Lymphocyte Differentiation, Recognition, and Regulation

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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

Katz, David H
Lymphocyte differentiation, recognition, and regulation.

(Immunology: an international series)
Bibliography: p.
Includes index.
1. Lymphocytes. 2. Cellular control mechanisms. 2. Cellular control mechanisms. 3. Immune response. I. Title. [DNLM: 1. Lymphocytes. 2. Immunity, Cellular. WH200 K195L]
QR185.8.L9K38 599'.08'765 77-7952
ISBN 0-12-401640-5

PRINTED IN THE UNITED STATES OF AMERICA

Preface

Basic immunology and, in particular, cellular immunology, has attracted an enormous amount of attention during the past decade. In fact, it seems clear that the great excitement generated in the 1960's and early 1970's about the structure and function of immunoglobulin molecules, which was the subject of another monograph in this series by Nisonoff, Hopper, and Spring, has translated itself into a similar level of excitement about the properties and functional characteristics of the cells comprising the immune system. An important difference, however, in the nature of the work concerning cell function and properties, which is the current focus of attention, and the studies on immunoglobulin structure and function of several years ago, is the character of the field itself. Hence, the number of active investigators and students in immunology has expanded severalfold during the past decade, a fact which should be a gratifying testament to the outstanding scientists who have worked so hard to lay the foundations and establish immunology as an important scientific discipline. This also, however, has created the inevitable consequence of opening many new subareas of active research and increasing the degree of specialization in which many of us find ourselves involved.

This monograph represents an attempt at broadening my own knowledge and perspective about modern cellular immunology. Quite frankly, I set out originally to update a review published in 1972 in *Advances in Immunology* on T and B cell regulatory interactions, and found that it was no longer feasible for me, or fair to a prospective reader, to approach the topic as an isolated one set apart from many other facets of lymphocyte biology. Indeed, I quickly realized that in many respects my own detailed knowledge of important, although at times peripheral, subjects was inadequate to perform the original task in a justifiable manner.

What appears in the 15 chapters of this book is, therefore, a product of my own learning exercise during the past months. It has covered many, but far from all, aspects of cellular immunology. It concentrates far more on animal

work than on studies in man, although in certain areas human lymphocyte biology has been discussed in some detail. I have attempted to be as thorough as possible in most areas discussed and, in particular, to cite the published work of many investigators, but inevitably I am certain that inadvertent errors were made. Since the monograph is intended to be a detailed review and reference source of the topics covered for students and investigators actively working in immunology, it has not been written in textbook style. As a result, anyone wishing to obtain a more basic background in fundamentals of immunology will be disappointed as well as perhaps slightly overwhelmed by the complexities of the issues discussed herein.

I sincerely appreciate the efforts of Baruj Benacerraf, Harvey Cantor, Herman Eisen, George Schreiner, Barry Skidmore, and Emil Unanue who critically read selected chapters and, where appropriate, made constructive suggestions for improvement of them. Jerry Reicher and his staff at Arrco Medical Illustrations deserve considerable credit for the artwork. I also wish to thank the other members of my laboratory who bore with me during the preparation of this monograph. I cannot overstate the phenomenal efforts of Marsha Goldman and Charlene Small who typed the manuscript and helped me enormously in the organization of it. And, finally, I wish to express my gratitude to my wife, Lee, and our daughters, Lisa and Danica, who endured prolonged periods of my absence from home and graciously made other sacrifices that permitted me to finish this task on time.

DAVID H. KATZ, M.D.

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I

Introduction

For the past 7 to 8 years, the phenomena of cell interactions in the development and regulation of immune responses have been extensively investigated. During this time, much has been learned about the importance of such cell interactions in regulating the immune system, and also about the possible mechanisms by which these interactions take place. During this same period, remarkable advances have been made in furthering our knowledge and understanding of cell surface membrane molecules, some of which perform receptor functions, either clonally restricted or nonclonal, and others which appear to be integrally associated with activation and differentiation events in lymphoid cells. Although it is true that much has yet to be determined, particularly at the molecular level, about the precise pathways involved in the development of immunocompetent cell functions and interactions, it seems fair to state that our insights on these events, derived to a great extent from phenomenological observations, have led to various working hypothetical models on which to proceed, eventually, and hopefully, to some concrete solutions.

Nearly five years ago, we undertook the task of reviewing the areas concerned with T cell regulation of antibody responses and the significance of cell interaction phenomena for the regulatory processes of the immune system (1051). Since then, there has continued to be an avalanche of new and exciting observations by many active investigators resulting in increasingly provocative modifications in certain basic concepts, and the strong indications that much of what is being analyzed in immunological systems may have considerably broader implications for developmental and molecular biology. Thus, the knowledge obtained about the fundamental regulatory control of lymphocyte and macrophage functions and the events initiating and perpetuating differentiation of such cells may now, or very shortly, be appropriately interpreted in the context of developmental functions of eukaryotic cells in general.

This monograph was prepared in an attempt to provide some insight on the systems and data on which the above statements are based. In so doing, I hope to

integrate information from diverse areas of cellular immunology, immunogenetics, and immunochemistry to form some cohesive concepts that can be perhaps utilized as a working foundation for students and investigators in various areas of immunology. The main points that emerge from such analyses to be presented herein are, in my view, that the immune system is an infinitely complex and finely tuned network of cells, receptors, and molecules which interact with one another in a genetically controlled manner that is manifested ultimately in the process known as differentiation.

In order to develop meaningful insights into the immense complexity of the integrated systems governing immune defenses of the individual, it seems appropriate to consider in some detail certain aspects of lymphocyte differentiation, mechanisms underlying specific recognition by such cells and the processes concerned with regulation of lymphocyte function. The following chapters in this monograph are directed to these aspects of cellular immunology and are not intended to represent the entire spectrum of present day immunology. Notably absent, for example, are discussions of the complement system or details of immunoglobulin structure, various aspects of immunopathological processes or tumor immunology, among other things.

Before going into any detailed analysis of our current knowledge of lymphocyte differentiation, receptor function, and regulatory processes, it may be helpful to those readers who are not very familiar with some of the systems frequently employed in cellular immunology to provide at the outset a general description of these techniques and the principles underlying them. I will return to them in greater detail in appropriate subsequent chapters.

THE TWO MAJOR CLASSES OF IMMUNOCOMPETENT LYMPHOCYTES

One of the major advances in immunobiology in the past two decades has been the recognition of two pathways for the differentiation of antigen-reactive cells. It is generally accepted that a class of bone marrow lymphocytes migrates to the thymus where these small lymphocytes adapt to certain specific immune functions by virtue of some crucial influence of the thymus. These thymus-derived lymphocytes, referred to as *T* cells, are responsible for the various phenomena of cell-mediated immunity, such as delayed hypersensitivity, transplantation reactivity, including cell-mediated cytotoxicity and mixed lymphocyte reactions, and cell-mediated resistance to infection; T cells are also perhaps the predominant cell type concerned with regulation of other lymphoid cells in the immune system. The second lymphocyte cell type, referred to as *B* cells, arises also in the bone marrow and settles ultimately in distinct anatomical

sites in peripheral lymphoid tissues where they give rise to the precursors of antibody-secreting cells (339, 448, 1051, 1381).

Among the more helpful tools available to the cellular immunologist are experimental systems in which analyses can be made of the respective functions of T cells and B cells, either independently of one another or in an interdependent manner. The introduction of defined haptenic determinants onto immunogenic carriers by Landsteiner (1201) has provided a most convenient method for the analysis of specific interactions between antigens and specific cells of the immune system. For many years it has been known that immunization with a hapten elicits anti-hapten antibody responses only when the hapten is coupled to a carrier substance which is itself immunogenic; nonimmunogenic substances serve only poorly, or not at all, as functional carriers for haptens. Moreover, as mentioned above, optimal hapten-specific secondary responses require challenge with the hapten–carrier conjugate used for primary immunization (i.e., the "carrier effect"). Since the anti-hapten antibodies produced by such immunizations were highly specific for the haptenic determinant employed (e.g., little, if any, contribution to binding energy was attributable to determinants on the carrier) and since the assumption was made that the specificity of antibody accurately expresses the specificity of the antigen-binding receptor molecules on the precursors of antibody-forming cells, then these observations suggested the operation of an additional recognition mechanism for the carrier molecule. Indeed, the demonstration that cooperative interactions between distinct lymphocytes respectively specific for carrier and haptenic determinants are essential for the development of anti-hapten immune responses validated this interpretation (reviewed in 1051; see Chapter X). Two basic *in vivo* experimental models have been employed to establish the latter point:

1. The adoptive secondary anti-hapten response following transfer of hapten-primed and carrier-primed cells into irradiated recipient mice.
2. The use of preimmunization or supplemental immunization with free carrier to enhance primary and secondary anti-hapten antibody responses in guinea pigs and rabbits.

The adoptive transfer experimental system is based on the use of a lethally or sublethally irradiated animal as a *relatively* immunologically inert recipient of primed or unprimed lymphoid cells whose functions is under analysis. Under usual circumstances inbred animals are employed in this technique, and the cell donors and recipients are syngeneic at the major histocompatibility locus. Modifications from this usual approach will be discussed in Chapters X and XII.

A schematic illustration of the basic approach for studying cooperative lymphocyte interactions in responses to hapten–protein (carrier) conjugates in mice is shown in Fig. I.1. In such systems, two types of donor syngeneic spleen cell

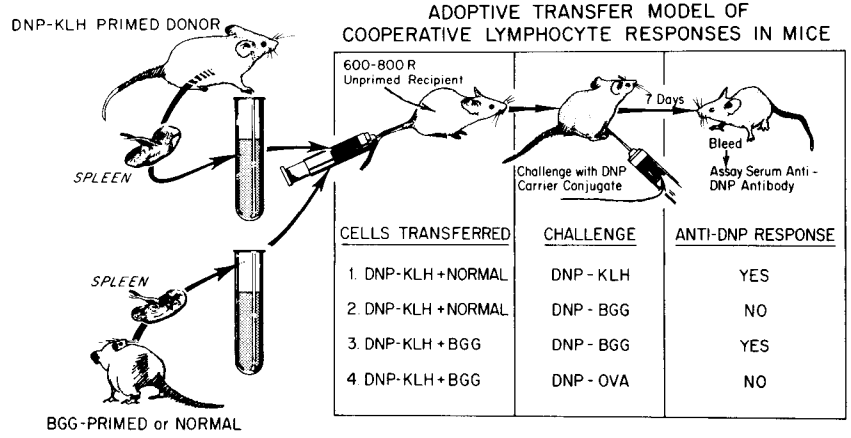


Fig. I.1. See text for explanation.

populations are employed: One of these is obtained from donors previously immunized with a hapten–carrier conjugate, in this case, 2,4-dinitrophenyl (DNP)–keyhole limpet hemocyanin (KLH); the second donor population is obtained from donors previously immunized with an unrelated carrier alone, in this case bovine γ-globulin (BGG), or from normal unprimed donors as a control. Appropriate numbers of the respective spleen cell populations are mixed together and injected intravenously into unprimed, irradiated syngeneic recipients. These recipients are then challenged intraperitoneally with one of several DNP–carrier conjugates and bled at an appropriate time thereafter (7 days in Fig. I.1). The serum obtained from such recipients is then titrated for levels of anti-DNP antibodies; anti-carrier antibodies can also be titrated if desirable. Moreover, other variations of this scheme include repetitive sequential bleeding of recipients for antibody titrations and removing recipient spleens for analysis of DNP-(or protein)-specific antibody-secreting cells in a localized hemolysin-in-gel assay for plaque-forming cells (PFC).

The basic results obtained in this system, as shown in Fig. I.1, demonstrate that recipients of a mixture of DNP–KLH-primed and normal spleen cells develop secondary anti-DNP antibody responses when challenged with the *homologous* (immunizing) conjugate, DNP–KLH, but not when challenged with a *heterologous* (unrelated carrier) conjugate, DNP–BGG (cf. groups 1 and 2); however, when the second donor inoculum consists of BGG-primed spleen cells, DNP–BGG challenge elicits a secondary anti-DNP antibody response (group 3). The capacity of BGG-primed spleen cells to assist the DNP–KLH cells in responding to DNP–BGG is highly specific as indicated by the failure of such cells

to assist in the development of a response to DNP coupled to a third unrelated carrier, ovalbumin (OVA, group 4).

As will be discussed in detail in Chapter X, the failure to obtain responses to DNP–BGG in group 2, the phenomenon known as the "carrier effect," reflects the absence of carrier (BGG)-specific T cells necessary for regulating the response of the DNP-specific B lymphocyte precursors of anti-DNP antibody-secreting cells. Spleen cells from BGG-primed donors are one such source of BGG-specific "helper" T lymphocytes and, hence, enable recipients in group 3 to develop responses to DNP–BGG (but not to an unrelated carrier, DNP–OVA).

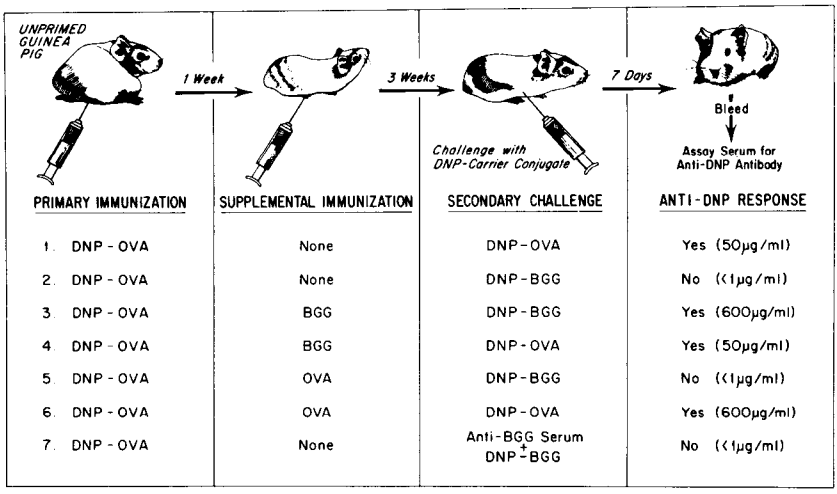
A similar type of adoptive transfer model can be used for analysis of responses of unprimed lymphocyte populations, mixtures of primed and unprimed lymphoid cells, or mixtures of lymphocyte populations originating in different lymphoid organs. For example, the classical experiments on T–B cell cooperation in responses to sheep red blood cell antigens (SRBC) were conducted with mixtures of unprimed thymus lymphocytes (thymocytes) and bone marrow lymphocytes in a system similar to that shown in Fig. I.1 (see Chapter X).

A somewhat different approach demonstrating the same cooperation phenomenon between carrier-specific T cells and hapten-specific B cells is that involving supplemental immunization of an intact animal with free carrier. This approach does not involve adoptive cell transfer into irradiated recipients and, hence, can be used in outbred as well as inbred animal populations such as rabbits, guinea pigs, rats, and mice. A schematic illustration of this approach in guinea pigs as studied in our own laboratory several years ago is presented in Fig. I.2. Guinea pigs primarily immunized with DNP–OVA will develop secondary anti-DNP antibody responses upon challenge with DNP–OVA, but not to secondary challenge with DNP–BGG (cf. groups 1 and 2). This "carrier effect" is circumvented, however, when DNP–OVA-primed animals are given a supplemental immunization with BGG. The higher magnitude of response obtained when such animals are challenged with DNP–BGG (group 3), as compared to the responses obtained in groups challenged with the homologous antigen, DNP–OVA (groups 1 and 4), reflects the mode of supplemental immunization which consists of BGG emulsified in complete Freund's adjuvant (CFA); indeed, when DNP–OVA-primed guinea pigs are given a supplemental immunization of free OVA in CFA, anti-DNP antibody responses to DNP–OVA are considerably increased (group 6).

The results of group 5 illustrate that the supplemental immunization with OVA did not prepare such animals for a secondary response to DNP–BGG; this also points out that *nonspecific* stimulation due to mycobacteria in CFA is not itself responsible for circumventing the carrier effect in this way.

The effect of supplemental immunization with BGG as shown in Fig.I.2 is not related to circulating anti-BGG antibodies, as shown by the failure to duplicate this effect by passive transfer of anti-BGG serum (group 7), and, indeed, has

PROTOCOL FOR CIRCUMVENTING THE "CARRIER EFFECT" BY SUPPLEMENTAL CARRIER IMMUNIZATION



PRIMARY IMMUNIZATION	SUPPLEMENTAL IMMUNIZATION	SECONDARY CHALLENGE	ANTI-DNP RESPONSE
1. DNP-OVA	None	DNP-OVA	Yes (50μg/ml)
2. DNP-OVA	None	DNP-BGG	No (<1μg/ml)
3. DNP-OVA	BGG	DNP-BGG	Yes (600μg/ml)
4. DNP-OVA	BGG	DNP-OVA	Yes (50μg/ml)
5. DNP-OVA	OVA	DNP-BGG	No (<1μg/ml)
6. DNP-OVA	OVA	DNP-OVA	Yes (600μg/ml)
7. DNP-OVA	None	Anti-BGG Serum DNP^{+}-BGG	No (<1μg/ml)

Fig. I. 2. See text for explanation.

been shown to be precisely comparable to the situation described in Fig. 1 in which a second donor inoculum of BGG-primed spleen cells assist DNP–KLH-primed cells to respond to DNP–BGG. In other words, supplemental immunization of guinea pigs with BGG (in this way) primes a "second" population of BGG-specific helper T lymphocytes which then cooperate with DNP-specific B lymphocytes in response to DNP–BGG. This phenomenon is not restricted to secondary responses, since under appropriate conditions of antigen dose and timing, animals which have been preimmunized with free carrier, e.g., BGG, manifest enhanced primary anti-DNP antibody responses following primary immunization with DNP–BGG. The kinetics as well as the magnitude of anti-DNP antibody production are sharply augmented under such conditions.

These experiments (Figs. I.1 and I.2) demonstrate that in hapten-specific antibody responses, an interaction of carrier-specific T cells with the hapten–carrier conjugate is required for optimal stimulation of the B cell precursors of anti-hapten antibody-producing cells. The complex regulatory role exerted by carrier-specific T cells in such antibody responses will be discussed at greater length in Chapter X. However, since such systems have been used in many studies directed to questions that will be discussed throughout this monograph, it is pertinent to establish the essential working "vocabulary" at this point. It should also be stated briefly here that a well-established property of carrier-primed T cells is that their capacity to function as regulatory *helper* cells is relatively radioresistant (see Chapter VIII). Accordingly, one not infrequently

utilized modification of the adoptive transfer model in mice shown in Fig. I.1 is to employ carrier-primed animals as irradiated recipients of hapten-specific B cells. Thus, in contrast to what occurs in an unprimed, irradiated recipient, DNP–KLH-primed spleen cells will develop a secondary anti-DNP antibody response to DNP–BGG following adoptive transfer to an irradiated, BGG-primed recipient, *without transfer of an additional inoculum of donor cells*. Moreover, conditions have been established to perform similar types of experiments *in vitro;* for purposes of this introductory discussion, simply visualize the recipient mouse in Fig.I.1 as a culture dish.

Another system frequently employed, and for which the reader should have an appreciation before proceeding, is the preparation of antigen-specific *activated* T cells (ATC). Since T cells stimulated by antigen respond by a clonal expansion and differentiation, on the one hand, and, on the other hand, by being activated to perform their specific function (i.e., helper cells, killer cells, etc.), we have elected to refer to the former as *primed* T cells, and the latter as *activated* T cells. As will be apparent throughout various sections of this monograph, *activated* T cells may result from stimulation by either specific antigen or agents other than specific antigen.

The preparation of antigen-specific ATC, as schematically depicted in Fig. I.3, is usually accomplished in the mouse by intravenous adoptive transfer of a suitable number of unprimed donor thymocytes into lethally or sublethally irradiated syngeneic recipients. Such recipient animals are then immunized with

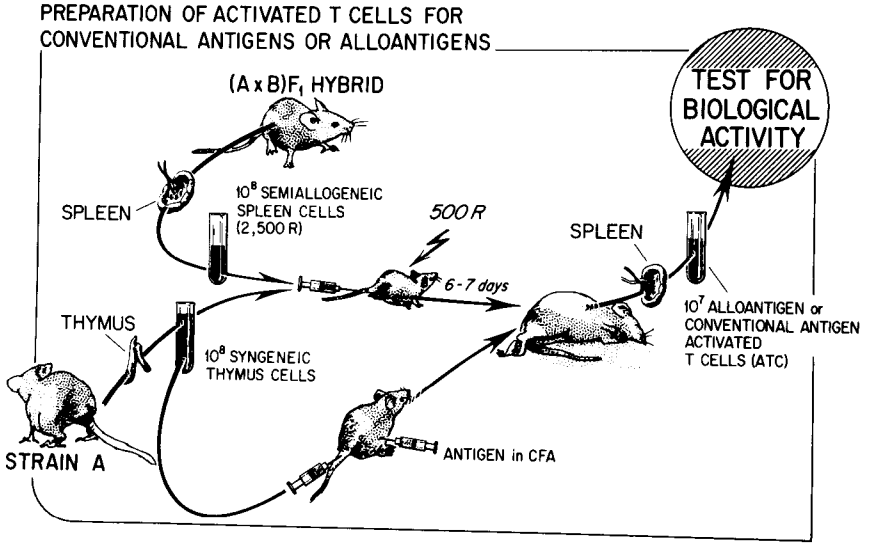


Fig. I. 3. See text for explanation.

the desired antigen. With soluble proteins or erythrocyte antigens this is usually done intraperitoneally, and, in the former case, either emulsified in CFA or administered with another adjuvant [aluminum hydroxide gel (alum), *Bordetella pertussis* or both]; when ATC are prepared against alloantigens of a histoincompatible strain, the target cells are irradiated and usually administered intravenously. After a suitable interval (6–8 days), the spleens of recipient mice are removed and processed according to experimental design, e.g., for functional analysis in either *in vivo* or *in vitro* systems. The main point here is that a substantial proportion of the viable, mature donor T lymphocytes present in the recipient spleen consists of specifically primed ATC; it should be noted, however, that, although this is an enriched population of antigen-specific T cells, it is by no means an exclusive population.

Finally, mention must be made of the fact that throughout the remainder of this monograph, it will be apparent to the reader that a considerable number of uncertainties exist in all of the various areas of cellular immunology; indeed, it is perhaps more accurate to state that very few certainties can be cited with any comfortable degree of assurance that a given one will not be perceived as, or delineated to be, something different in a matter of time. This is not at all surprising for primarily two reasons: (1) The field is filled with an ever-growing body of active experimentalists and clinicians whose collective excitement about the seemingly expanding horizons of immunobiology into broader areas of biology and medicine has created an enormous momentum of inquiry into fundamental aspects of the system; inherent in this situation are the difficulties that arise as a consequence of our creative ideas running, at times, ahead of our current level of technological capabilities. (2) Most importantly, the immune system itself and, particularly, its cellular and molecular components, is so enormously complex that it defies any *single* answer as appropriate for explaining any part of its machinery; indeed, one of the lessons learned in reviewing the literature for preparing this monograph has been that evolution of the immune system has built into it an incredible degree of flexibility. Rarely, does it seem, has the system created a single pathway to an end with no alternative avenue to take when a biological detour becomes advantageous. Hence, one is almost safer to assume that only a few absolutes exist in the immune system.

I have attempted, therefore, to present both sides of the story in appropriate instances of debate, sometimes at the expense of redundancy in various spots. Also, because I am impressed by the overwhelming amount of detail that has arisen in recent years concerning cell surface markers of the lymphoid system and the confusion in many people's minds of when such markers do or do not appear in ontogeny and/or functional stages of differentiation, an effort was made to synthesize the current body of knowledge in this area, realizing that it will change in many instances within a short time.