The Macrophage

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Preface

THE UBIQUITOUS phagocytic cells that we know as macrophages vary in many of their characteristics, depending on their location, physiological state, and function. Although Metchnikoff appreciated many of their potentialities nearly a century ago, most early investigators regarded macrophages primarily as scavenger cells. Research in many fields has made it increasingly obvious that macrophages do, in fact, have a multitude of important functions above and beyond their ability to scavenge and dispose of effete cells and extraneous matter.

Several decades ago it was realized that macrophages are the chief agents of antimicrobial cellular immunity. Only during the past few years has the importance of the macrophage-cytophilic antibody system in cellular immunity been appreciated. The maturation of monocytes to macrophages, then to epithelioid cells, and finally to giant cells, has been described both *in vivo* and *in vitro*. The contribution of this sequence of events to cellular immunity is not fully apparent but is of great interest.

Recent research has led to an awareness of the extensive synthetic capabilities of macrophages. It has been shown that these cells can synthesize interferon, components of complement, and numerous other biologically active substances, including a wide array of enzymes.

Macrophages are important in allograft rejection. They function in delayed sensitivity reactions and in the pathogenesis of autoimmune diseases. They are probably often essential to antibody formation by their activities of trapping and processing antigen, and perhaps by virtue of the activity of their ribonucleic acid. In addition, macrophages are able to detoxify both exotoxins and endotoxins, as well as other injurious compounds.

Thus, macrophages have emerged from their historical role as simple scavenger cells to take their place, with lymphocytes, as mobile cells with a wide spectrum of functions of primary importance to body economy.

The possible relationships between macrophages and lymphocytes, and between macrophages and other cell types, remain controversial. However, it is probable that one vital function of macrophages is to regulate the

proliferation and differentiation of other cell types, and, conversely, that other cells contribute to macrophage homeostasis.

Recent rapid advances in research concerning macrophages have resulted in a tremendous increase in the literature, scattered throughout a wide variety of publications. The object of preparing this monograph is twofold: first, to consolidate available information in order to provide a comprehensive characterization of the macrophage for those unfamiliar with it; and, second, to review some of the most recent work in this area for the benefit of those who are already familiar with the field. Wherever possible, reviews are quoted. Many recent findings, not summarized elsewhere, are reviewed in detail. It is hoped that the references cited will provide a point of departure for gaining further information on subjects of special interest to the reader.

Even though much is known about the macrophage and its functions, many problems of great interest remain unsolved. For example, consideration of the control mechanisms which operate in the proliferation and differentiation of macrophages offers a challenge for future research. In addition, the molecular events concerned in macrophage-cytophilic antibody activity are of the utmost importance and are incompletely understood. These and similar problems provide promising areas for further investigation.

There are many who have participated, directly or indirectly, in the preparation of this monograph. Although it it not possible to thank each one individually, our debt to them is great, and we are appreciative of their contributions. We are grateful to our colleagues who have read portions of the manuscript and offered valuable suggestions. Special thanks are due to Dr. Q. N. Myrvik and Mrs. E. S. Leake, not only for their criticisms of the manuscript, but also for a number of electron micrographs.

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Chapter 1 | Introduction

THE TERM *macrophage* will be used in the following pages to define a ubiquitous large mononuclear cell type characterized by the ability to phagocytize particulate material and to store vital dyes. Among the many names that have been used to designate cells fulfilling these criteria are the following, for which complete references may be found in the review by Sacks (1926): clasmatocytes (Ranvier, 1900), adventitial cells (Marchand, 1901), Kupffer cells (Ribbert, 1904), rhagiocrine cells (Renaut, 1907), mononuclear leukocytes (Aschoff, 1924), pyrrol cells (Goldmann, 1909), histiocytes (Kiyono, 1914; Maximow, 1924), and reticular cells (Aschoff, 1924). In addition, Hortega cells of the central nervous system are generally regarded to be macrophages (de Asúa Jiménez, 1927), and septal cells of the lung are sometimes classified as macrophages (Robertson, 1941). This list, although incomplete, illustrates the great diversity of cells defined as macrophages. The diversity of this cell type accounts for many of the controversies which have arisen during investigations conducted over the past hundred years.

Near the turn of the century, Metchnikoff (1905) discussed the concepts of immunology current at that time, including his own avant-garde ideas concerning phagocytosis. He reviewed some of the earliest reports on phagocytosis by mammalian leukocytes, published by Grawitz in 1877 and 1881, and also discussed his own experiments on macrophages, which began during the same decade.

Metchnikoff was a zoologist who was primarily interested in determining the function of the mesoderm. Although at the time it was widely accepted that the ectoderm supplies the cutaneous layers and the entoderm the digestive organs of multicellular animals, the function of the mesoderm was completely obscure. The work of Metchnikoff with sponges and other lower animals revealed that ameboid cells of their mesoderm could actively ingest, and subsequently digest, foreign materials. Even though Metchnikoff was not medically trained, he had encountered Cohnheim's writings on pathology and had been particularly impressed with the descriptions of "ameboid cell" infiltrations and with his theories on inflammation in the human. He was struck by the similarities between leukocytes in inflam-

matory exudates of higher animals and phagocytic ameboid cells of the echinoderms, and hypothesized that cells of this type have the important function in body defense of ingesting and digesting extraneous material. To test this hypothesis, he inserted rose thorns into the transparent bodies of starfish larvae. Metchnikoff postulated that if ameboid phagocytes are important in defense they should accumulate at sites of injury. Much to his delight, as he watched the transparent animals, masses of ameboid cells collected around each inserted thorn. Since the starfish larvae had neither blood vessels nor a nervous system, he concluded that this cellular exudation must represent a primitive basic defense mechanism. Metchnikoff speculated that the ingestion and digestion of extraneous materials by human ameboid phagocytes represents an analogous mechanism which has been retained throughout evolution because it offers a highly efficient defense against invasion by foreign agents.

Virchow (1885) was interested in, and favorable to, Metchnikoff's proposal that phagocytes in an inflammatory exudate function as agents to destroy invading microorganisms, even though during his time pathologists generally accepted the view that cells which ingest bacteria play a harmful role in infection because they serve as vehicles of microbial dissemination. This concept had developed because microbes could be seen within motile phagocytes, but no evidence had been found that they were destroyed intracellularly.

Metchnikoff (1884a) was able to show that materials are digested within phagocytes of various species and that engulfment of anthrax bacilli (1884b) by phagocytes of vertebrate animals leads to, in his words, "a desperate struggle" between bacilli and the ameboid cells.

The principal tenet of "the theory of phagocytes," formulated by Metchnikoff, was that phagocytes are solely responsible for immunity to harmful agents. This led to a controversy between supporters of this theory and proponents of the "humoral theory," who maintained that humoral factors alone account for immunity. Each group was confident that its own concept was correct and was unable to perceive that both of these agencies, and others as well, can interact to constitute the total immune response.

Metchnikoff (1905) concluded that "there is only one constant element in immunity, whether innate or acquired, and that is phagocytosis . . . phagocytes . . . ingest micro-organisms and absorb soluble substances . . . seize microbes whilst these are still living . . . and bring them under the action of their cellular contents, which are capable of killing and digesting the micro-organisms or of inhibiting their pathogenic action. Phagocytes act because they possess vital properties and a faculty for exerting a fermentative action on morbific agents. The mechanism of this action is not definitely settled, and we can foresee that for future researches there will be a vast and fertile field to be reached by pursuing this path."

Over half a century later most of these statements are still apropos, and have been confirmed by a tremendous amount of data which has accumulated during the interim, largely as the result of advances in techniques. For example, electron microscopy has permitted study of the ultrastructure of cells, the ultracentrifuge has allowed separation and characterization of cellular components, and the development of radioactive-labeling techniques has provided a tool for studying the sources and metabolism of various cell types.

Subsequent chapters are devoted to some of the most important evidence pertaining to the sources and characteristics of macrophages. Limitations of space do not permit a complete review of the vast literature on these subjects. However, the references listed offer additional information on the topics discussed.

Metchnikoff was, indeed, correct when he foresaw a "vast and fertile field" of research into the activities of macrophages. The works presently discussed give further insight into the scope of this field and afford indications of the large amount of investigation that remains to be done.

SUMMARY: The macrophage is defined as a ubiquitous large mononuclear cell type, capable of phagocytizing particulates and of storing vital dyes. Some of the basic contributions of Metchnikoff, which led to an appreciation of the importance of the macrophage and gave indications of the mechanisms of its activity, are discussed. The perspicacity of Metchnikoff's observations pointed the way to much of the subsequent work in this field, which has been greatly facilitated by technological advances. In subsequent chapters, information will be presented concerning macrophage sources and characteristics.

Chapter 2 | The Structure of Macrophages

MACROPHAGES are found in all organs of the mammalian body. When the marked differences in environment within various organs are considered, the finding that the morphology of these cells varies according to their location is not unexpected. Moreover, the morphology of macrophages varies depending on their state of activity.

A. *Monocytes*

A classical description of monocytes, seen in stained smears of peripheral blood examined with light microscopy, was presented by Bloom (1938a). The blood monocyte, which normally constitutes 3 to 8% of the circulating leukocytes, is a spherical cell as large or larger than the granulocyte, approximately 10 to 11 μ in diameter. Its rounded nucleus is usually indented, and the cell contains a more abundant cytoplasm than do lymphocytes. The characteristics of the monocyte nucleus are thought to reflect the state of maturity of the cell, an elongated nucleus with peripherally distributed clumped chromatin being found in more mature cells. The cytoplasm is slightly basophilic and is often finely reticulated. It has numerous vacuoles and may contain many small azurophilic granules.

Living monocytes observed with phase microscopy frequently exhibit a finely granular cytoplasm resembling "ground glass," in contrast to the more refractile homogeneous cytoplasm of lymphocytes. Supravital staining with Janus green reveals the presence of a large number of short rodlike mitochondria, which frequently occur in a group near the centrosphere. With neutral red supravital staining, a rosette of neutral red vacuoles is often seen in the cytoplasm near the nuclear indentation. The Golgi apparatus also is found in this region.

Bessis (1956), by use of phase microscopy, demonstrated that monocytes move in the same manner as tissue macrophages. The cell assumes a triangular shape, with one angle pointing towards the rear and a hyaloplasmic veil along the advancing margin. The undulating cytoplasmic veil is extremely active under appropriate conditions, as can be seen by cinematography. Phase contrast microscopy also reveals granules, vacuoles, and

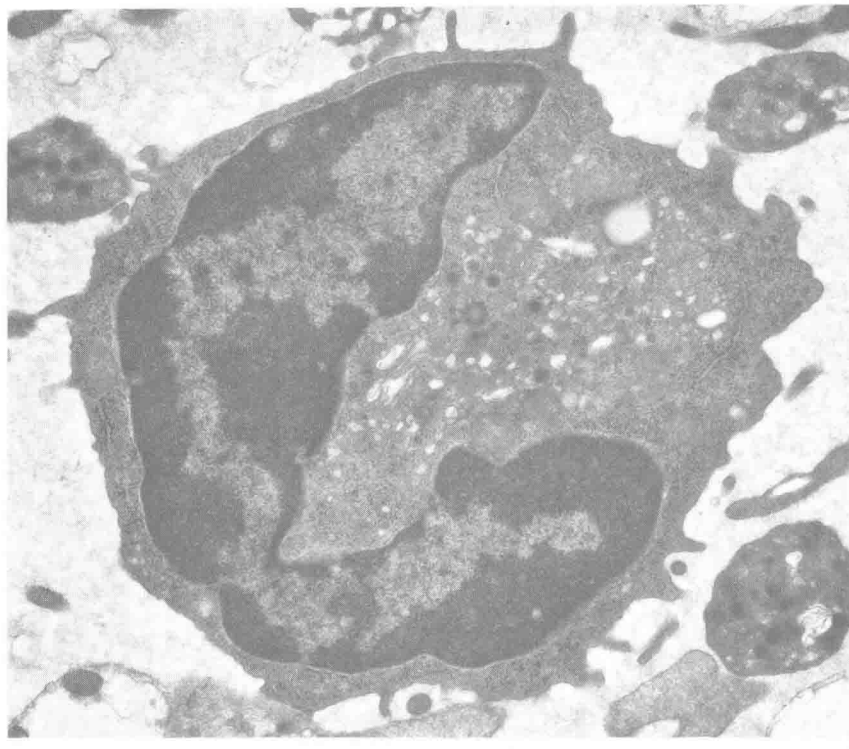

FIGURE 2-1. Electron micrograph of a normal blood monocyte from a guinea pig. Magnification: 12,865. (Courtesy of David Simpson and Russell Ross.)

rod-shaped mitochondria in an abundant light-gray cytoplasm, and one or two nucleoli may be seen in the nucleus. The cells have a distinct tendency to adhere to and spread on glass (Forteza, 1964).

Electron microscopy (EM) has served to emphasize certain characteristics of blood monocytes which aid in distinguishing them from large lymphocytes (Low and Freeman, 1958). Monocytes contain numerous small circular or rod-shaped mitochondria, many small round or oval profiles of endoplasmic reticulum (ER), and some ribosomes in an abundant cytoplasm. Lymphocytes, on the other hand, contain but few mitochondria, little ER, and few ribosomes in a sparse cytoplasm. Differences between the nuclei of monocytes and lymphocytes, as seen by EM, are particularly striking. Monocytes have a characteristic dark, irregular band of chromatin adjacent to the inner margin of the nuclear membrane, while the chromatin of lymphocytes is regularly dispersed throughout the nucleus.

B. *Peritoneal Macrophages*

Peritoneal macrophages are easily obtained from experimental animals and hence have been studied more extensively than other macrophages. In

Giemsa-stained or May-Grünwald-Giemsa-stained preparations examined by light microscopy, peritoneal macrophages frequently measure 10 to 30 μ in diameter, and have nuclei approximately 6 to 12 μ in diameter. The abundant cytoplasm is slightly basophilic, may contain many vacuoles and granules, and gives evidence of being actively ameboid. The eccentric nucleus is oval or kidney-shaped and stains more lightly than the nucleus of a lymphocyte (Maximow, 1924; 1932).

By phase contrast microscopy, peritoneal macrophages are seen to contain abundant light-gray diffuse cytoplasm with darker-gray rod-shaped mitochondria frequently grouped near the centrosphere. Granules and vacuoles occur in the cytoplasm in varying numbers depending on the physiological state of the cell. The pronounced tendency of macrophages to adhere to and spread on glass surfaces creates hyaloplasmic flow which gives rise to expansions of the characteristic veil-like undulating membrane (Policard, 1957).

North and Mackaness (1963a) examined peritoneal macrophages with the electron microscope. Normal peritoneal macrophages from nonimmunized mice were shown to have characteristics which are typical of the peritoneal macrophages of many species. The cytoplasm is enclosed by a three-layered unit membrane, approximately 80 A thick, with many protuberances and invaginations indicating a high degree of activity. Peripheral cytoplasm in the numerous cellular processes is finely granular and

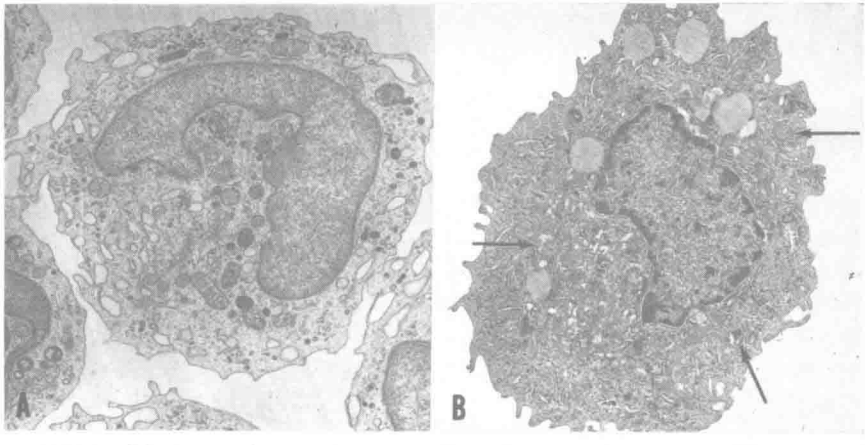

FIGURE 2-2. Electron micrographs of peritoneal macrophages. A, Nonactivated macrophage washed directly from the peritoneum of a germ-free rat, without an inducer of exudate. The cytoplasm contains relatively few ribosomes and electron-dense lysosomes. Magnification: 5,060. B, Activated macrophage washed from the peritoneum of a rabbit 4 days after the injection of Bayol F. The nuclear material is aggregated adjacent to the nuclear membrane. The cytoplasm contains innumerable ribosomes, a substantial amount of rough endoplasmic reticulum, large phagosomes filled with the injected oil, and many small lysosomes and phagolysosomes (arrows). Magnification: 5,060. (Courtesy of E. S. Leake and Q. N. Myrvik.)

usually lacks the structures seen in the rest of the cytoplasm, i.e. membranes of ER with and without attached ribosomes, many free ribosomes approximately 30 Å in diameter often arranged in polysomes, and cylindrical mitochondria 0.3 to 0.5 μ in diameter and 1.5 to 2.0 μ long. Cytoplasmic vesicles 300 Å to 0.5 μ in diameter, enclosed by a unit membrane, are of three main types: small pinocytic vesicles, various-sized organelles containing a fine granular material, and larger, denser vacuoles. The nucleus is oval with one or more indentations and is enclosed by a porous double-unit membrane. Nucleoli are sometimes seen. Ribosomes are attached to the external portion of the nuclear membrane, which appears to be continuous with the ER. The centrosome, opposite the nuclear indentation, is a spherical region of about 5 cu μ of finely granular cytoplasm containing one or more fibrous centrioles and is surrounded by groups of Golgi membranes. Many investigators have reported similar observations on the ultrastructure of mouse peritoneal macrophages (e.g. Carr, 1967).

Stimuli of various kinds, such as those which accompany the phagocytosis of bacteria, can cause "macrophage activation." An "activated macrophage" is one which is metabolically highly active and contains many organelles, including lysosomes rich in hydrolytic enzymes. Following ingestion of *Listeria monocytogenes* by mouse peritoneal macrophages, North and Mackaness (1963a) observed the bacteria within phagocytic vacuoles (phagosomes), surrounded at first by a clear area bounded by a unit membrane. Later, the clear area was filled with an amorphous material which apparently had been transferred from cytoplasmic vesicles (lysosomes) into the phagosomes to form phagolysosomes.

The peritoneal macrophages of mice that have survived an initial infection with *L. monocytogenes* are regarded to be "immune" since they can kill the specific organism rather than support its intracellular growth. North and Mackaness (1963b) compared the ultrastructure of peritoneal macrophages from mice immunized with *L. monocytogenes* with that of peritoneal macrophages from nonimmunized mice. The cytoplasmic membrane of immune macrophages is smoother, and has fewer protuberances and invaginations than the membrane of nonimmunized macrophages. There are many free ribosomes but very few profiles of ER. The mitochondria are smaller, more numerous and contain more cristae. Compared with nonimmune peritoneal macrophages the cytoplasm of immune macrophages is less dense and appears to be highly hydrated. It contains fewer vesicles and a very extensive Golgi apparatus. Nucleoli are usually seen within the nucleus. It was suggested that these differences between immune and nonimmune cells probably result from the emergence in immune mice of a new population of peritoneal macrophages which are in the process of differentiation.

Peritoneal macrophages from nonimmunized hamsters are similar to those of the mouse described above, as evidenced by the EM studies of