ADVANCES IN IMMUNOLOGY

VOLUME 31

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Immunology

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CONTRIBUTORS TO THIS VOLUME

HOWARD FLEIT
PAUL W. KINCADE
ROBERT W. KOZAK
IRA S. MELLMAN
CHARLES E. MOODY, JR.
FRANCIS W. RUSCETTI
KENDALL A. SMITH
EMIL R. UNANUE
JAY C. UNKELESS
MARC E. WEKSLER

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EDITED BY

HENRY G. KUNKEL
The Rockefeller University
New York, New York

FRANK J. DIXON
Scripps Clinic and Research Foundation
La Jolla, California

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HOWARD FLEIT, *Department of Cellular Physiology and Immunology, The Rockefeller University, New York, New York 10021* (247)

PAUL W. KINCADE, *Sloan-Kettering Institute for Cancer Research, Rye, New York 10580* (177)

ROBERT W. KOZAK, *Department of Medicine, Cornell University Medical College, New York, New York 10021* (271)

IRA S. MELLMAN,* *Department of Cellular Physiology and Immunology, The Rockefeller University, New York, New York 10021* (247)

CHARLES E. MOODY, JR., *Department of Medicine, Cornell University Medical College, New York, New York 10021* (271)

FRANCIS W. RUSCETTI, *The Laboratory of Tumor Cell Biology, The National Cancer Institute, Bethesda, Maryland 20205* (137)

KENDALL A. SMITH, *The Immunology Program, Dartmouth Medical School, The Norris Cotton Cancer Center, Hanover, New Hampshire 03755* (137)

EMIL R. UNANUE, *Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115* (1)

JAY C. UNKELESS, *Department of Cellular Physiology and Immunology, The Rockefeller University, New York, New York 10021* (247)

MARC E. WEKSLER, *Department of Medicine, Cornell University Medical College, New York, New York 10021* (271)

* Present address: Section of Cell Biology, Yale University School of Medicine, New Haven, Connecticut 06510.

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PREFACE

Immunology has been a cyclical and opportunistic field, constantly exploiting widely divergent but developing areas. Nowhere is this better illustrated than in the diversity of experimental animal systems utilized. Several decades ago the immunized rabbit was the primary tool of the immunologist. Then, with the beginning of interest in delayed-type hypersensitivity, the guinea pig became uniquely prominent. During the era of the immunoglobulins, the human system with its large quantities of myeloma proteins dominated the picture. On the other hand, the immunologist of the decade of the seventies and the early eighties has been virtually completely preoccupied with the murine system. The development of genetics as an integral part of immunology is in considerable part responsible for the current overwhelming use of this clearly advantageous experimental animal. However, occasionally and perhaps increasingly so recently, developments originating in the human system are having a significant impact. Three of the chapters in this volume illustrate this point and cover subjects that initially arose from discoveries in the human; the autologous MLC is perhaps the most striking example.

The first chapter of this volume is written by Dr. Unanue who has become one of the leaders in macrophage research. He presents a very comprehensive review of the important regulatory role of these cells in a great variety of cellular immune reactions. The very different functions of macrophages continue to be a surprising feature of these cells. These range from the presentation of antigen to T cells to the release of prostaglandins and mediators such as LAF. Perhaps the most interesting aspect of the macrophage is its key position in *Ir* gene control; this topic and its controversial aspects are very thoroughly discussed. Other accessory cells such as the Langerhans cells, the dendritic cell, and the thymic "nurse" cells are also discussed in considerable detail, although their exact relationship to the macrophage remains to be established.

No review could be more timely than the second chapter on cloned T cells and T cell growth factor. The two authors, Smith and Ruscetti, coming from the two pioneering laboratories in this field, combine their knowledge and experience in this article. New T cell lines with different functions are appearing in publications almost daily, and this system is proving to be of tremendous aid to the cellular immunologist. It provides the much needed capacity to dissect T cell

responses in detail from a functional standpoint. The T cell growth factor or TCGF which is primarily responsible for the propagation of these clones has been isolated, and its characteristics are described in considerable detail. It is apparent that this protein is much more than just an essential for the growth of these clones and plays a most important role in T cell proliferative responses under natural conditions.

The third chapter deals with B lymphocytes, with special emphasis on their culture and precursor characteristics. The technology for continuous B cell lines has not developed to the degree described for T cells in the previous chapter. However, progress has been made and Dr. Kincade's methods show considerable promise. A very diverse literature on the relationship of B cell precursors to various known stem cells is thoroughly reviewed along with the author's observations utilizing his semi-solid agar culture system. Progress in this area has been substantially accelerated by new monoclonal antibodies which help to identify B cell precursors prior to their identification through Ig production.

There have been few areas of immunology in which so much controversy exists as for the question of the character of the Fc receptor. However, Dr. Unkeless and his associates have clarified the field markedly recently through the use of monoclonal antibodies. It still remains mystifying just how different laboratories report such different molecular weights for Fc receptors even when the same cell type is analyzed. These different results are reviewed and related to the new observations that have resolved some of the issues. It is evident from the review that much of the problem relates to the low binding affinity of IgG, aggregated IgG, and immune complexes to Fc receptors. By obtaining monoclonal antibodies to the Fc receptor directly, a much higher binding affinity is obtained that permits clear identification at the molecular level. Although most of the work concerns the macrophage, a considerable portion of the review concerns B lymphocytes and other cells with Fc receptors.

The last chapter reviews in considerable detail the important new area of immunology concerning the autologous MLC. Dr. Weksler is one of the discoverers of this unexpected reaction that appears to have profound significance in the cellular immune response. It is now evident that stimulation of autologous or syngeneic T cells results from Ia-positive non-T cells in the native state and that it is not due to modification of the stimulating cell as some critics postulated. In the mouse it is clear that the dendritic cell is the primary stimulator; in the human this question remains unsettled. Defects in this response are

striking in certain diseases, especially in patients with systemic lupus erythematosus.

The Editors wish to express their gratitude to the authors for the fine chapters they have written and thank the publishers for their constant cooperation.

HENRY G. KUNKEL
FRANK J. DIXON

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The Regulatory Role of Macrophages in Antigenic Stimulation Part Two: Symbiotic Relationship between Lymphocytes and Macrophages

EMIL R. UNANUE

Department of Pathology, Harvard Medical School, Boston, Massachusetts

I. Historical

In 1972, I reviewed for this series the regulatory role of phagocytes in antigenic stimulation, i.e., in immune induction (Unanue, 1972). The information gathered at that time clearly pointed to a highly significant role of accessory phagocytic cells in the multiple cellular interactions taking place during the early development of an immune response. To recapitulate briefly, a number of *in vivo* experiments had indicated a direct relationship between the uptake of antigens by the phagocyte system and the extent of the immune response. Thus, changes in antigen molecules that resulted in enhanced or decreased uptake by the phagocytes resulted in higher or lower immune responses, respectively. After the development of methodologies for obtaining live exudate cells rich in phagocytes and for pulsing these with antigen, the immune response to macrophage-associated antigens was

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possible to assay, using combinations of *in vivo* and *in vitro* methods. This approach led to the result that the presentation of antigen bound to live macrophages to the lymphocytes was a highly efficient mode of generating an immune response. Invariably, a strong antibody response was produced to antigen molecules transferred to a host in syngeneic, live macrophages. Concurrently with this approach, the analysis of cellular interactions employing culture methods pointed to a requirement for adherent phagocytic cells in order for lymphocytes to proliferate or to make antibody. In 1972, the basis for the strong immunogenicity of macrophage-associated antigens and for the requirements of macrophages for *in vitro* immune responses were not clear, nor were the cellular and molecular events understood. We felt strongly, based in part on our own studies on antigen handling, that antigens associated with macrophage surface were involved in antigen presentation, yet how, in what form, and with what class of lymphocyte remained unexplained. Whether this antigen simply served as a device to focus T and B cells or played a more essential role was not known. The role of macrophages in directly regulating growth and differentiation of lymphocytes could only be speculated, based on suggestive experiments on the effects of adjuvants on macrophages. Although great steps had been taken in our basic knowledge of the biology of phagocytes, many issues were still to be resolved, most notably those of macrophage differentiation, antigen handling, and secretion.

The 8 years after the earlier review witnessed an incredible number of studies on phagocyte biology and on macrophage-lymphocyte interactions. These studies have placed the macrophage, now more than ever, as a critical regulatory cell with functions never before suspected. Our progress in this field came as the result of improvement in tissue culture methods, in techniques for isolating and identifying lymphocytes, in procedures to probe various cellular interactions, plus major advances in macrophage biology, and, most notably, in basic cellular immunobiology. Progress in immunobiology developed in parallel in the 1970s in various areas, i.e., transplantation genetics, immune response genetics, T-B cell collaboration, cytolytic T cell–tumor effects, and macrophage–lymphocyte interactions. The major findings and discoveries are now beginning to be integrated with each other, but it is obvious that the advances in immunogenetics and in histocompatibility have been of major impact in all of immunology. Indeed, in the course of a brief period of time, three basic cellular interactions, that of macrophages with T cells (Rosenthal and Shevach, 1973), T cells with B cells (Kindred and Shreffler, 1972; Katz *et al.*,

1973), and cytolytic T cells with their targets (Zinkernagel and Doherty, 1974), were found to be regulated by the major transplantation locus of the species. I consider the series of studies by Alan S. Rosenthal and Ethan M. Shevach published in 1973 the most important study on macrophage–lymphocyte interaction in the 1970s and perhaps one of the most seminal ones regarding the genetic control of immune responses. These studies indicated a role of macrophages in events controlled by the *I* region of the major histocompatibility gene complex, a feature never previously suspected, and signaled an *essential* interaction between macrophages and the thymus-derived T lymphocyte. The explosive series of studies in macrophage biology that followed represented, to a great degree, extensions of their basic findings. It may turn out that the common denominator for all the phenomena of cell-to-cell interaction is the process of antigen handling regulated by the transplantation gene locus.

Phagocytes are now viewed as cells capable of exerting a fine control on their environment, particularly on the lymphoid system. This control is exerted not only by carrying out antigen presentation, an essential function, but also by regulating growth and differentiation of lymphoid cells by way of a number of secretory products. Clearly, immune induction depends on a critical interrelationship between the phagocytes and the lymphocytes, the former being the nonspecific cell probably descendent of the primitive amebocytes, whereas the latter are cellular elements that give specificity to the immune response. Both cell types control and regulate each other, and the key in this regulation are the *I*-region products.

This chapter is organized into two major sections. The first reviews the various experimental systems in which macrophages have been involved, such as T-cell proliferation to antigen and lectins, T-B cell interactions, cellular immunity reactions. Some of these I have described more extensively than others. For example, T-cell proliferation and T-B cell interaction are analyzed in depth inasmuch as they were the basic systems for study of macrophage–T cell effects. In contrast, the involvement of macrophages in the development of the cytolytic T-cell response to viruses and tumors is treated briefly, this being a highly complex response involving a multiplicity of interactions and still very much under current study. The second part analyzes the biology of the phagocytes, the synthesis and expression of *I*-region-associated antigen, the issue of macrophage heterogeneity, and the function of antigen presentation and secretion of active products.

II. Analysis of the Regulatory Role of Macrophages

A. Interactions with T Cells in Antigen-Induced Proliferation

The proliferative response of T cells to antigen has been one of the systems most extensively employed for studying macrophage–lymphocyte interactions in culture. The first series of analyses in man (Hersch and Harris, 1968; Cline and Sweet, 1968) and in the guinea pig (Seeger and Oppenheim, 1970) on lymphocyte proliferation have already been reviewed (Unanue, 1972). Most recent experimental studies have used the guinea pig and mouse as the species of choice. Populations of lymphoid cells rich in T lymphocytes were obtained from lymph nodes, draining the depot of antigen, spleen, or the peritoneal cavity. The cells from the peritoneal cavity were an excellent source of T cells developing a very strong and notable antigen-specific response much higher than that shown by lymph node T cells (Rosenstreich *et al.*, 1971; Rosenstreich and Rosenthal, 1973). This may be because the immune T cell may migrate selectively into sites of inflammation as indicated by the studies of Koster and associates (1971) in antibacterial T-cell immunity carried out at the Trudeau Institute. Accordingly, in our own studies examining the proliferative response of T cells to *Listeria monocytogenes*, we found that an injection of proteose peptone intraperitoneally was *essential* in order to induce the exudate rich in strong T cells (Farr *et al.*, 1979a). Other studies in the mouse used thioglycolate broth (Schwartz *et al.*, 1978). Mineral oil was the choice inflammatory agent in experiments using the guinea pig (Rosenstreich and Rosenthal, 1973). It is worth recalling that the first successful transfer of contact sensitivity, the classical experiments of Landsteiner and Chase (1942), utilized lymphoid cells harvested from the peritoneal cavity.

Regardless of the source, the T cells have been isolated by separating out the phagocytes and B cells using combinations of brief culture on dishes to remove the bulk of adherent cells, followed by passage through glass beads or, preferably, nylon-wool columns as per the technique described by Julius *et al.* (1973). The technique of Julius requires a critical amount of nylon wool per input number of cells; if carried out correctly, it results in yields of about 25–35% of lymph node or splenic lymphocytes, the bulk of which bear T-cell markers. A good separation results in about 95% T cells, without phagocytes, and, at the most, 2–3% B cells.

Using stringent procedures to remove the macrophages and to en-