridomas, and there are mono-

clonal products (lymphokines or "factors") which are not immunoglobulins but display immunologic functions. These latter monoclonal products are synthesized and secreted by non-B cells which are hybrids between a T cell, or a macrophage, and a malignant lymphoid cell (myeloma or lymphoma), or which are immortal lymphoid cells (not hybrids deliberately constructed in the laboratory) (12,27,28,33,35,51) (see Chapters 9 and 10). Within the category of monoclonal antibodies from nonhybrid cell lines, one can include those produced by B lymphocytes transformed by the Epstein–Barr virus (33,35) and those produced by human and mouse myelomas (27,51). Monoclonal antibodies of this kind displaying specificity for bacterial antigens have been found. Also, monoclonal products relevant to antibacterial immune responses have been reported which are not antibodies and are secreted by T-cell clones or T-cell hybridomas (12,28) (see Chapter 10).

While monoclonal antibodies against bacteria produced by B-cell hybridomas may represent the best example of monoclonal probes for diagnostic purposes, other monoclonal products provided by non-B-cell clones (hybridomas or not) may become the best agents for prevention and treatment of infectious diseases. Immunodeficiencies leading to bacterial infections may be amenable to correction by administration of lymphokines obtained in large quantities from monoclonal cell lines maintained in culture.

IV. WARNINGS

Monoclonal antibodies and lymphokines do indeed have a promising future in bacterial immunology. However, overoptimism concerning the future applications of these molecules may be dangerous.

Experience already shows that there are problems still unresolved concerning hybridoma technology. For example, the type of immunogen utilized to immunize the prospective donor of lymphocytes for fusion may influence significantly the outcome of fusion (see Chapter 3). One should be fully aware of this phenomenon when planning the construction of hybridomas. The immunogenic preparation may have to be entirely different, although from the same strain, depending on which antigens one wants to study by means of monoclonal antibodies.

The importance of choosing the appropriate assay for screening hybridomas in search of the desired antibodies cannot be overemphasized (see Chapters 4, 6, and 7). If one is looking for monoclonal antibodies that fix complement (C′) screening of hybridomas must be done with an assay that demonstrates the C′-fixing ability (or inability) of the antibodies. If antibodies recognizing the antigen molecule in its native configurations are sought, one cannot use denatured antigen in the test system. Examples of this kind are numerous, and they are dis-

cussed throughout this treatise. As a general rule, one should bear in mind that whenever possible one should use more than one type of assay for screening hybridomas and more than one antigen, e.g., the immunizing antigen and a cross-reacting one.

Mixtures of monoclonal antibodies as polyvalent reagents of a well-established specificity spectrum may become very useful for diagnostic purposes, for elucidating complex microbial communities, and for serotherapy (see Chapters 3, 8, and Volume II). It should be recognized, however, that antibodies in such mixtures may behave differently than when used individually. Interantibody influences have been demonstrated (24,46,64) which indicate that before preparing a mixture in quantities for massive use, each component must be studied individually and in the presence of the other components. This would be the best way of preparing mixtures in which only the correctly chosen antibodies are included in the right proportions to obtain unequivocal results.

The increasing number of laboratories that produce monoclonal antibodies and lymphokines as well as the growing number of bacterial species being studied are bound to yield a great number of these immunologically active molecules and a great deal of data. Much confusion can be expected (42). Perhaps some of this confusion, which will slow progress in the field, could be avoided if the investigators would collaborate with the organizers of a data bank (see Chapter 12).

Serotherapy with monoclonal antibodies will have to be carefully evaluated. The risk of injecting infectious agents such as viruses along with the antibodies will have to be considered since malignant cell lines are usually involved in the preparation of monoclonal antibodies and lymphokines (see Chapters 9 and 10).

The list of difficulties, drawbacks, and limitations concerning the preparation and utilization of monoclonal antibodies and lymphokines is much longer than suggested by the few examples discussed in this section. A better and more complete understanding of the problems inherent in hybridoma technology and monoclonal antibodies against bacterial antigens can be gained from reading the chapters in this and subsequent volumes. The authors have made an effort to highlight the limitations of their procedures and results while manifesting their hopes for the future concerning the potential benefits of the routine use of monoclonal products.

V. TOMORROW

Examination of data available at the present time concerning monoclonal antibodies and lymphokines against bacteria, many of which are described and discussed in this treatise by pioneers in the field, suggests a number of conclusive comments. The data also indicate developments for the near future.