The Antigens

VOLUME IV

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ACADEMIC PRESS New York San Francisco London 1977
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. 2. Immunochemistry. 3. Immune response. I. Arnheim, Norman. II. Title.
[DNLM: 1. Antigens. 2. Immunity. QW570 S464a]
QR186.5.S44 574.2'92 73-799
ISBN 0-12-635504-5

PRINTED IN THE UNITED STATES OF AMERICA

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Preface

This is the fourth 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 chapter describes a particular subject, presenting historical background and introducing recent developments. Its ultimate purpose is to give an integrated picture that may help better understand immunologic phenomena.

The first chapter complements those in the previous volumes which dealt with chemically defined antigens since it presents a comprehensive and critical review of lipids as antigens. The next chapter, on the immunology of antibiotics, belongs in the same category. The following two chapters are concerned with more complex antigens: bacteria and fungi. No effort is made to classify the huge number of bacterial antigens, but an attempt is made to correlate immunity and infection. Fungi are of great biologic importance, and progress in the elucidation of their antigenic structure is an exciting challenge to the immunologist.

The last three chapters deal with three important and timely areas of immunology: antigenic competition, adjuvants, and lectins. The lectins are plant proteins reacting specifically with markers on cell surfaces, and they resemble antibodies from several points of view. Adjuvants help the expression of antigens, or unique antigenic determinants, and they may influence also antigenic competition, that interesting immunologic phenomenon in which antigenic determinants within the same molecule, or in different molecules, compete for an efficient immune response.

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

MICHAEL SELA

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CHAPTER 1

Immune Reactions of Lipids and Lipid Model Membranes

CARL R. ALVING

Abbreviations

BAθ	brain-associated θ antigen
BSA	bovine serum albumin
CFA	complete Freund's adjuvant
CRP	C-reactive protein
CTH	ceramide trihexoside (see Fig. 2)
DDA	dioctadecylammonium bromide
DNP	2,4-dinitrophenyl
DTH	delayed-type hypersensitivity
L-BSA-DNP	lipid (dodecanoic acid)-BSA-DNP conjugate
LPS	lipopolysaccharide
PFC	plaque-forming cells
STS	serologic test for syphilis
Thy-1; θ	θ antigen
VDRL	Veneral Disease Research Laboratory serologic test for syphilis

Phospholipids (see Fig. 1):

CL	cardiolipin
GPE	glycerophosphorylethanolamine (completely lacks fatty acids)
lysoPE	lysophosphatidylethanolamine (contains only one fatty acid)
PA	phosphatidic acid
PC	phosphatidylcholine (lecithin)
PE	phosphatidylethanolamine
PG	phosphatidylglycerol
PI	phosphatidylinositol
PS	phosphatidylserine

Phospholipid conjugates (Six *et al.*, 1973; Nicolotti and Kinsky, 1975):

DNP-Cap-PE, **DNP-Cap-lysoPE**, **DNP-Cap-GPE**: dinitrophenylated aminocaproyl conjugates of PE, lysoPE or GPE

ABA-Tyr-PE, **ABA-Tyr-GPE**: mono(*p*-azobenzenearsonic acid)tyrosyl conjugates of PE or GPE

Gangliosides: G_{M3}, G_{M2}, G_{M1}, G_{D1a}, G_{D1b}, and G_{T1} are illustrated in Fig. 5

I. Introduction

A. Definitions

It is well known that lipids are fats and fatlike substances, but the limits of this class of compounds have never been distinct. A good general description is that given by Duell (1951): "The lipids include all those substances which are insoluble in water but soluble in the so-called fat solvents (diethyl ether, petroleum ether, chloroform, hot alcohol, benzene, carbon tetrachloride, acetone, etc.) which are related either actually or potentially to fatty acid esters, and which at the same time are utilizable by the animal organism. The latter qualification is essential to exclude mineral oil derivatives. This definition should not be interpreted too rigorously, or it would exclude certain compounds long considered to be members of this group. Thus, . . . lecithin is insoluble in acetone, cephalin in alcohol, while the sphingomyelins and the cerebrosides are insoluble in such a widely accepted fat solvent as diethyl ether." The above reference should also be consulted for a complete classification of lipids. Some judgment has to be used in this area, since it is clear that a large number of natural and synthetic drugs in the modern pharmacopoeia, and even certain hydrophobic membrane-associated proteins or oligopeptides now would fall under this definition. Because of these developments, older distinctions have become somewhat blurred. This review, therefore, will consider generally only the classic lipids, both as antigens and in ancillary roles, and this includes phospholipids, simple glycolipids (usually not more than five sugars), neutral lipids (such as cholesterol), fatty acids, and fat-soluble vitamins.

In Figs. 1 and 2, the structures of some of the common phospholipids (often referred to also as phosphatides) and neutral glycosphingolipids are illustrated. The glycerophosphatides have phosphatidic acid as a basic structure, while the sphingolipids, such as sphingomyelin or glycosphingolipids, all contain sphingosine. Ceramide is sphingosine to which an amide-linked fatty acid has been attached. Other lipids will be shown in appropriate sections.

B. Historical Aspects and Recent Developments

Until recently the field of lipid immunology has been greatly dependent on the contributions of several outstanding chemists and immunochemists whose major interests involved research on brain

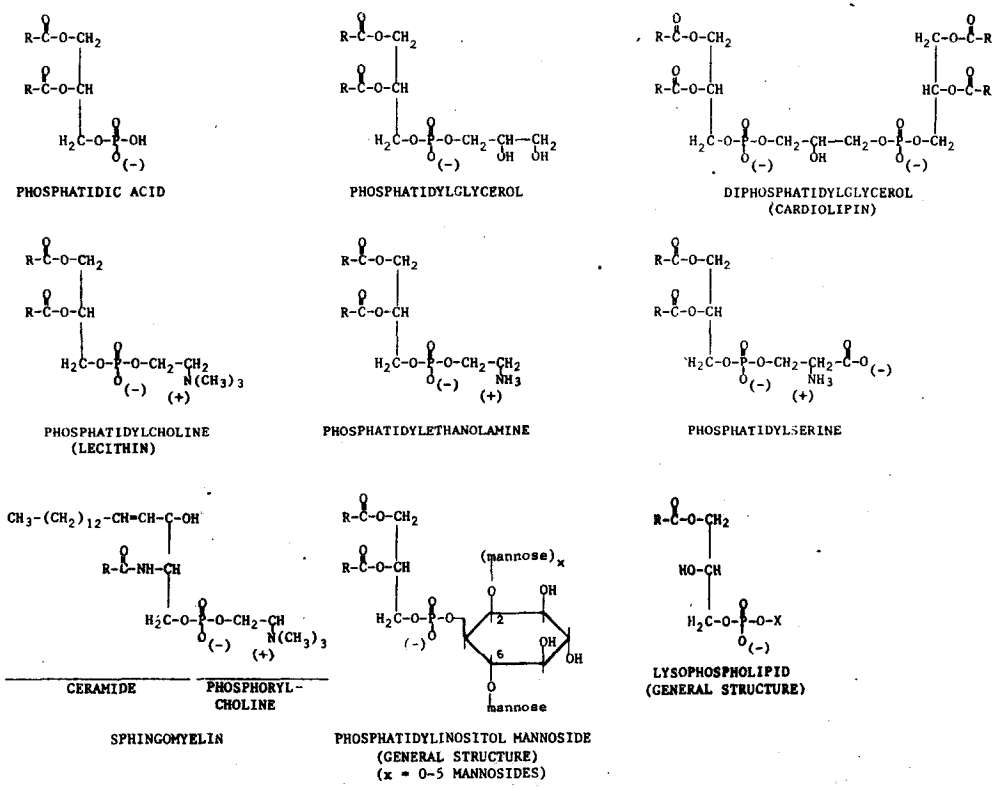

Fig. 1. Structures of phospholipids.

tissue, syphilis antigen, or red blood cells. Many of the lipids and lipid antigens that have been historically important were detected long ago, and have been the subjects of prolonged, and sometimes controversial, investigations.

Perhaps the largest early debt is owed to the great neurochemist, J. L. W. Thudichum, who, beginning in 1874 during the course of studies on brain tissue, discovered, characterized, and named many of the lipids which have been found to be most important in lipid immunology. These include phosphatides (as a type of compound), and specifically phosphatidic acid, sphingomyelin, and cephalin (now called phosphatidylethanolamine), and glycosphingolipids (as a type of compound), and specifically galactocerebroside and sulfatide (galactocerebroside sulfate) (Thudichum, 1884).

One of the most important early discoveries was the finding by Wasserman (1906) that antibodies against lipoidal extracts of various

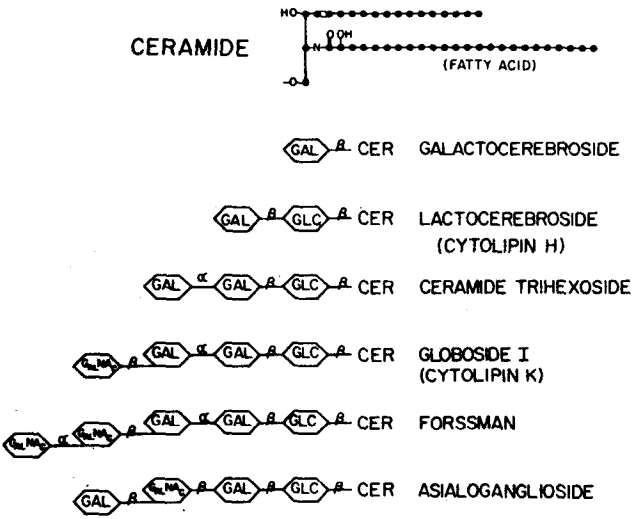

Fig. 2. Structures of neutral glycosphingolipids. Abbreviations used: GLC, glucose; GAL, galactose; GalNAc, *N*-acetylgalactosamine; CER, ceramide.

tissues appeared during the course of syphilitic infection. This discovery became the basis of a standard serologic test for syphilis. Subsequently, the most important haptenic lipid involved in this reaction was identified as a phospholipid, called cardiolipin (Pangborn, 1942). As will be discussed later, research on cardiolipin immunology still is thriving and, if anything, is becoming more important from a biologic viewpoint.

Another landmark discovery of an important antigen was made by Forssman (1911). The "Forssman antigen" is mainly responsible for hemolysis of sheep erythrocytes in the presence of antiserum (hemolysin) and complement. This has served for decades as the basis for most clinical and experimental complement fixation assays. Forssman antigen is a heterophilic substance found in sheep erythrocytes and a variety of tissues from other species and has been recognized for a long time as a lipid (Taniguchi, 1921a,b; Landsteiner, 1945), but has been characterized only recently (Section IV,B,3).

The common characteristic of these lipid substances is that they are associated predominantly with membranes. This was largely responsible for their early detection (i.e., by hemagglutination, hemolysis, flocculation, etc.), but this also was responsible partly for the notable difficulties that have been experienced in their isolation and complete description of their chemical and immunologic properties. Furthermore, the lipids have structures that frequently are sufficiently simple

to allow cross-reactivity with a number of other substances. An example of this is cardiolipin, which may cross-react not only with many cardiolipin analogues, but also with phosphatidylinositol, and even with DNA and RNA (Guarnieri and Eisner, 1974) (Section V,C).

The greatest barriers to advancement in the field of lipid immunology have always been the chemical and physical properties of the lipids themselves, particularly water insolubility. The problem of reactivity of soluble antibodies with lipid antigens was partially overcome by the inclusion of "auxiliary lipids" such as lecithin and cholesterol in the antigen suspension (reviewed by Landsteiner, 1945; Rapport and Graf, 1969; Niedieck, 1975a). This technique is still commonly employed for "solubilizing" lipid haptens into a membrane, or membranelike, surface which holds the haptenic polar portion of the lipid in such a configuration as to make it accessible to antibody binding (reviewed by Rapport and Graf, 1969; Niedieck, 1975a).

As will be shown below, many studies have demonstrated that mixtures of lipids in the form of "liposomes," consisting of concentric spherules of lipid bilayer membranes, may serve similar functions as auxiliary lipids, and can mimic closely, or exactly, many of the immunologic aspects of intact cell membranes (Haxby *et al.*, 1968). Using these model membranes, it has been possible to probe certain aspects of membrane function that could not otherwise be as easily investigated, such as the roles of membrane composition on antibody binding, complement activation, membrane damage, and immunogenicity of membrane-associated lipid haptens. The invention of liposomal membranes thus has turned the problem of insolubility of lipids into a virtue.

In the past few years, remarkable technological advances have been made in the field of lipid chemistry. This has led to increased interest in studies on the recognition, isolation, purification, characterization, and even synthesis of many simple lipoidal substances that are important in immunology. A few of the many interesting examples of recent technical and theoretical advances include total synthesis of galactocerebroside (Shapiro and Flowers, 1959), cytolipin H (lactocerebroside) (Shapiro and Rachaman, 1964) and related simple sphingolipids; total synthesis of cardiolipin (de Haas and van Deenen, 1965); complete characterization of Forssman antigen as a ceramide pentahexoside (Siddiqui and Hakomori, 1971); identification of theta-like antigens of mouse brain and mouse thymocytes as two closely related gangliosides, G_{D1b} and G_{M1} (Esselman and Miller, 1974a; Miller and Esselman, 1974, 1975); and synthesis either of protein–fatty acid, or hapten–protein–fatty acid conjugates (Coon and Hunter, 1973, 1975; Dailey and Hunter, 1974), or simple hapten–phospholipid conjugates

(Nicolotti and Kinsky, 1975; Kochibe *et al.*, 1975), which have been used in methods to study the molecular basis of trafficking patterns of antigens, and immunogenicity of antigens with regard to humoral versus cellular immunity.

II. Nonliposomal Lipid Model Membranes

A. *"Auxiliary" Lipids in Immune Reactions*

It has been recognized for more than 60 years that "auxiliary" or "activator" lipids, such as phospholipids and cholesterol, are necessary in order to create optimum conditions for immune reactivity of most simple lipid haptens (Browning *et al.*, 1910; Brown, 1944; reviewed by Landsteiner, 1945; Rapport and Graf, 1969). Although additional lipids are not always absolutely required for antibody binding, almost invariably a marked stimulatory effect is observed in immunologic assay procedures. This can be detected by precipitation or flocculation phenomena, and the auxiliary lipids are particularly important for complement fixation (Browning *et al.*, 1910; Taniguchi, 1921a,b; Kent, 1940; Brown, 1944; Maltaner and Maltaner, 1945; Papirmeister and Mallette, 1955; reviewed by Rapport and Graf, 1969; Niedieck, 1975a). In most studies lecithin and cholesterol have been used as auxiliary lipids, but a variety of other simple lipids have been effective, including: other phospholipids, galactocerebroside, sulfatide, globoside I, fatty acids, and lipid detergents (Mægli and Mallette, 1964; Makita *et al.*, 1966; Rapport and Graf, 1969; Niedieck, 1975b). These auxiliary lipids were used either by themselves, or in combination with cholesterol, but cholesterol alone was ineffective (Maltaner and Maltaner, 1945; Makita *et al.*, 1966).

For many years the exact role of auxiliary lipids was poorly understood, although certain empirical reasons for using them were clear. In order to obtain measurable precipitation due to antigen–antibody binding, lecithin (or other auxiliary lipid) was needed, although cholesterol was not necessarily required. Furthermore, with certain acidic lipid antigens, such as cardiolipin, the antigen alone was "anticomplementary," and this deleterious effect was eliminated by the inclusion of lecithin or lecithin and cholesterol (Maltaner and Maltaner, 1945; Niedieck, 1967). When combined with lecithin and lipid hapten, cholesterol also had a stimulatory effect on antibody-dependent complement activation (Browning *et al.*, 1910; Kent, 1940; Rapport and Graf, 1969), and possible mechanisms for this will be discussed in detail later (Section III,C,5).