# Immunobiology of the Macrophage

*Edited by*

*David S. Nelson*

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# Preface

In recent years an improved understanding of the roles played by macrophages in the immune system has led to an increased interest in these cells by immunologists in general and, inevitably, to an increase in the volume of literature concerning them. In this book a group of immunologists of diverse scientific and geographic backgrounds present an account of the present state of knowledge of the immunobiology of the macrophage. The authors have been encouraged to give personal accounts of current developments in their special fields of interest as well as critical surveys of the backgrounds leading to these developments. Some topics have been considered from differing viewpoints in the different chapters so that the reader may sense the continuing activity in areas of controversy. We hope that this work will provide both knowledge and stimulation for further research.

Most scientists—especially those who edit books and write reviews—have a burden of gratitude to many people. Acknowledgment of all one's pastors and masters, colleagues, assistants, and critics would be well-nigh impossible (as well as being of little interest to the reader), but I cannot let the opportunity pass to thank the following people: Dr. Maurice Landy, who suggested the production of this book and who, with Drs J. J. Oppenheim and A. S. Rosenthal, helped mold its shape; the staff of Academic Press for their patient assistance; my colleagues in the Kolling Institute, especially Katherine Carson, Jean Penrose, and Cynthia Shneider, for administrative and scientific assistance and for tolerating my short temper while in literary labor; and my wife, Dr. Peggy Nelson, for her forebearance, encouragement, and support, both domestic and scientific, in this as in so many other ventures.

*David S. Nelson*

# Introduction

Many would argue that the golden era of phagocyte biology occurred at the turn of the century. Highlighted by the discoveries of Ilya Metchinokoff and his contemporaries, this exciting period saw the process of phagocytosis placed in a broad and physiological perspective, spanning the phylogenetic tree from protozoa to man. Largely focused on infectious processes, this work progressed with the simplest of technologies and laid the groundwork for all that was to follow. Aided by the brilliance of the continental schools of organic chemistry, vital and supravital dyes were synthesized and rapidly employed by cytologists in their search for those cells which would eventually be categorized as belonging to the reticuloendothelial system. "Cytases" and intracellular digestion took their place in the medical literature, neutral red and Janus green reached their acme, chick embryo extract and Carrell flasks flourished, hosts and parasites began their struggle for dominance, and learned colleagues argued endlessly over the relative importance of humoral and cellular factors. Ferments and intermediary metabolism were rather vague notions and herd immunity and antitoxins were in vogue. But in spite of the uncertainties of biochemical and immunological knowledge, the cellular pathologists of the day, those visionaries of disease pathogenesis, were incorporating the microphages and macrophages with their enzymes and antienzymes into a framework of inflammation, tissue injury, degenerative disease, and wound repair. A framework which even today with ever increasing sophistication is current and timely.

This era passed and with it the verve and interest in the study of the phagocyte—in part, because investigators were diverted into other exciting areas of contemporary biology; in part, because of the advent of chemotherapy and the control of bacterial disease; in part, because the bright young investigators of the day became the Deans and Department Chairmen; in part, because departments of pathology, particularly experimental pathology, lost their regal and central role in the medical curriculum. In any event, in spite of a few dedicated phagists, the doldrums were upon us.

Yet, even a brief perusal of this volume indicates that the tides have turned and the fortunes of the macrophage are once again ascendant; a transition which has occurred within the last decade and one with uncertain origins. Spurred by all the tools and concepts of modern science, one could mention the tritiation of thymidine, the latency of rat liver phosphatases, the Millipore filter, the flying coverslip, buffered osmium, the sheep erythrocyte, zymosan and its humoral companions, the first and sixth carbons of grape sugar, bleaches and horseradishes, polystyrene and mayonnaise, ammonium sulfate and pepsin, beans and stalks. All played a role in the renaissance and the revisitation of Metchinkoff.

But if the zenith has not been reached, what of the future? What new insights can be obtained and how will they mesh with the new cell biology? Where do the answers lie which will elucidate the current phenomenon of chemotaxis and motility, endocytosis, immunological receptors, activation, secretory products, the containment of neoplasms, and the regulation of the immune response?

They lie in the wasteland of the plasma membrane and contractile proteins, in the discovery of new and meaningful genetic models, and in the development of a novel sociology to explain the interactions of the macrophage with its molecular and cellular neighbors. David Nelson and his gifted colleagues have set the stage.

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# The Role of Macrophages in Antibody Responses *in Vitro*

*Carl W. Pierce and Judith A. Kapp*

## I. INTRODUCTION

During the last decade, our understanding of the cells and mechanisms involved in the development and the regulation of antibody responses has increased at an astonishing pace. Distinct pathways for the differentiation of two classes of clonally restricted, antigen-specific, immunocompetent lymphocytes from hemopoietic stem cells are now well recognized, as are the distinctive properties and functions of these lymphocytes. Although the precise pathway(s) of differentiation of the precursors of antibody-producing cells, or B cells, in mammals is still the subject of intensive investigation, the immunologic function of B cells is incontrovertible. B cells, after interaction with antigen via membrane immunoglobulin (Ig) receptors specific for that antigen, and under appropriate regulatory influences of thymus-derived lymphocytes, develop into plasma cells that secrete antibody molecules uniquely capable of combining with the antigenic moiety which initially stimulated the B cells. Thymus-derived lymphocytes, or T cells, after interaction with antigen via a still undefined membrane receptor, do not synthesize nor secrete antibody. T cells, however, are responsible for the various phenomena of cell-mediated immunity including the tissue rejection phenomena, such as graft versus host reactions and allograft and tumor rejection, and the production of a variety of biologically active molecules, "lymphokines," which are responsible for the inflammatory response and the tissue damage characteristic of delayed hypersensitivity reactions. T cell function is also critical for immunity and resistance to certain infectious microorganisms. Furthermore, from the experiments of numerous investigators, it is now clear that T cells, in addition to their function as effector cells for cell-mediated immune reactions, are the critical regulators of both B cell and T cell responses to antigen. T cells mediating positive regulatory functions are referred to as "helper cells" in antibody responses and "amplifier cells" in cell-mediated immune responses. Negative regulatory functions in both T cell and B cell responses are ascribed to "suppressor T cells." The relationships between and the mechanisms of action of the T cells mediating these opposing regulatory functions are currently under intensive investigation in numerous laboratories.

The antigens that stimulate antibody responses may be divided into two broad and probably artificial classes: T cell-dependent and T cell-independent antigens. T cell-dependent antigens are complex multideterminant antigens, such as heterologous erythrocytes and hapten–protein conjugates. The development of antibody responses to these antigens by B cells is strictly dependent on the concomitant positive regulatory influence, or helper effect, of T cells. T cell-independent antigens are generally polymeric molecules with repeating chemical subunits, such as pneumococcal polysaccharides, lipopolysaccharides, flagellin, and polyvinylpyrrolidone. These antigens are capable of stimulating antibody responses without the helper effect of T cells and thus derive their classification as T cell-independent antigens. However, in antibody responses to these types of antigens, the suppressive effects of T cells are most easily demonstrated and their classification as T cell-independent antigens becomes considerably less meaningful and valid. Several review articles are available which delve into this background material in greater detail (Katz and Benacerraf, 1972; Claman and Mosier, 1972; Gershon, 1974; Warner, 1974; Pierce and Benacerraf, 1975; Coutinho and Möller, 1975; Nossal and Schrader, 1975).

The macrophage is a third type of cell that is intimately involved in the development and expression of humoral and cell-mediated immune responses. In contrast to T cells and B cells, macrophages are neither clonally restricted nor antigen specific, but function as nonspecific accessory cells. Their functions in antigen uptake, catabolism, and presentation to T and B cells in the initiation of immune responses have been reviewed (Unanue, 1972), as have their roles as accessory effector cells in both cellular and humoral immune responses (Benacerraf and Green, 1969). Considerable attention has also been devoted to the physiology of macrophages, the mechanisms of phagocytosis and pinocytosis, and their functions in the inflammatory response (Cohn, 1968; Nelson, 1969; van Furth, 1970; Gordon and Cohn, 1973; Steinman and Cohn, 1974a; Ebert and Grant, 1974). The reader is referred to these articles and appropriate chapters of this book for detailed information on these aspects of macrophage function.

In this chapter, we will attempt to present a cogent overview of the functions of macrophages in the initiation and regulation of antibody responses *in vitro*. We realize some of our views may be controversial and biased by our experiences. However, if we succeed in acquainting the uninitiated reader with the complexities of these macrophage functions, and, at the same time, stimulate others to perform experiments to clarify those areas where controversy exists, we will have accomplished our goals.

## II. ANTIBODY RESPONSES *IN VITRO*

### A. Methodology

Two similar culture systems are used to study antibody responses *in vitro*. In both culture systems, dispersed, single spleen cells, or appropriate combinations of "purified" T cells, B cells, and macrophages are suspended in a completely supplemented culture medium containing selected fetal calf serum at a cell density between $10 \times 10^6$ and $20 \times 10^6$ cells/ml. In the system developed by Mishell and Dutton (1967), the cells are incubated with antigen in small plastic petri dishes at 37°C in a humidified atmosphere of 7% $O_2$, 10% $CO_2$, and 83% $N_2$ on a slowly rocking platform to facilitate interactions among the cells. Each culture is supplemented daily with a nutritional "cocktail" and fetal calf serum. In the system developed by Marbrook (1967), the cells and antigen are placed in a glass tube closed at the bottom with dialysis membrane. The bottom portion of the tube is immersed in culture medium contained in a larger vessel, and the apparatus is incubated stationary at 37°C in the humidified atmosphere used in the Mishell–Dutton system. The density of cells on the dialysis membrane is sufficient for required cell interactions, and the cultures do not require the daily supplementation with nutritional cocktail and fetal calf serum. After the desired incubation period, the cells are harvested and assayed for plaque-forming cells (PFC) to the stimulating antigen by one of the variations of the hemolytic plaque technique of Jerne *et al.* (1974). By employing appropriate modifications of this assay procedure (Pierce *et al.*, 1971), IgM, IgG, and IgA PFC responses can readily be measured.

In addition to heterologous erythrocytes [usually sheep erythrocytes (SRBC)], hapten–protein conjugates, bacterial proteins, polysaccharides, lipopolysaccharides, serum proteins, viruses, and synthetic polypeptide antigens, such as the random terpolymer of L-glutamic acid$^{60}$-L-alanine$^{30}$-L-tyrosine$^{10}$ (GAT), stimulate both primary and secondary antibody responses in tissue culture systems. In these latter cases, the determinant of interest, the hapten, protein, or GAT, is coupled to the indicator erythrocyte used in the hemolytic plaque assay and, with appropriate controls, determinant-specific PFC responses can readily be measured.

### B. Advantages and Disadvantages of Tissue Culture Systems

These two culture systems support the development of primary and secondary antibody responses with approximately equal efficiency. PFC

responses that develop in culture are comparable in magnitude, kinetics, Ig class, and dependence on antigen dose to responses in intact animals during the first 7 days after immunization (Claman and Mosier, 1972; Pierce, 1974). This parallelism between responses *in vivo* and *in vitro* has been observed for most antigens that have been studied thoroughly. It also has been the general experience that those antigens that stimulate poor primary responses *in vivo* also stimulate poor primary responses *in vitro*. Since culture systems are closed systems and one has more precise control of the components of these systems than is possible *in vivo*, they are well suited for investigations of the critical factors in the induction of the antibody response. For example, the numbers, types, and immune reactivities of cells from the immune system that are added to the cultures and the interactions among these cells can be rather precisely controlled. The concentration and physical form of the antigen and the interaction of the various types of cells with antigen in the cultures can also be controlled. Furthermore, specifically reactive cells or reagents, such as antimetabolites, antibodies to antigen, or membrane molecules on the responding cells, can be added to the system at defined times in known numbers or concentrations (Pierce, 1974). Although antibody responses *in vitro* are comparable in many respects to *in vivo* responses, and although tissue culture systems offer many advantages in experimentation not possible *in vivo*, one must be extremely cautious when extrapolating from one system to the other, and one should avoid making dogmatic statements based on results from one system which have not been confirmed in the other.

Tissue culture systems are not without disadvantages. Spleen cells or partially purified T and B cells from mice are most commonly used in these culture systems; lymphoid cells from rabbit, rat, and chicken have been used successfully only by a few investigators. Most of the data we will discuss have been derived from cultures of mouse cells. However, even with mouse lymphoid cells, considerable strain to strain variation exists, and no amount of cajoling or uttering of incantations, friendly or hostile, can convince spleen cells from some strains of mice to respond faithfully on a regular basis. Cell survival in these cultures is not good; only approximately 25% of the initial cells survive for 5 days. Thus, we are dealing with a dying system, and the effects of products released from dead or dying cells on the surviving cells are still largely unknown. The tissue culture environment itself is artificial and may not provide optimal conditions in terms of nutrients. In addition, fetal bovine serum, not required for antibody responses *in vivo*, is a necessary ingredient of the culture medium. Fetal bovine sera vary in their capacity to support antibody responses *in vitro;* some are nonsupportive, and others are non-

specifically cytotoxic. This variability is an annoyance that could readily be done without. Also absent from tissue culture systems are the multitude of *in vivo* homeostatic mechanisms, such as the influences of circulating immunocompetent cells, the influx of virgin precursor cells from central lymphoid tissues, and the feedback regulation by other stimulated lymphocytes and previously synthesized antibodies, all of which obviously affect development and expression of antibody responses. Last, antibody responses in tissue culture systems are subject to a variety of maladies that we call, in all seriousness, gremlins (C. W. Pierce and J. A. Kapp, unpublished observations, 1975). These include the propensity of the investigator to put fingers in the cultures or to drop the cultures and various acts of God and man, such as the tides, phases of the moon, rocket launches, and the changes of the seasons. Nevertheless, tissue culture systems have been extremely useful for investigating many facets of the antibody response; the function of macrophages has been one of these facets.

## III. INTRODUCTORY IMMUNOGENETICS

We will restrict our discussion of immunogenetics to the major histocompatibility, or *H-2*, complex of the mouse as it relates to antibody responses, and will not consider genetics of immunoglobulins. The *H-2* complex, a small segment of genome in the ninth linkage group on chromosome 17, has become the focus of great interest to immunologists with the recognition that this gene complex controls a variety of functions of immunological significance in addition to histocompatibility antigens. The *H-2* complex has been divided into four regions on the basis of the antigens or immunologic functions each region controls. At the two ends of the *H-2* complex are the *K* and *D* regions, which encode serologically detectable transplantation antigens. These antigens are present on membranes of macrophages, T cells, B cells, and nonlymphoid cells; these antigens not only stimulate T cells in the various tissue rejection responses but also are the membrane target antigens of the effector T cells in these responses. Inside the *H-2* complex and adjacent to the *D* region is the *S* region, which encodes serum proteins in the complement sequence, but is otherwise unrelated in function to other regions of the complex. The *I* region lies between the *S* and *K* regions and encodes a family of antigens, Ia antigens, which are on the membranes of T cells, B cells, and macrophages and are also involved in the stimulation of T cells in tissue rejection responses. Recent observations indicate that syngenicity of *I* region antigens is necessary for optimal cooperative interactions between