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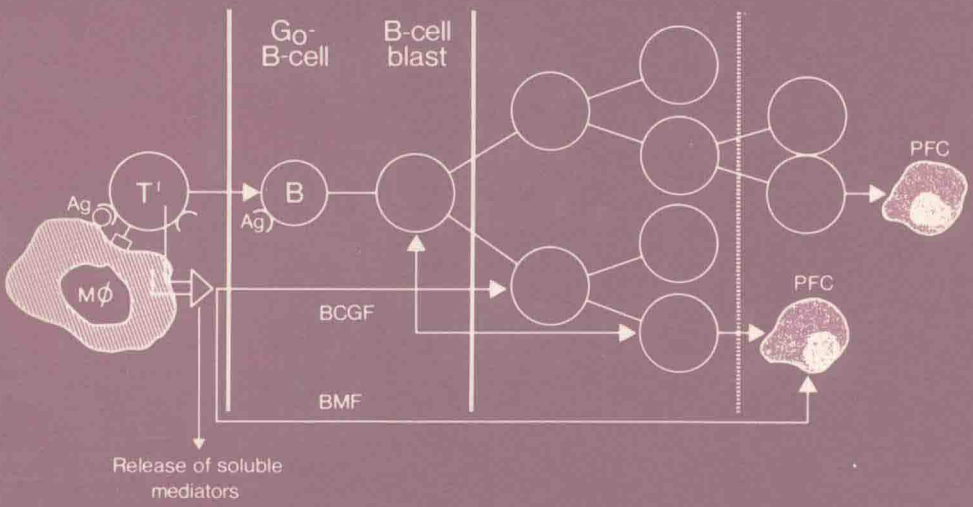
Immunopharmacology and the Regulation of Leukocyte Function

edited by
David R. Webb



Immunopharmacology and the Regulation of Leukocyte Function

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Foreword

During the past 3 years immunopharmacology has entered the immunological thesaurus. One international congress has already been held and a second is just around the corner. Three new journals devoted to immunopharmacology appeared within a single year and are already filled with articles of both basic and clinical significance.

The term "immunopharmacology" denotes different things to different people. To some it means the application of drugs to modify the immunological response, to others it connotes the identification of immunologically generated factors that produce pharmacological actions. To the basic biologist, immunopharmacology implies the use of drugs to dissect the steps involved in the developing immunological reaction. To the clinician the term suggests the treatment of patients with immunological diseases. The editor and his contributors have taken the most generous view that immunopharmacology includes everything from studies of immunological modifiers to the characterization of colony-stimulating factors. The book emphasizes the almost limitless horizons of immunopharmacology.

As one contemplates the sudden emergence of pharmacology as a distinct subdiscipline, one gets the impression it sprang, fully armed, like Minerva from the head of Jove. Did it not have a gestation period? As one looks back over the history of immunology, the embryonic and fetal development of immunopharmacology becomes evident. It has long been with us, although not clearly delineated. What was the moment of its conception? Probably it should be dated just over a century ago from the studies of a young medical student, Paul Erhlich, on the effects of aniline dyes on cells. The story is told by his secretary, Martha Marquardt, that when Robert Koch was first introduced to the young student, he was told that "this 'little Erhlich' is very good at staining, but he will *never* pass his examinations." Like Ehrlich, immunopharmacology has now come of age and it has *passed* its examinations.

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Preface

Historically, the subject matter of research in immunology has moved from the serological and biochemical analysis of immunoglobulins to a consideration of the cellular components of the immune system. In the last 15 years our understanding of the nature of the cells involved in immunity and the complex cell interactions which occur has grown enormously. Concomitantly, this has led to the opportunity for molecular geneticists, cell biologists, and pharmacologists to exploit the immune system for their own interests. The molecular geneticists have been particularly successful in unraveling the unique mechanisms used by immunoglobulin genes to generate diverse sequences. As immunologists begin to dissect cell behavior and study the regulation and physiology of immunocompetent cells, they are relying to an increasing degree on drugs and natural products to help dissect the mechanisms which control immune responses. The usefulness of immune modulating agents in deciphering the cellular immune system has led to an awareness of the power of a pharmacological approach to research problems. Many immunologists now consider such an approach to be worthy of a specific subdisciplinary designation, hence the name immunopharmacology. Insofar as this writer is concerned, the name implies the use of drugs or natural products to modify the function of immunocompetent cells in order to learn how such cells function and what sort of regulatory mechanisms are operative in the immune system. In the present volume I have asked a number of investigators whom I consider experts in the use of this approach to present a review of their respective research areas. Few, if any, would probably call themselves immunopharmacologists, yet I have become increasingly persuaded that the term accurately reflects what many of us practice.

The contributors to this volume cover several of the areas of interest in cellular immunology which have benefited from what is in essence an immunopharmacological approach. Drs. Schreier and Cammisuli and Watson et al. present their studies on the regulation of T and B cell function using in vitro models. Cohen and Crnic give a broad overview of the role glucocorticoids play as physiological regulators of the immune system. There follow several chapters which reflect my own biases and research interests, namely the regulation

of leukocyte function by products of arachidonic acid metabolism. These ubiquitous substances, the prostaglandins, the hydroxy-fatty acids, etc., seem to play a role in fine tuning an extraordinarily diverse range of cell and tissue activities. Macrophages and lymphocytes may be studied not only by using drugs but also through the use of a fascinating group of plant substances, the lectins. The results of studies using lectins are presented in the chapters by Edelson and Olfant and by myself and my associates. In addition to plant lectins, the bacterial lipopolysaccharides have provided an interesting group of substances with selective effects on the immune system. Dr. Rietschel and his associates provide a detailed account of the chemistry and biology of these substances, and this is complemented by the studies reported by Dr. Jacobs which focus more on the effects of lipopolysaccharides on lymphocyte function. Lastly, Dr. Reynard reviews the complex area of the complement system and the kinds of substances which can modulate the activity of the various complement components.

This volume is by no means complete in its coverage of topics which may be rightfully considered as immunopharmacology. Rather it is offered as a sampler of the kinds of approaches which can be taken which result in meaningful revelations concerning the functioning of the immune system.

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Contents

1

Antigen Induction of Specific and Nonspecific Signals in the Humoral Immune Response

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I. Introduction

According to the concept of clonal selection (1,2), B lymphocytes synthesize and express on their surface genetically determined recognition structures, the immunoglobulin molecules. The prediction was that interaction of antigen with these surface-expressed receptors would lead to selective proliferation and maturation of lymphocytes within the total repertoire of 10^5 to 10^6 different specificities, whereby the antibody secreted by the progeny of stimulated cells would retain its original specificity.

This simple concept of antigen-specific induction of a humoral immune response became more complex when it was found that the specific interaction of antigen with surface-immunoglobulin-bearing B lymphocytes was not a sufficient signal to induce an antibody response. For the majority of antigens, the participation of T lymphocytes (3-6) and of a third, nonspecific cell type, the macrophage (7) (accessory cell, adherent cell), turned out to be a stringent requirement for the induction of a specific antibody response. These cellular interactions are not only mediated by specific antigen but are also restricted by structures encoded in the major histocompatibility complex (8,9). Helper T cells can only exert their function in B cell induction if they are compatible in the I region with the B cells and/or macrophages (10,11).

In vitro studies over the last 10 years indicated that T cells can be replaced by soluble products which are found in the supernatants

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of mixed lymphocyte cultures (allogeneic effect factors; 12,13) or antigen- or mitogen-stimulated T cells (14,15). No consensus could, however, be reached about the cellular origin of these factors, their antigen specificity, and their H-2 restricted or unrestricted mode of action, nor is there full agreement whether the same rules hold for all types of antigen.

In the last several years the number of possibilities for interpreting the sequence and mode of cellular interactions in the antibody response was further increased by reports about T cell subpopulations which either enhance or suppress the antibody response (16-18). Helper T cell subpopulations with synergistic effects and with isotype and idiotype specificity have been described (19-26). Moreover, it was shown that T cell help is counteracted by the concomitant induction of specific and nonspecific suppressor T cells (16,27,28). This increasing number of T cell subpopulations and soluble mediators with all their potential mechanisms of interactions makes the integration of all the described phenomena confusing for the outsider and leaves the investigator with much freedom to interpret experimental results.

Therefore, our own approach to deal with this complex problem aimed primarily at the establishment of experimental systems in which the number of variables was greatly reduced and controlled. To this end we have applied, wherever possible, culture conditions which allow discrimination between antigen-induced and nonspecific effects exerted by medium components or spontaneously released cell products during in vitro culture (29-31). We have used pure or highly enriched populations of specific helper T cells (31,32), and we have attempted to dissect the system by studying interactions between T cells and B cells and T cells and macrophages separately (31,33-39).

Many of these studies have been carried out with IgM antibody responses directed against heterologous erythrocytes. These particulate antigens have been shown to have less stringent requirements for B cell induction as compared with soluble antigens (40,41). In this review we will try, therefore, to trace the common features and basic differences between these antigen classes on the basis of our own experiments without reviewing the overwhelming literature in this controversial field. At this stage of rapid development, based on cloned cells, better defined biologically active mediators, and improved culture technology, we find it challenging to state the issues in their simplest form and to make the basis for present and future contradictory claims more translucent.

II. Experimental Systems: Critical Features and Limitations

The study of interactions between lymphocyte subsets and accessory cells at the cellular or molecular level requires appropriate cell culture

techniques. When mixtures of lymphoid cells as present in spleen or lymph nodes are placed in culture, the behavior and interaction of these cells depend, more than in any other cell biological system, on the culture conditions employed by the experimenter (42). Constituents of the cell culture medium, as required for survival and growth of cells in vitro, may exert a variety of effects on T cells, B cells, and macrophages during the culture period (28,43). Serum components and cellular products from disintegrating cells may exert stimulatory and inhibitory effects which under physiological conditions are only induced by specific antigen (29,30). The magnitude of these complex effects depends on the batch and concentration of serum, the cell density, and previous immunological experience of the cell donor, which differs for individual experiments. Therefore, within different experimental systems as used in different laboratories, the same signal may be revealed as specific or nonspecific, may be identified as an absolute requirement, or may escape detection altogether. The interpretation of results which are obtained from in vitro studies, as well as the choice of suitable culture systems for studying a given problem, requires, therefore, some basic understanding of the critical features and limitations inherent in in vitro techniques (30).

A. The Primary Antibody Response In Vitro

Cell culture systems which promote a primary in vitro antibody response of dispersed spleen cells in vitro have been described by Mishell and Dutton (44,45) and Marbrook (46) and have been widely used since with minor modifications. Dissociated spleen cells of unprimed mice, cultured together with particulate antigens [i.e., heterologous erythrocytes like sheep red blood cells (SRC) or horse red blood cells (HRC)] give rise to a specific IgM response with similar kinetics as observed in the intact animal upon immunization with the same antigen. For the induction of a significant antibody response, it is mandatory to fulfill at least two criteria. The cell concentration must be high ($5\text{-}10 \times 10^6$/ml), and the culture medium has to be supplemented with selected batches of fetal bovine serum (29,30,42,45). Depending on the actual cell density, the mouse strain, the health status of the animal, and the batch-number and concentration of fetal bovine serum (FBS) employed, the number of plaque-forming cells (PFC) which arise after 4-5 days of culture may vary between very few and several hundred thousand. This quantitative variation is not found in the intact animal, where 1 mouse spleen (about 10^8 cells) yields, quite consistently, 10^5 specific antibody-forming cells 4-5 days after immunization with a "saturating" dose of SRC (47). Extensive studies have traced, at least in part, the reasons for this variability inherent in Mishell-Dutton culture systems and have provided explanations why high cell density and selected serum supplements are