ADVANCES IN
Immunology

EDITED BY

FRANK J. DIXON HENRY G. KUNKEL

VOLUME 28

1979

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New York, New York

VOLUME 28

1979

ACADEMIC PRESS

A Subsidiary of Harcourt Brace Jovanovich, Publishers

New York London Toronto Sydney San Francisco

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 7DX

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 61-17057

ISBN 0-12-022428-3

PRINTED IN THE UNITED STATES OF AMERICA

79 80 81 82 9 8 7 6 5 4 3 2 1

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PREFACE

The subjects reviewed in this volume range over the field of Immunology from basic experimental aspects to clinical considerations. The initial areas include the genetic basis of immunologic function and the substances involved in its control. Next is an overview of basic immunopathologic processes responsible for immunologic disease. Finally reviewed are two of the most common and important environmental stimuli, bacterial endotoxins and parasites, that interact with immunologic defenses. Characteristic of current thought in Immunology, the authors of this volume have emphasized the molecular and cellular aspects of their subjects, thereby providing fundamental explanations of the complicated events they discuss.

The extreme complexity of the regulatory mechanisms by which cells of the lymphoid system interact was not anticipated a few years ago. Now it is clear that both antigen-specific and nonspecific stimulation and suppression are part of the immunologic scheme. In the first article, Drs. Tada and Okumura draw extensively on their own research in presenting a clear and detailed account of antigen-specific T cell regulatory factors. Particularly helpful is the perspective in which the authors place the many and often conflicting published reports on this subject. Also, their consideration of the contributions of the genes from both the Ig V and the I regions in determining antigen-specific T cell regulatory function provides an attractive hypothesis and indicates the further experimentation necessary for confirmation or disproof.

It is becoming clear that immune complexes, the inevitable consequence of an antibody response, are important in immune processes far beyond their well-established role as mediators of tissue injury. In the second article, Drs. Theofilopoulos and Dixon describe the interaction of immune complexes with complement and with the cells of the immune system, the functions of which they profoundly influence. As might be anticipated in immunologic events, the effect of immune complexes depends to a great extent on their antigen–antibody ratio so that their influence, either stimulatory or suppressive, is itself modulated by quantitative aspects of the immune response. The development of numerous techniques for the detection and quantitation of immune complexes has stimulated clinically related research and expanded the list of diseases in which immune complexes appear to play an important role. An extension of this diagnostic technology is the ability to isolate immune complexes and, in turn, their antigenic com-

ponent, thereby making it possible to identify the antigens involved in immune processes of a great many diseases, including those of unknown etiology.

The human major histocompatibility complex-controlled equivalents of the murine Ia antigens are restricted in cellular distribution, related to differentiation, and strikingly associated with susceptibility to certain diseases. Drs. Winchester and Kunkel in the third article discuss conceptual and technical developments in the field of human Ia antigens by describing the chemical structure and immunologic relationship to their murine counterparts. The distribution of human Ia antigens is limited primarily to the B lymphocyte series, stimulated T cells, and stem and precursor hematopoietic cells, suggestive of both cell type and differentiation specificity. Finally, the association of certain Ia antigens with susceptibility or resistance to a variety of diseases, including rheumatoid arthritis, systemic lupus erythematosus, idiopathic thrombocytopenic purpura, rheumatic fever, multiple sclerosis, and diabetes, provides both a valuable clinical predictive measure and a lead for the future study of pathogenetic factors possibly related to the particular Ia genes involved.

Exposure to bacterial endotoxins results in a prompt and profound immune reaction, a response of obvious survival value. The nature of endotoxins and their interactions with cells of the immune system are the subjects of the fourth article, by Drs. Morrison and Ryan, both prominent investigators in this field. The direct interaction of endotoxin with B lymphocytes, leading to the formation of antibodies to endotoxin as well as a spectrum of other immunoglobulin molecules, is considered in detail. In addition, the interactions of endotoxins with T cells and macrophages and the associated immunoregulatory effects are discussed. The central role of the lipid A portion of the endotoxin molecule in cellular activation and the molecular events occurring at the cell surface in the course of stimulation are presented. Finally, the potential of endotoxins as therapeutic manipulators of the immune response in man is evaluated.

Immunoparasitology, a field of great potential importance, is just beginning to attract attention. In the last article of this volume, Dr. Mitchell discusses this subject, emphasizing the general elements involved in resistance to parasitic infection and the characteristics of these infections that make them likely targets of immunodiagnosis, immunoprophylaxis, and/or immunotherapy. Consideration of work using current immunologic technology in the study of immune responses to parasitic infections is limited primarily to the mouse. The characteristic elements of these responses, such as hypergamma-

globulinemia and eosinophilia, are discussed in detail, as well as the less well-recognized immunosuppression, which accompanies a number of parasitic infections. This article should provide important background material for immunologists who might consider turning their attention to this new field.

We wish to thank the authors for their efforts in writing these reviews and the publishers for their painstaking preparation of this Volume.

FRANK J. DIXON
HENRY G. KUNKEL

CONTENTS

ADVANCES IN IMMUNOLOGY, VOL. 28

The Role of Antigen-Specific T Cell Factors in the Immune Response

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I. Introduction

We are now just beginning fully to realize that the immune response is achieved via a complex series of interconnected cellular events initiated by antigenic stimulation rather than the activation of a single isolated clone of lymphocytes. The initial concept of interaction of thymus (T)-derived cells and bone marrow (B)-derived cells has been considerably broadened to incorporate macrophage–T cell, T cell–T cell, and T cell–B cell interactions, which as a whole constitute the network of an immunoregulatory system.

ISBN 0-12-022428-3

There has been a well known dichotomy in explaining the interconnectedness of the immunocompetent cells. The network concept originally formulated by Jerne (1972, 1975, 1976) explains it by idiotype–anti-idiotype interactions between lymphocytes. On the other hand, there have been abundant experimental data indicating that cell interactions are governed by genes in the major histocompatibility complex (MHC) (reviewed by Katz and Benacerraf, 1976; Benacerraf and Katz, 1975; Benacerraf and Germain, 1978; Rosenthal and Shevach, 1977; Miller and Vadas, 1977). One of the central issues in both concepts is the molecular mechanism whereby antigen-specific signals are conveyed from one component to the other. On the basis of the above dichotomy, it is predicted that if such mediators do exist, they should be able to recognize both antigen and target cells to which they make meaningful and unmistakable communications. Investigations performed in the last decade, in fact, resulted in the identification and characterization of such biologically active molecules produced by T cells, which we call antigen-specific T cell factors. They are able to bind to antigen (or perhaps to idiotype in some cases) and are capable of activating other cell types of the restricted nature (either B or T cell syngeneic or allogeneic). Some of them enhance the antibody responses by B cells, whereas others suppress both humoral and cell-mediated immune responses. In certain experimental systems, there have been found strict genetic restrictions imposed by genes in MHC, whereas in other conditions only the antigen specificity is the restricting element. There are overt similarities and differences between the factors reported by different investigators, which may primarily be caused by the structure of molecules, experimental conditions, and even by artifactual observations. In addition, there are many other "factors" that nonspecifically modulate the immune response, whose participation in the observed net effect of antigen-specific factors is obvious. We shall, however, restrict ourselves to describing the T cell factors that unequivocally possess antigen specificity as determined by the criteria given below.

The term "factor" is by no means a clear definition of the molecules. It merely designates a heterogeneous group of molecules having distinctive immunologic functions whose molecular entity is still ambiguous. In fact, none of these antigen-specific factors has been either isolated or characterized chemically beyond localization of activity in column effluents. Nevertheless, these antigen-specific factors seem to provide important clues for solving most currently controversial problems in immunology. Since it is unlikely that T cells would release antigen-specific molecules other than T cell surface recognition struc-

tures, they are obviously the most likely candidates for the antigen receptor of T cells. Their definite functions in positive and negative immunoregulation also suggest that they represent physiologically legitimate mediators of specific T cell functions.

The purpose of this review is to bring together the available information on these T cell factors, albeit controversial and conflicting at the moment, and to examine their roles in cell interactions in the immune response. We shall, therefore, confine the discussion to considerations of those factors defined by their definite antigen specificity and functional characteristics rather than those separated and characterized by immunochemical means.

II. An Overview

Shortly after the discovery of T–B cell collaboration in the antibody response, several investigators predicated the antigen-specific receptor on helper T cells, which acts to focus antigen onto corresponding B cells (Mitchison and Rajewsky, 1974; Mitchison *et al.*, 1971; Taylor, 1969). Such a conceptual receptor of helper T cells is sometimes designated as IgX and has provided a basis for the development of the theory of "carrier antibody" (Bretscher and Cohn, 1968). By extension, Bretscher (1972) proposed a hypothesis in which he assumed the presence of a T cell immunoglobulin (Ig) or a factor that gives a second signal to B cells that have reacted with antigen (two-signal hypothesis). This hypothesis postulates that both the Ig receptor and some receptor for T cell-derived helper factor as part of B cell surface membrane are capable of signal transmission. These receptors differ from each other insofar as the Ig receptor conveys a tolerogenic signal by the binding of the haptenic determinants of the antigen, whereas the other receptor, being capable of interacting with the factor by virtue of the antigen-binding capacity of the factor, stimulates B cells toward differentiation and antibody synthesis. This hypothesis certainly stimulated the survey for the molecule relevant to the theory. A similar concept as a negative counterpart of IgX was presented by Gershon and Kondo (1971), when they first demonstrated the infectiousness of tolerance caused by suppressor T cells. As the suppression they observed was clearly antigen specific and mediated by T cells, they called the possible mediator for the suppression as IgY as opposed to IgX.

An actual demonstration of an antigen-specific factor having biologic activity was made by Feldmann and his co-workers (reviewed by Feldmann and Nossal, 1972; Feldmann, 1974a,b). They described

several features of an antigen-specific molecule of the immunoglobulin nature produced by T cells. The molecule termed IgT was capable of mediating specific helper activity in the *in vitro* antibody response, and almost ideally fit the idea of Bretscher and Cohn. It carries κ- and μ-chain determinants, and is considered to be the monomeric form of IgM. Coinciding with the detection and isolation of monomeric IgM from T cells of some animal species (Marchalonis *et al.*, 1972; Cone and Marchalonis, 1974; Szenberg *et al.*, 1974), the model of T–B cell collaboration and regulation by IgT attracted much attention from immunologists. The extensive studies by Feldmann and co-workers, indeed, gave very clear accounts of various facets of cell interactions involved in antibody formation, immunologic tolerance, antigenic competition, and specific suppression, as will be examined in Section IV. This attractive model, however, confronted serious difficulty as several other investigators failed to detect μ and κ chains on T cells by very sensitive methods. In addition, serum IgM antibody in a small quantity was also found to enhance the antibody response under certain circumstances (Henry and Jerne, 1968). However, we think that these do not constitute major objections to the presence of IgT, since other biologically active T cell factors discussed in this review are also undetectable by usual chemical means. It would be fair to state that there exist certain difficulties in concluding that IgT is the only or most important mediator from helper T cells. We should only remain alert for an alternative explanation of IgT, since there are a number of similarities between IgT and other helper factors, including that recently described by Feldmann and co-workers (1977b).

The second category of antigen-specific T cell factors comprises mostly, if not entirely, a small molecular weight protein of nonimmunoglobulin nature having either helper or suppressive activity. Such molecules are released or extracted from antigen-stimulated T cells of known biologic functions. One of the first demonstrations of such factors was made in the suppression of IgE antibody response in the rat (Tada *et al.*, 1973). This was followed by a demonstration of antigen-specific helper or cooperative factor by Taussig (1974) in a much better defined experimental system of mouse IgM antibody formation. Both suppressor and helper factors were first demonstrated in *in vivo* experiments, and later extensively analyzed in *in vitro* experimental systems. Thus the relevance of these factors in *in vivo* immune responses is obvious.

The most remarkable feature of this category of T cell factors is that they possess no known immunoglobulin constant region determinants despite their exquisite antigen specificity. They are able to bind anti-

gen with specificity and affinity comparable to those of serum antibodies and can be eluted similarly from antigen-coated columns to purify antibodies. Further analysis of these T cell factors by different investigators eventually led to the same conclusion—that the factors possess determinants coded for by genes mapped in the *I* region of the mouse major histocompatibility complex (MHC). Since several workers in this field have utilized well defined synthetic polypeptides to which immune responses are under the control of Ir genes, the argument concerning the relationship between these *I* region determinants (Ia) bearing T cell factors and functionally defined Ir genes has been reiterated during the last several years. However, this argument was unfortunately not very fruitful, since we now notice much more confusion than agreement on this issue (see Sections III,C and IV,D). Nevertheless, we have been considerably enlightened with respect to the genetics of immunoregulation by studies on these T cell factors, as the structural genes coding for the determinants on helper and suppressor factors have been unambiguously mapped in *I-A* and *I-J* subregions, respectively. Furthermore, there have been some important discoveries on the consequences of cellular events initiated by these T cell factors. We discuss diverse pathways of the action of T cell factors with respect to their cellular targets, subsequent activation processes, and genetic requirements in Section V.

Similar antigen-specific T cell factors have been demonstrated in the regulation of cell-mediated immune responses as well. These include factors involved in the delayed-type hypersensitivity to chemical compounds and in the cytotoxic T cell response to tumor antigens. These experiments allow us to envisage the widely diverse range of regulatory effects of very similar factors. It again stresses the possibility that T cell-derived factors represent the physiologically legitimate mediators in various types of cellular interactions.

Having established that the T cell factors discussed herein have exquisite antigen specificity, what picture emerges of the structural and genetic relationship between antigen-binding sites of the T cell factors and immunoglobulins? Recent studies from at least two major laboratories indicate that some of the T cell factors bear idiotype determinants identical or similar to those of antibodies against the same antigen (see Section VII). More recently, the presence of immunoglobulin *V* region structure has also been demonstrated. The chemical approach to purify and characterize these T cell factors has, however, long been hampered by the low recovery of the materials. One important strategy has recently been provided by making hybrid cell lines continuously producing some of the T cell factors in a quantity suffi-

cient for chemical analysis. The materials obtained from hybridomas would allow us far more precise analysis of their function, genetics, and chemistry.

There have been a number of reports on soluble antigen-specific T cell factors during the past 10 years other than those discussed in this review. We must confess that we have tried to put all the factors in order without any success, and therefore we have gathered information only on the factors that have been extensively characterized as to their biologic and immunologic properties. In addition, we are aware that it is still premature to draw any definite conclusions in this review. At this time, when absolutely revolutionary findings could appear at any moment and old erroneous experiments are being discredited every second, it is almost impossible to write a comprehensive and meaningful review on such a complex subject as this. We must be very careful, for erroneous rumors tend to spread rapidly throughout the scientific world. Thus, it is fair to reiterate that some of the presently empirical results should be reinterpreted in the future, old problems will disappear, and new ones will certainly emerge.

III. Antigen-Specific T Cell Factors That Augment the Antibody Response

A. Problems in T–B Cell Collaboration

One of the central functions of T cells is their collaborative or helper function in the synthesis of antibodies by B cells. Even with extensive studies performed during the last decade, the precise mechanism of T–B cell interaction is still unclear. However, we are now quite certain that the mode of T–B cell interaction for antibody synthesis is not a uniform process, but may have diverse pathways. In the antibody response against hapten carrier conjugates, it is generally accepted that helper T cells recognize carrier determinants and then assist in the stimulation of B cells by haptenic determinants. This is supported by a number of experimental studies both *in vivo* and *in vitro* and has created a number of theories, such as antigen-focusing (Mitchison *et al.*, 1971), carrier antibody (Bretscher and Cohn, 1968), two-signal and one-signal concepts (Bretscher, 1972; Coutinho and Möller, 1974). One important and widely accepted feature is that in order to induce optimal antihapten antibody response, haptenic and carrier determinants should be present on the same molecule, i.e., linked or cognate interaction (Mitchison, 1971a,b; Rajewsky *et al.*, 1969). This was shown not only in the hapten-carrier systems, but also in the response against multideterminant protein molecules. In such a situation, one

can imagine that antigen-specific helper factors, which recognize carrier determinants and interact with hapten-binding B cells, are ideal devices for envisaging the molecular bases of T–B cell interactions.

On the other hand, there are experimental data in which hapten-specific B cells can be triggered by T cells that are stimulated by unlinked carrier (Hamaoka *et al.*, 1973; Kishimoto and Ishizaka, 1973; Tada *et al.*, 1978a). Many antigen-nonspecific helper factors have also been reported. However, under physiologic conditions the stimulus by specific antigen is undoubtedly required for the induction of final antigen-nonspecific signals. Since the induction of helper T cells is also regulated by other T cells (Feldmann *et al.*, 1977a), there is no objection to the notion that antigen-specific factors are involved in the generation of antigen-nonspecific signals. Such an antigen-specific augmenting T cell factor mediating T–T cell interaction has been described by Tokuhisa *et al.* (1978). Furthermore, a number of recent reports suggest that there are more than two types of helper T cells with distinct functional and phenotypic expressions whose recognition structures are apparently different from each other (Marrack and Kappler, 1976; Janeway, 1975; Janeway *et al.*, 1977; Tada *et al.*, 1978b).

In general, helper T cells belong to the subclass of Lyt-1^+,2^-,3^- T cells (Cantor and Boyse, 1975). Accordingly, the source of antigen-specific augmenting T cell factors has been shown to be Lyt-1^+,2^-,3^- T cells (Feldmann *et al.*, 1977b; Tokuhisa *et al.*, 1978). One of the major controversies concerns the finding that these helper T cells do not express detectable immunoglobulins nor Ia antigens, which are possessed by antigen-specific helper factors derived from them. Although some helper T cells have been shown to possess Ia determinants detectable by cytotoxic treatment with anti-Ia and C (Okumura *et al.*, 1976; Tada *et al.*, 1978a), the determinants are apparently not related to those of helper T cell factors. Whether this is due to the rapid shedding of the helper molecules from cell surface membrane or to a quantity too small to be detected by serologic methods is presently unknown.

There is another theoretical difficulty in understanding the antigen specificity of existing T cell factors. Recent reports on the condition for T cell stimulation indicate that T cells recognize not only antigen itself but also self-antigens, namely the Ia antigens on macrophages (Rosenthal and Shevach, 1977; Paul *et al.*, 1977; Schwartz *et al.*, 1978; Rosenthal, 1978; Singer *et al.*, 1977; Kapplar and Marrack, 1976, 1977; Miller *et al.*, 1976a,b; Erb and Feldmann, 1975; Pierce and Kapp, 1976; Yamashita and Shevach, 1978). Whether helper T cells recognize antigen and macrophage Ia separately or in associated form has

not yet been settled. This problem should seriously be considered, if we admit the requirement of identities of certain critical genes in *I* region for effective T–B cell collaboration (Kindred and Shreffler, 1972; Katz *et al.*, 1975; Kappler and Marrack, 1977; Sprent, 1978a,b; Yamashita and Shevach, 1978). In addition, under certain experimental conditions, the identity of Ig allotype or idiotype genes between helper T cells and responding B cells is required (Herzenberg *et al.*, 1976; Woodland and Cantor, 1978). Thus, T–B cell collaboration is not merely a matter of bridging of T and B cells by antigen.

In cognizance of these problems, we should ask critically how the antigen-specific T cell factors described below can entertain the apparent complexity of the above phenomena. Although the activation of B cells by free molecules excreted from the membrane may be different from that induced by the T-B cell contact, the properties of helper T cell factors have to meet some of the requirements in addition to their antigen specificity.

B. Antigen-Specific T Cell Factor Bearing Immunoglobulin Determinants (IgT)

The first demonstration of the antigen-specific T cell product having immunoglobulin structure was made by Feldmann and Basten (1972) using Marbrook culture vessels with a double-chamber separated by a cell-impermeable nucleopore membrane. Activated T cells specific for a carrier, such as keyhole limpet hemocyanin (KLH) or fowl γ-globulin (FγG) were placed in one chamber and hapten, 2,4-dinitrophenyl (DNP)-primed B cells in the other, with antigen (DNP–KLH or DNP–FγG) in both compartments. The nucleopore membrane prevents the direct contact of B and T lymphocytes, while enabling the protein molecules to migrate freely from one compartment to the other. Feldmann and Basten described that activated T cells were capable of providing helper activity for the DNP-primed B cells to respond to DNP–KLH or DNP–FγG across the nucleopore membrane, depending on the specificity of activated T cells. Responses to unrelated antigens, such as donkey erythrocytes (DRBC), even if present in the same culture were either unaffected or only slightly increased. The marked specificity of the collaborative factor for both T and B cells mimics the rigid requirements for the "linked recognition" of determinants on the same antigen molecule by T and B cells as in the adoptive secondary response observed by Mitchison (1971a,b).

Feldmann and co-workers characterized the active principle in the supernatant of activated T cells. It was found that the specific coopera-