Antigens, Lymphoid Cells, and the Immune Response

G J V NOSSAL and G L ADA

IMMUNOLOGY *An International Series of Monographs and Treatises*

ANTIGENS, LYMPHOID CELLS, AND THE IMMUNE RESPONSE

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FOREWORD

This book inaugurates the international series of monographs and treatises on "Immunology" which deals with major topics of current interest in the ever-expanding field of immunology. It marks a new venture by the Editors of the *Advances in Immunology* which aims to provide an adjunct series covering in greater depth the most timely and most important subjects in this discipline. Many of the outstanding immunologists who will be involved have already contributed chapters to the *Advances*. Immunology has become such a hybrid science, impinging on such variegated areas, that a distinct need has developed among its students for detailed coverage of central topics.

It is amply fitting that the series begins with a volume from one of the world's foremost immunology laboratories. The subject, too, is most appropriate since it represents such a central and fundamental theme of immunology. A high standard has been set and, if similar realization stems from subsequent volumes, the success of the series is virtually guaranteed.

F. J. Dixon, Jr.
H. G. Kunkel

PREFACE

The aim of this book is to present an up-to-date picture of what is known about the manner in which antigens stimulate an immune response. Such a monograph seems necessary because a large amount of precise and valuable information has accumulated in a short time and has not before been fully collated. We hope that the volume will be of value to a diverse group of readers. It is primarily directed to research workers, post-doctoral fellows, and graduate students in the field of cellular immunology. However, the field of immunology has such wide ramifications into spheres such as clinical medicine and surgery, biochemistry, and microbiology that few professional biologists or doctors can afford to ignore it entirely. We hope that our analysis of the interactions between antigen molecules and lymphocytes will suggest to students analogies to other biological information and control systems. We hope, also, that it will demonstrate to the biochemists and immunochemists who have so revolutionized the antibody problem over the last decade that their biological counterparts have also not been idle, and that the two schools can learn from one another.

Our volume deals with the nature and properties of antigens and with the functional anatomy and cell physiology of the mammalian lymphoid

system which responds to antigens. The focal point of our attention is the confrontation between antigen molecule and responsive lymphocyte, and the crucial decision which must then be made between immunity or immunologic tolerance. While there is a conscious bias toward studies conducted in our own laboratories, we hope that we have summarized and integrated all the key information gathered on these topics since 1963. The scope and treatment of the subject matter and the kinds of technical information which are presented are fully summarized in Chapter 1. A speculative synthesis of the field in the final chapter should, we hope, be of fairly general interest.

It became clear to us in writing the volume that one of the key ways in which this book differs from most others in immunology is that it and the work it summarizes represent real collaboration between biologist and biochemist. We feel that this combined focusing of different background skills has not only enriched our professional lives, but represents the key to progress in our understanding of the life process for the foreseeable future.

G. J. V. Nossal
G. L. Ada

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CHAPTER 1

INTRODUCTION: CENTRAL QUESTIONS IN CELLULAR IMMUNOLOGY

The decade of the 1960's brought a remarkable change to immunology. From the discovery of the multichain nature of the immunoglobulins, progressively more detailed knowledge of their structure soon followed, and the realization that Bence-Jones proteins were homogeneous immunoglobulin light chains stimulated extensive investigations into the amino acid sequence of first light and later heavy immunoglobulin chains. In 1969, the first sequence analysis of a complete immunoglobulin molecule became available, so that it is reasonable to conclude that the problem of antibody structure is nearing a definitive solution. In fact, it is becoming evident that many investigators who have contributed to our knowledge of antibody structure are turning their attention to cellular immunological problems. The way in which antigen affects lymphocytes and activates antibody production is still far from being fully understood, but methods of increasing sophistication have recently become available to help accelerate progress. While the main chemical truths about antibody structure are generally known to and accepted by cellular immunologists, it appears that the key discoveries about lymphocytes and antibody-producing cells made during the 1960's have

been less fully digested by immunochemists. Thus we felt that, both from the point of view of advanced students in immunology, and of workers in immunochemistry wishing to delve deeper into cellular problems, the time was ripe for a book which summarized current knowledge about antigens, lymphoid cells, and the immune response. In particular, we will concentrate on the induction of antibody formation. We felt 1970 to be an appropriate publication time because studies on the fate of antigen reached a new stage of precision and value when radioautographic techniques were applied to them. This approach has now been in wide use for about 7 years, and while it is clear that it will continue to yield fruitful information, we believe that the broad principles determining the localization of injected antigen molecules have now been fairly fully uncovered and should be summarized.

The dominant research theme in the authors' laboratories over the past several years has been to seek an answer to the question: how does antigen work? In any broad overview of the problem of antibody formation it is usual to divide the immune response into three major compartments: (1) afferent; (2) central; (3) efferent. The first compartment is concerned with the mechanisms by which the "antigenic message" is brought to the lymphoid system which eventually responds by antibody production. The second deals with the origin, nature, and function of lymphoid cells and with the key question of the genetic basis of the information necessary for the synthesis of antibodies. The third covers the cellular events which follow an effective encounter between antigen and reactive lymphocyte, including the intervening steps of cell multiplication, differentiation, and migration. In this context, the main emphasis in this book will be on the afferent component. However, as our story unfolds, it will become increasingly clear that the above separation of the problem into three compartments is highly arbitrary and that the inductive function of antigen can only be understood if due regard is given to certain aspects of the central and efferent limbs as well.

When we commenced our series of investigations on the role of antigens in immunity in 1963, the tracing of radioactively marked antigen molecules through the body of an injected animal already had a long history. Yet, the yield of information truly relevant to inductive mechanisms had been slender. Nevertheless, it seemed to us that a detailed knowledge of where antigen was, and (perhaps more importantly) where it *was not*, during critical stages of immunological events, constituted information worth possessing. If nothing else, such information was a *sine qua non* for an eventual complete appreciation of inductive

mechanisms. However, as we thought about the matter in 1963, two flaws in the majority of the then available literature on distribution of radioactive antigens became apparent to us. First, far too little attention appeared to have been given to questions of the nature, dose, and specific activity of the labeled antigens. Frequently large quantities of lightly labeled material had been injected with obvious loss of sensitivity; and the antigens chosen were materials of low inherent immunogenicity and possessing a tendency to permeate widely through extracellular fluids, resulting in high "noise levels." Second, relatively little use had been made of the very powerful tool of radioautography, let alone quantitative or high-resolution variants of the technique. We thus decided to design an extensive set of investigations using chiefly a pure protein antigen, flagellin, and its various polymerized and fragmented versions, rendered radioactive to as high a substitution level as practicable with the external label ^{125}I. The advantages and disadvantages of this type of labeling will be discussed in Chapter 4.

We will make no attempt at an exhaustive review of the extensive early literature on antigen tracing, as this has already been dealt with by Campbell and Garvey (1963) and we will not cover problems of delayed hypersensitivity or allograft rejection. However, immunological memory and immunological tolerance will naturally be considered. The many uncertainties which continue to surround cellular immune phenomena complicate considerably the task of authors wishing to deal logically with the subject. It is clear that the two broad categories of cells involved in interacting with antigens are phagocytic and lymphocytic cells, but the detailed relationships *between* the two categories remain problematical. For this reason we have deemed it best to discuss these two cell categories separately before attempting any speculative synthesis. Thus Chapters 4, 7, and 8 will be chiefly concerned with the reticuloendothelial cells which capture antigens, and 5, 6, 9, and 10 with lymphocytes and the effects which antigens may have on them. These seven chapters are preceded by two fairly general ones which discuss the nature of antigens and antibodies from the viewpoint of their function in the afferent limb of the antibody response. There has been a great expansion of interest in antibody production *in vitro* over recent years; while certain *in vitro* studies will be considered in Chapters 2 through 10, and particularly in Chapter 8, we hope to summarize most of the new knowledge on this subject in Chapter 11.

In Chapters 2 through 11 we will concentrate mainly on experimental findings and their immediate implications. In Chapter 12, we present a speculative synthesis of currently available information on mechanisms