VOLUME III

# Th
# Ant

Edit

Micha

# The Antigens

## VOLUME III

*EDITED BY*

MICHAEL SELA

*Department of Chemical Immunology*
*The Weizmann Institute of Science*
*Rehovot, Israel*

ACADEMIC PRESS New York San Francisco London 1973

*A Subsidiary of Harcourt Brace Jovanovich, Publishers*

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

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

Library of Congress Cataloging in Publication Data

Sela, Michael.
The antigens.

Includes bibliographies.
1. Antigens and antibodies. 2. Immunochemistry.
I. Title. [DNLM: 1. Antigens. 2. Immunity. QW570 S464a H2 V2 v. 1-2)
QR186.5.S44 574.2'92 73-799
ISBN 0-12-635503-7

PRINTED IN THE UNITED STATES OF AMERICA

# List of Contributors

*Numbers in parentheses indicate the pages on which the authors' contributions begin.*

G. L. ADA (189), Department of Microbiology, The John Curtin School of Medical Research, The Australian National University, Canberra, Australia

ANTHONY C. ALLISON (479), Division of Cell Pathology, Clinical Research Centre, Middlesex, England

WILLIAM H. BURNS (479), National Institute for Dental Research, National Institutes of Health, Bethesda, Maryland

P. L. EY* (189), Department of Microbiology, The John Curtin School of Medical Research, The Australian National University, Canberra, Australia

S. FERRONE (361), Department of Molecular Immunology, Scripps Clinic and Research Foundation, La Jolla, California

JOEL W. GOODMAN (127), Department of Microbiology, University of California, San Francisco, California

KLAUS JANN (1), Max-Planck-Institut für Immunbiologie, Freiburg-Zähringen, Germany

GEORGE E. KENNY (449), Department of Pathobiology, School of Public Health and Community Medicine, University of Washington, Seattle, Washington

DAVID G. MARSH (271), Division of Clinical Immunology, Department of Medicine, The Johns Hopkins University School of Medicine at the Good Samaritan Hospital, Baltimore, Maryland

* Present address: Max-Planck-Institut für Immunbiologie, Freiburg-Zähringen, Germany

M. A. PELLEGRINO (361), Department of Molecular Immunology, Scripps Clinic and Research Foundation, La Jolla, California

R. A. REISFELD (361), Department of Molecular Immunology, Scripps Clinic and Research Foundation, La Jolla, California

OTTO WESTPHAL (1), Max-Planck-Institut für Immunbiologie, Freiburg-Zähringen, Germany

# Preface

This is the third volume of a comprehensive treatise that covers all aspects of antigens and related areas of immunology, focusing its attention on the chemistry and biology of antigens as well as on their immunologic role and expression. Each contribution describes a particular subject in depth, keeping a historical perspective rather than dealing exclusively with developments of the past few years. It gives the reader an adequate key to the literature and at the same time summarizes succinctly the present status of the subject. Its ultimate purpose is to give an integrated picture that may help better understand immunologic phenomena.

The general plan of "The Antigens" is described in the Preface to Volume I. The first two volumes of this work were devoted to defined macromolecules as antigens and to immunoglobulins. The next two volumes are devoted primarily to more complex antigens and to antibodies.

The first chapter of this volume complements the antigenic macromolecules discussed in the previous volumes, as it gives a comprehensive and critical review of microbial polysaccharides as antigens. The second chapter deals with antigenic determinants and their specific reaction with antibody combining sites. The third chapter is devoted to the reaction of antigens with their specific receptors on lymphocytes. The next four chapters are concerned with several categories of more complex antigens which, in most cases, have not as yet been characterized adequately at a molecular level—allergens, histocompatibility antigens, antigens of Mycoplasmatales and chlamydiae, and animal viruses. They are all of crucial biologic importance, and progress in the elucidation of their structure and their biologic function is one of the exciting challenges to the immunologist.

It is a pleasure to acknowledge also on this occasion the cooperation of the staff of Academic Press in the preparation of this treatise.

*MICHAEL SELA*

# Contents of Other Volumes

## Volume I



## Volume II

# Contents

CHAPTER 1

# Microbial Polysaccharides*

KLAUS JANN AND OTTO WESTPHAL



## I. Introduction

Microbial polysaccharides are located on the cell surface and are, therefore, of importance in recognition and immune response of a

* This article is dedicated to Professor Michael Heidelberger, New York, the great pioneer of polysaccharide immunochemistry.

higher organism to microbial infection. These polysaccharides are either an integral part of the cell wall, as this is known to be the case with the somatic lipopolysaccharides of gram-negative Enterobacteriaceae, or they may form large extracellular capsules like those of *Pneumococcus*, *Klebsiella*, and many *Escherichia coli*.

Polysaccharides of microorganisms have been studied chemically for a long time and much is known about their structure. They can be pure polysaccharides or complex ones. The most prominent and best studied complex polysaccharides are the somatic lipopolysaccharides. The biosynthesis of a number of microbial polysaccharides and lipopolysaccharides has been studied, and a coherent picture of the mechanism of polysaccharide biosynthesis has emerged from these studies.

The first immunologic approach to bacterial polysaccharides was achieved by Avery (1915), Goebel (1935, 1938, 1939), and Heidelberger (Heidelberger and Avery, 1923, 1924; Heidelberger and Kendall, 1929; for a review, see Heidelberger, 1960) at the Rockefeller Institute between 1920 and 1940. This was when, after the predominance of bacterial toxins, the importance of bacterial polysaccharides as antigens was shown in the studies of the capsular polysaccharides of *Pneumococcus*. The protective effect of pneumococcal polysaccharide against pneumonia infection (Heidelberger and McPherson, 1943a,b; MacLeod *et al.*, 1946; Heidelberger *et al.*, 1946, 1947) was proof of the immunologic importance of bacterial polysaccharides. The detection of oligosaccharidic serologic determinants was followed by their use as artificial antigens when coupled to protein (Goebel *et al.*, 1934a,b). Antisera obtained with these artificial antigens reacted with the native polysaccharides and also with the whole bacteria.

The promising start of the immunochemistry of microbial polysaccharides coincided with the beginning era of antibiotics. There is no doubt that antibiotics solved the problem not only of infection due to *Pneumococcus* but also of many other infections, but by the same token they created the severe problem of bacterial resistance to antibiotics, thus limiting their application. The early and quite spectacular results of antibiotic treatment, which seemed so simple, slowed down further development of the immunology of bacterial polysaccharides.

The topic of microbial polysaccharides as antigens gained renewed interest when it was found that the serologic classification of *Salmonella*, as laid down in the Kauffmann–White Scheme (Kauffmann, 1954, 1961) was based on the fine structure of the bacterial surface

polysaccharides (for a review, see Lüderitz *et al.*, 1966a, 1968a, 1971). The pioneering work of the Rockefeller group was taken up again and extended; new techniques were introduced and new perspectives opened.

In recent years important contributions were made to the problem of immunogenicity of polysaccharides, mainly on dextrans (Kabat and Bezer, 1958) and pneumococcal capsular polysaccharides (Howard *et al.*, 1971a,b,c). It was found that most microbial polysaccharides can not be digested by mammalian enzymes so that they remain in the circulation and tissue for a long time. This became an important aspect in the immune response to microbial polysaccharides. Furthermore, antigenic specificity and the antigen–antibody reaction were analyzed in detail, which led to a better understanding of immunodominant sugars (Lüderitz *et al.*, 1966a) and antigenic determinants. In the course of these studies, which were carried out by many research groups, antibodies with restricted heterogeneity (Kunkel *et al.*, 1962; Krause, 1970; Haber, 1970) and myeloma proteins (Cohn, 1967; Cohen and Milstein, 1967; Potter, 1970) were found which are directed against microbial polysaccharides. This, of course, was a great help in studies of the kinetics of the antigen–antibody response and will be of importance with respect to its genetics. Also, the role of microbial polysaccharides in infection became more and more a topic of research, and in this context their relatedness to the polysaccharides of mammalian cells and to histocompatibility antigens has to be considered.

We review here the present state of microbial polysaccharide immunology by quoting relevant examples which, taken together, may give an up-to-date picture of results and problems in this field.

## II. Chemistry of Microbial Polysaccharide Antigens

### A. Methods

Chemical and physical methods may contribute to answer two questions: (1) What makes a polysaccharide immunogenic? (2) What is the chemical basis of its antigenicity (serologic specificity)? In any case it is desirable to know the structure of a polysaccharide as precisely as possible. Structural analysis has to clarify size and shape of the polysaccharide, its sugar composition, the sequence of

sugar components, and the nature of their linkage (position of substitution and anomeric configuration). In the following the most widely used methods are mentioned.

*Size and Shape.* In general use are the Svedberg method (Kabat and Mayer, 1961; Svedberg and Pedersen, 1940; Schachman, 1959; Jann *et al.*, 1965, 1968) in which sedimentation and diffusion constants are measured with the ultracentrifuge, and the molecular weight is calculated from the data obtained. Similarly, sedimentation equilibrium may be used (Kabat and Mayer, 1961; Jann *et al.*, 1965, 1968; Archibald, 1947; Yphantis, 1960). For estimation of molecular weight, comparative gel chromatography may be used, but this is not reliable, since, in contrast to proteins, polysaccharides are mostly extended and their shape may vary greatly. The shape of polysaccharides can be assessed using their intrinsic viscosity (Kabat and Mayer, 1961; Jann *et al.*, 1965, 1968) or light scattering. In contrast to these physical methods there exist chemical methods for the determination of the molecular weight (chain length) of polysaccharides such as reaction with [$^{14}$C]cyanide (Isbell, 1951; Moyer and Isbell, 1958) or periodate oxidation (Abdel-Akher *et al.*, 1951; Unrau and Smith, 1957).

*Sugar Composition.* For qualitative determinations paper or thin-layer chromatography on total hydrolysates are run. According to more recent recommendations, the liberated sugar components are reduced to polyols, peracetylated, and then determined by gas–liquid chromatography (Sawardeker *et al.*, 1967). With the use of admixed reference substances, such as peracetyl-xylitol, this method is frequently performed on a quantitative basis. For enzymatic analysis total hydrolysates can be used.

*Sequence.* In order to gain information as to the sequence of the sugar constituents, oligosaccharides have to be isolated by partial acid hydrolysis or special techniques like Smith degradation (Goldstein *et al.*, 1959, 1965), acetolysis (Hanessian and Haskell, 1964; Kocourek and Ballou, 1969), or continuous hydrolysis and dialysis of the products (Painter, 1960; Painter and Morgan, 1961; Galanos *et al.*, 1969a) followed by chromatographic separation. The oligosaccharides are then analyzed using a variety of chemical and enzymatic methods that depend on the nature of the material under study. Their structure can also be established by methylation and gas chromatography followed by mass spectrometry (Kärkkäinen, 1970). From the results of oligosaccharide analyses the sequence in the polysaccharide can be reconstructed. Frequently, the oligosaccharides are methylated, hydrolyzed, and after subsequent reduction

and acetylation subjected to combined gas chromatography and mass spectrometry (Björndal *et al.*, 1970a).

*Nature of Linkage.* In a polysaccharide each sugar unit may be linked in one of two anomeric configurations ($\alpha$ or $\beta$) to any of the free hydroxyl groups of the next sugar unit. Thus, the variety is so large that reliable methods for linkage analysis are necessary. One of them consists in the oxidation of the polysaccharide with metaperiodate, followed by measurement of consumption of the reagent and determination of the reaction products, such as formic acid, acetaldehyde, etc. This method, which has been reviewed extensively (Goldstein *et al.*, 1959; Smith and Montgomery, 1956; Hay *et al.*, 1965), is rather complex and leads to ambiguous results. Nevertheless, it is useful and, in combination with other methods, widely in use. Presently the best method for linkage analysis is a combination of methylation, gas chromatography, and mass spectrometry. The methylation technique for polysaccharides and oligosaccharides has been repeatedly modified and is now a simple and reliable method (Hakomori, 1964; Hellerqvist *et al.*, 1968; Björndal *et al.*, 1970a). The methylated polysaccharide is hydrolyzed and the products are transformed into volatile derivatives which are subjected to gas chromatography. The various peaks that are eluted from the column with an inert carrier gas can be directly analyzed in a mass spectrometer (Kärkäinen, 1970; Hellerqvist *et al.*, 1968). For certain components, such as hexuronic acids or amino sugars (Sandford and Conrad, 1966; Tarcsay *et al.*, 1971; Lindberg *et al.*, 1973a), special techniques have been worked out. In general it can be stated that linkage analysis in polysaccharides today has nearly reached perfection.

It is fair to say that the elaboration of the complete structure of any given polysaccharide is possible, in principle, and simply a matter of the availability and application of suitable refined analytic techniques.

### B. Bacterial Lipopolysaccharides

Lipopolysaccharides (LPS) are found in the outer membrane (plastic layer of the cell envelope) of gram-negative bacteria, where they form complexes with protein and phospholipid of the kephaline type (Romeo *et al.*, 1970, Rothfield and Romeo, 1971). They are the somatic antigens, and are thus the chemical basis for serologic classification of gram-negative bacteria (Kauffmann, 1966). Furthermore, they are receptors for many bacteriophages (Lindberg, 1973) and, therefore, play an important role in bacteriophage typing. Both properties of LPS are due to the polysaccharide moiety. Additionally,

LPS exert endotoxic activities for which the lipid moiety is responsible (Westphal *et al.*, 1952b; Eichenberger *et al.*, 1955; Lüderitz *et al.*, 1973).

Due to their manifold chemical and biologic aspects, LPS have been the subject of extensive studies through the last two decades. Consequently, many reviewing articles have been written (Lüderitz *et al.*, 1966a, 1968a, 1971; Nikaido, 1968, 1974; Osborn, 1969; Mäkelä and Stocker, 1969). For detailed information the reader is referred to these reviews.

## 1. Structural Features of LPS

It was found that the LPS of gram-negative bacteria, and especially those of enterobacteria are composed of three structural regions, I, II and III (Lüderitz *et al.*, 1971), as indicated in Fig. 1.

*Region I* is represented by the O-specific polysaccharide. This consists of oligosaccharide repeating units—a structural feature that seems to be common for most bacterial polysaccharides. This structural region of the LPS is distinguished by a great variability: many different sugar residues may be present in many combinations and glycosidic linkages.

*Region II* is an oligosaccharide that originally was found to be common for all *Salmonella* LPS and, therefore, called the "common core." It was found later that a number of different core oligosaccharides exist which are structurally closely related. These are but few, and there is a lower degree of structural freedom in region II than there is in region I. Through the core oligosaccharide the serologic R specificity is expressed.

*Region III* is the lipid moiety of the LPS which was termed lipid A. It is thought to be a structural component of the outer membrane of gram-negative bacteria. Thus the LPS are anchored in the outer membrane via their lipid component. Lipid A is a unique glycophospholipid, containing glucosamine, fatty acids, and phosphate. The structure of lipid A is practically the same in all enterobacteria.

As one compares the structure of many bacterial LPS, it will be obvious that structural variability becomes increasingly greater going from inner to outer parts of the molecule.

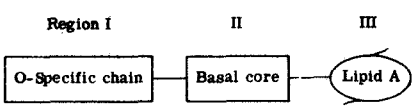


*Fig. 1.* Schematic diagram of the general structure of bacterial lipopolysaccharides. From Lüderitz *et al.* (1971).