# 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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# Contents

# 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.

Macrophages of the thymus resemble those of lymph nodes in structure (Clark, 1963). Kostowiecki (1963) extensively reviewed the literature on thymic macrophages.

### F. *Liver Macrophages (Kupffer Cells)*

Kupffer cells constitute approximately 30% of the nucleated cells of the liver! Various methods have been described for separating them from liver parenchymal cells (e.g. St. George, 1960; Garvey, 1961; Bennett, 1966; Pisano, Filkins, and DiLuzio, 1967; Mills and Zucker-Franklin, 1969). Many years ago, Rous and Beard (1934) described a method for separating Kupffer cells from nonphagocytic cells in liver perfusate. The macrophages were allowed to ingest minute particles of iron prior to liver perfusion and were subsequently removed from suspension with a magnet. Beard and Rous (1934) cultivated the isolated Kupffer cells *in vitro* and characterized them. They recognized morphological similarities between liver macrophages and macrophages from other sources and grouped all of these cells together as members of the reticuloendothelial system. They also stressed that certain differences exist between fixed Kupffer cells and free macrophages of exudates or macrophages of the spleen and other organs. For example, they reported that the surfaces of Kupffer cells are considerably stickier than those of other macrophages and hence these cells are difficult to handle *in vitro*. It was their opinion that Kupffer cells are easily distinguishable from other macrophages.

Kupffer cells were visualized by EM in the investigations of deMan *et al.* (1960), who reported that these irregularly shaped cells project into the lumen of the sinuses that they line and have fine cytoplasmic extensions inserted between adjacent parenchymal cells. The surface area of the cells is large because of the many projections and invaginations of these cells. Portal blood flows directly through the liver sinuses and over the large surface area of the Kupffer cells, providing them with the opportunity to adsorb and dispose of substances from the gastrointestinal tract. The cytoplasm of these cells contains many mitochondria and electron-dense bodies, which deMan *et al.* (1960) called cytosomes; however, these enzyme-rich bodies probably represented lysosomes and phagolysosomes. The intensive phagocytic activities of Kupffer cells are reflected in the EM observations made by Rouiller (1962), who found that they contain many vesicles, lysosomes, phagosomes, and some erythrocytes in various stages of digestion.

Matter and co-workers (1968) made a stereological analysis of the fine structure of the "micropinocytosis vermiformis" in Kupffer cells of the rat. This structure, said to be characteristic of all macrophages, is made up of tubular and lamellar portions, usually parallel to the cell surface, which join to form an intricate labyrinth. About one tenth of the tubular structures communicate with the pinocytic system; the rest form a dense layer



around the pinocytic system, which communicates with the extracellular environment. This "micropinocytosis vermiformis" affords a means of facilitating the entrance of materials into the cell.

### G. *Central Nervous System Macrophages (Microglia Cells)*

Microglia cells (Hortega cells, mesoglia) have long been considered to be macrophages, since in conditions of trauma they become typical ameboid phagocytic cells (Truex, 1959). Under normal conditions, resting microglia cells do not store intravenously injected dyes because the wall of astroglia surrounding blood vessels prevents dye from reaching them. If the wall is injured to allow dye penetration, it can be shown that microglia cells will store dyes. Hortega cells which have lost their processes and have transformed to free macrophages have been called "compound granular corpuscles," *Gitterzellen,* or lipophages. *Gitterzellen* are very similar in appearance to Kupffer cells (Jaffé, 1938).

Hosokawa and Mannen (1963) and Honjin (1963) studied neuroglia cells by light and electron microscopy and tabulated the characteristics of the different types. They reported that microglia cells are found in both gray and white matter. The average cell length for human resting microglia is 61.6 $\mu$, for monkey 52.2 $\mu$, and for rabbit 53.2 $\mu$, with respective nuclear lengths of 12.6 $\mu$, 10.6 $\mu$, and 8.1 $\mu$. The chromatin-rich nucleus is irregular or twisted in shape and presents one nucleolus. The cytoplasm contains a moderate number of mitochondria, many vacuoles and granules, a large amount of ER, and clumps of Golgi membranes.

Resting microglia cells have a rod-shaped or triangular nucleus with very thin, branching, spiny cytoplasmic processes. The bipolar microglial cell is called a "rod cell" because the nucleus assumes an elongated rod form between two thin bipolar cytoplasmic processes, which may be branched at each end. Other forms with angular nuclei have three, four, or occasionally more processes arising from the angles around the nucleus and are called tripolar, quadripolar, or multipolar respectively.

Microglia cells may be perineuronal or perivascular satellites, with spiny processes attached to either a nerve cell or blood vessel or connecting the two. The reasons for these cell associations are not known, since the microglia cells do not usually form foot processes, as do the astroglia cells which contribute to the blood-brain barrier.

As previously stated, following trauma the thin spiny processes of resting microglia cells are lost, the cytoplasm swells, and the cells resemble free macrophages found at other loci in the body.

### H. *Macrophages of Other Organs and Tissues*

In addition to macrophages of blood, peritoneum, lungs, spleen, lymph nodes, liver, and the central nervous system, macrophages are present in virtually every other organ and tissue. They are ultrastructurally similar,

macrophages of granulomas of rats fuse to form giant cells. Labeling studies of Silverman and Shorter (1963) gave no evidence for division of nuclei following cell fusion. However, this question remains unsettled.

*SUMMARY:* Macrophages are found throughout the mammalian body. Those found in many locations are similar in morphology. However, alveolar macrophages exhibit striking structural differences from most other macrophages, presumably due to the uniqueness of the lung environment; they are easily distinguished by virtue of their smooth oval nucleus and high cytoplasm-to-nucleus ratio.

In general, macrophages have eccentrically located, indented, reniform nuclei, frequently with peripheral chromatin and one or two nucleoli. In activated macrophages the cytoplasm is abundant and contains many vesicles, including lysosomes, phagosomes, and phagolysosomes. Epithelioid and giant cells apparently are terminal forms of macrophages which give morphological evidence of having been extremely active. Epithelioid cells contain lysosomes but no intact phagosomes, and giant cells often lack both lysosomes and intact phagocytic vacuoles.

octophs

# Chapter 3

# The Phylogeny and Ontogeny of Macrophages

## A. *Phylogeny*

Macrophages of various animal species were studied extensively by Metchnikoff (1905), whose descriptions of the digestion of red blood cells (RBCs) by macrophages in the flatworm are highly graphic. When the worms were fed goose RBCs the nuclei and hemoglobin made it possible to trace the fate of the ingested cells. Phagocytic cells in the worm intestinal epithelium rapidly ingested the RBCs, and subsequently digested them slowly but completely. In the Coelenterata, intracellular digestion could be observed directly *in vivo,* since these animals are transparent. The actinians, or sea-anemones, also proved to be good subjects for study. These animals eat many types of food, including shrimp. The empty shrimp shells are ejected and particles of the meat are engulfed and digested by phagocytic cells in the intestinal epithelium, in a manner similar to that observed in the flatworm. Since these phagocytes can also take up neutral red and other dyes, they are classified as macrophages. Metchnikoff presented evidence supporting the concept that these cells are entirely responsible for the primitive digestion of lower animals. With increasing phylogenetic complexity, this primitive digestive function carried out by phagocytes is replaced by enzymes secreted by specialized cells of the alimentary system. The macrophages of higher animals, however, retain some digestive capabilities, which aid in tissue resorption and other functions.

The reticuloendothelial apparatus of a complex and highly developed invertebrate, the lesser octopus *Eledone cirrosa,* has been studied by Stuart (1968). This octopus lacks lymphatics and discrete foci of lymphoid tissue, but has a "white body" which serves as the leukoformative organ. Carbon particles injected into the octopus were observed to localize within large collections of phagocytic cells in the gill and the post-salivary gland, and in some phagocytes of the "white body." A single type of ameboid cell was found in the blood. It resembled typical macrophages seen in other animals, and was cultured *in vitro* for periods of up to 4 days. Although

the blood lacked agglutinins for bacteria, yeast, or RBCs, it contained opsonins (presumably nonspecific) for these particles, which greatly facilitated their phagocytosis by macrophages.

Metchnikoff also described polymorphonuclear neutrophils (PMNs) and macrophages in many higher animals, including mammals, the *Alligator mississipiensis,* and even one of the lowest vertebrate forms, the lamprey. Although PMNs of all these species can ingest foreign erythrocytes, macrophages show stronger positive chemotaxis toward foreign RBCs and are chiefly responsible for their destruction.

Antibacterial cellular immunity can be demonstrated easily in various species of fish, as shown by Mesnil (1895). Perch, gudgeon, and goldfish resisted intraperitoneal infection with *Bacillus anthracis* despite the fact that their peritoneal fluids readily supported growth of the bacilli *in vitro* at temperatures of 15 to 23° C. Mesnil clearly showed that the macrophages of osseous fishes are responsible for immunity to this organism even though it remains within the macrophages for long periods before being killed.

The temperature dependence of both phagocytosis and cellular immunity in various species was reviewed by Mesnil (1895), Metchnikoff (1905), and later by Bisset (1946, 1947).

Berry and Spies (1949) reviewed the literature on phagocytosis in certain lower animals, including invertebrates.

In recent studies on antigen clearance from the circulation by the macrophages of various species, Nelstrop, Taylor, and Collard (1968a, b, c) found that clearance of a second dose of $T_1$ bacteriophage was more rapid than clearance of a primary dose of the phage in the goldfish, lamprey, and shore crab, but not in the dogfish or land snail. They concluded that an immune response, phylogenetically more primitive than that concerned with humoral antibody formation, may exist in these animals. Apparently this postulated primitive response is concerned with a macrophage-associated cellular immunity, and is nonspecific. However, the possibility that macrophages of fishes may participate in specific immune reactions is suggested by the finding of Chiller *et al.* (1969) that macrophages from fish immunized with sheep RBCs can form rosettes, evidently because of surface cytophilic antibodies.

It is obvious that various activities of macrophages contribute to body economy throughout a wide spectrum of the phylogenetic scale.

## B. *Ontogeny*

The origin and development of the reticuloendothelial system (RES) in human embryos was described by Richter (1958), who also reviewed much of the earlier literature on this subject.

The ontogeny of the macrophage in human fetal tissues has been well described by Andersen and Matthiessen (1966), who reported that macrophages of typical morphology are widespread in fetal tissues. These cells exhibit ameboid motility, and have marked pinocytic and phagocytic capabilities. Their lysosomes contain an abundance of acid hydrolytic enzymes and fuse with phagosomes in characteristic fashion to form phagolysosomes. The cells appear to digest tissue, especially in certain areas undergoing embryonic development. In the early fetus they are found both within vessels and in perivascular connective tissue, but never in nonvascular mesenchyme. For this reason, Andersen and Matthiessen contend that these cells originate from "primitive leukocytes." Apparently they can differentiate into chondroclasts and multinucleated osteoclasts, as well as microglia cells.



The functional development of the RES in animal fetuses has been the subject of recent investigation. The phagocytic capacity of the RES develops progressively during fetal life of the rat (Reade and Jenkin, 1965) and the chick (Karthigasu and Jenkin, 1963), but fails to reach adult levels by the time of birth. Histological studies (Reade and Casley-Smith, 1965) revealed that Kupffer cells are capable of taking up intravenously injected particles from the circulation of fetal rats at the earliest time studied, 14 days after conception; however, their phagocytic efficiency increased with increasing age of the fetus. Karthigasu, Reade, and Jenkin (1965) examined the antibacterial capacity of fixed macrophages of both rats and chicks. They demonstrated that, even in the presence of specific antibodies, fetal macrophages generally lack the capacity to kill certain gram-negative bacteria following phagocytosis. It is probable that this attribute develops soon after birth, because the macrophages of neonatal animals were observed to possess bactericidal capabilities. However, the macrophages of adults are more active than those of newborn animals (Reade, 1968).

Certain components of C′, known to be synthesized by macrophages of adult animals (see Chapter 6), are also synthesized by the human fetus (Propp and Alper, 1968). Adinolfi, Gardner, and Wood (1968) demonstrated that macrophage-rich tissues, such as liver, lung, and peritoneal cells, taken from fetuses 10 weeks of age or older synthesize $\beta_{1C\text{-}1A}$ globulins *in vitro*. Tissues from 14-week-old fetuses made $\beta_{1E}$ globulin as well. These findings make it reasonably certain that macrophages acquire some of their specialized synthetic capabilities early in ontogeny.

*SUMMARY:* Macrophages are found in all animals. In the most primitive invertebrates they are totally responsible for the digestion of food and the disposal of foreign materials. In higher animals, some of their primitive digestive activities are replaced by enzymes secreted by other cells; however, macrophages retain digestive capabilities which aid in tissue resorption and other functions.

Antibacterial cellular immunity is a characteristic function of macrophages throughout a wide phylogenetic range. Whereas, in the lower animals, a primitive nonspecific response results in a macrophage-associated

cellular immunity, most of the vertebrate species can apparently supplement this by providing for specific immune activities of macrophages, through the agency of cytophilic antibodies or other means.

During the ontogeny of mammals, macrophages appear early and are active in resorption of tissue during embryonic development. They acquire many of their synthetic capabilities very early during ontogeny, as evidenced by their ability to synthesize hydrolytic enzymes and certain components of complement. The phagocytic capabilities of the RES increase progressively during fetal and neonatal life.

# Chapter 4

# Sources, Maturation, and Life Span of Macrophages

THE SOURCES, maturation, and life span of macrophages have been subjects of much conjecture. For many years, light microscopy was virtually the only tool available for studying these problems; in recent years, techniques for radioactive labeling and tracing of cell division and migration have provided better approaches.

## A. *Bone Marrow Stem Cells as Progenitors of Macrophages*

Experiments using radiation chimeras have provided evidence that the bone marrow is a major source of macrophage precursors. Indeed it has been suggested that bone marrow stem cells serve as the sole primary origin of macrophages (Virolainen and Defendi, 1968). Balner (1963) transplanted mouse bone marrow cells into lethally x-irradiated recipients. Cytotoxicity tests conducted with specific alloantisera demonstrated that at 6 weeks all of the free peritoneal macrophages in the resulting chimeras were derived from the transferred bone marrow cells. In similar experiments, Goodman (1964) showed that at various times, ranging from several months to over a year after transfer of bone marrow, virtually all of the peritoneal macrophages in the recipients were of donor origin.

Other experimental evidence likewise strongly supports the thesis that cells from the bone marrow serve as the principal progenitors of macrophages. For example, parabiosis experiments have demonstrated conclusively that many exudative macrophages are derived from blood-borne cells (Volkman and Gowans, 1965a). The common circulation between two parabiosed rats was interrupted long enough to label cells in one of the partners with a pulse of tritiated thymidine. Subsequent radioautography showed that a substantial number of macrophages in induced inflammatory exudates of the unlabeled partner were labeled, and hence were blood-borne, presumably monocytes from bone marrow. Studies with labeled cells further indicated that precursors of macrophages proliferate rapidly and continuously in areas distant from the inflammatory site, and release their progeny into the circulation. The extent and characteristics

of labeling of peritoneal exudate cells in rats given tritiated thymidine also indicated that a population of morphologically unidentified bone marrow cells provides precursors of peritoneal macrophages (Volkman, 1966). In addition, histochemical staining indicates that certain cells in bone marrow serve as precursors of monocytes and that there is a continuous flow of monocytes from the marrow (Braunsteiner and Schmalzl, 1968).

Still other evidence which supports the concept that bone marrow cells serve as principal progenitors of macrophages, as well as other cells, found in inflammatory lesions has come from the work of Spector and Willoughby (1968). These investigators transfused lymph node, thymus, or bone marrow cells into rats treated with 600 r of x-irradiation. It was found that, whereas rats restored with lymph node or thymus cells lacked the capacity to mount a cellular inflammatory response, animals which received bone marrow cells regained this capacity.

Macrophages in other locations have also been shown to be derived from cells in the bone marrow. Pinkett, Cowdrey, and Nowell (1966) used mice made chimeric by the transfer of marrow cells carrying the $T_6$ chromosome marker into syngeneic x-irradiated mice. Approximately two thirds of the alveolar macrophages arose from the transfused cells; evidently the remaining one third were derived from existing pulmonary tissue. It has been conclusively shown that macrophages in the liver normally develop mainly from bone marrow precursors, as reviewed by Boak *et al.* (1968).

### B. *Blood Monocytes as Circulating Immature Macrophages*

There is general agreement that blood monocytes can mature into tissue and exudative macrophages (Ebert and Florey, 1939; Tompkins, 1955; Lawkowicz and Krzeminska-Lawkowicz, 1957; Rebuck and LoGrippo, 1961; Spector, Walters, and Willoughby, 1965; Spector and Coote, 1965; Hurd and Ziff, 1968).

The recent radioautographic studies of Volkman and Gowans (1965a), Trepel and Begemann (1966), and Gillman and Wright (1966) have suggested that blood monocytes account for many of the macrophages in local inflammatory sites.

The importance of environmental stimuli in the differentiation of macrophages was stressed by Carrel and Ebeling (1926a, b) and Carrel (1934) many years ago. These investigators concluded that macrophages merely represent highly differentiated monocytes. Furthermore, they showed that differentiation can occur *in vitro* and depends on the medium used. Transformation of mouse monocytes into typical macrophages was observed *in vitro* and *in vivo* by Cohn and Benson (1965a).

The *in vitro* transformation of chicken monocytes into macrophages, then into epithelioid cells, and finally into multinucleated giant cells was described by Lewis (1925) and by Hetherington and Pierce (1931). Sutton and Weiss (1966) studied leukocyte cultures of cardiac blood of chickens by light and electron microscopy. The most active phagocytosis of particulates by mononuclear cells occurred between 6 and 24 hours. After one day in culture a monolayer of macrophages developed. Many of these cells transformed into epithelioid cells between days 3 and 6. Giant cells appeared as early as 2 to 4 days and were commonly present at 9 to 15 days.



That human monocytes readily transform into macrophages *in vitro* was shown by Lewis (1925) and by Berman and Stulberg (1962). The work of Rabinowitz and Schrek (1962) is particularly informative. These investigators used phase microscopy and cinemicrography to follow the transformation of human blood monocytes into mature macrophages; the transformation occurred in about one week.

Bennett and Cohn (1966) obtained relatively pure suspensions of monocytes from horse blood by flotation on dense albumin solutions. Further purification was achieved by allowing the monocytes to adhere to the culture vessel at 37° C for 2 hours, at which time most of the contaminating lymphocytes were washed away. Culture for 24 to 72 hours resulted in transformation of the monocytes into typical activated macrophages, as evidenced by their functional and biochemical characteristics. The authors, in agreement with van Furth and Cohn (1968), suggested that the probable development of macrophages from monocytes *in vivo* is as follows: blood monocytes are derived from the bone marrow and have a short half-life in the circulation before emigrating through capillaries into tissues, particularly in response to inflammatory stimuli; within tissues they mature to become typical macrophages.

### C. *Other Possible Sources of Macrophages*

In addition to bone marrow stem cells and peripheral blood monocytes, mature macrophages arise from other precursors. The possible transformation of lymphocytes to macrophages will be discussed in Section D of this chapter.

Early investigators believed that endothelial cells could give rise to macrophages. However, Sacks (1926) noted that Aschoff could find no evidence that phagocytes originate from vascular endothelium, but only from "specialized endothelium" in organs such as the liver and spleen. The present consensus is that certain of the cells adhering to endothelium of the sinuses of liver and spleen are inactive macrophages (littoral cells) and not true endothelial cells (Florey, 1962) and that vascular endothelium does not give rise to free phagocytes.

It has also been proposed that tissue mesenchyme is a source of macrophages. Libansky (1966) used the skin-window technique in an attempt

to define the source of macrophages at local inflammatory sites in a selected group of patients with various types of leukopenia. Although the intensity of neutrophil infiltration at the skin-window site correlated with the neutrophil count, no correlation was observed between the numbers of mononuclear cells at the site and in the circulation. Even when circulating mononuclear leukocytes were virtually absent the local inflammatory response was normal, provided the blood neutrophil count was normal. It was concluded that the macrophages at sites of inflammation in patients with decreased numbers of circulating mononuclear cells came from tissue mesenchyme. It was further suggested that tissue mesenchyme might serve as a source of macrophages in healthy individuals as well as in leukopenic patients.

Mobilization of histiocytes from tissues and from special organs and structures, such as the "milk spots" of the omentum, is commonly observed. On the basis of studies with the phase microscope, Felix and Dalton (1955) asserted that the majority of macrophages which accumulate in the ascites during inflammation of the peritoneum are mobilized from the omentum and that few, if any, come from the blood stream.

Peritoneal macrophages can proliferate (Felix and Dalton, 1955; Bloom and Fawcett, 1962; Forbes and Mackaness, 1963; Forbes, 1965). Their proliferation results from various stimuli including nonspecific bacterial products (see Chapter 5), antigen (Forbes, 1966), and growth factor produced by fibroblasts (see Chapter 5). Aronson and Elberg (1962) used tritiated thymidine to label the nuclei of rabbit peritoneal macrophages *in vivo* and *in vitro*. They observed that, although macrophages did not divide appreciably during the first 24 hours after injection of an irritant, many labeled cells appeared later suggesting that macrophages had proliferated in the peritoneum.

Virolainen and Defendi (1967) succeeded in inducing division in most of the cells of a macrophage monolayer. Other evidence which indicates that macrophages are capable of division and, furthermore, that they have a life span of weeks or months, has come from the *in vitro* studies of Chang (1964) who claims to have cultivated mouse peritoneal macrophages in good condition for 220 days. Mitotic division occurred early in the course of culture, but the percentage of dividing cells in this *in vitro* system was always less than 0.05%. Bennett (1966, 1967) also reported a low mitotic rate for macrophages *in vitro*. Epstein and Krasnobrod (1968) demonstrated that macrophages divide in experimental granulomas of man and concluded that these cells give rise to the epithelioid cells in the lesions.

Peritoneal macrophages of animals with delayed sensitivity to an antigen are stimulated to divide following contact with the antigen. Whereas only about 1% of peritoneal mononuclear cells from untreated mice synthesized desoxyribose nucleic acid (DNA), Forbes and Mackaness (1963) showed that more than 50% of peritoneal mononuclear cells (predominately

granulomas



macrophages) collected from sensitized animals 20 to 30 hours after challenge with specific antigen were synthesizing DNA. This suggests that division of macrophages mobilized locally is an important source of these cells in exudates associated with delayed sensitivity reactions.

It is conceivable that some of the macrophages associated with delayed sensitivity reactions could be derived from circulating lymphocytes which escape from antigen-stimulated lymph nodes. These lymphocytes are known to be responsible in some way for the development of delayed sensitivity. However, the labeling studies of Turk and Polák (1967) suggest that sensitized lymphocytes from draining lymph nodes do not directly transform or differentiate into macrophages. Turk and Polák found that, 8 days after giving a dose of allergen to produce delayed sensitivity, very few or none of the peritoneal macrophages were derived from cells of the draining lymph nodes.

Although it has been suggested that alveolar macrophages arise from septal cells of the lung (Robertson, 1941), this is only a remote possibility. The septal cells, also known as "great alveolar cells," "type II cells," and "granular pneumocytes," are secretory cells with "cytosomes" presumed to contain surfactant. Sorokin (1966) showed that alveolar macrophages and great alveolar cells differ cytochemically. Great alveolar cells also have limited phagocytic capabilities, as compared with alveolar macrophages. Thus, it is not probable that the two cell types belong to a single cell line.

Mitosis rates of rat alveolar cells were determined by Bertalanffy (1964). The life span of alveolar macrophages, from mitosis to the time of extrusion from the lung, was calculated to be about 27 days or less for the cells with many lipid-containing vacuoles and 9 days for those without large amounts of lipid. These results are essentially in agreement with those of Spencer and Shorter (1962) and Shorter, Titus, and Divertie (1964), who used tritiated-thymidine labeling to determine the turnover time of alveolar cells in mice. These investigators concluded that most alveolar macrophages have a life span of about 7 days, although some labeled cells remained in the lungs for 3 weeks and longer. Similarly, Bowden, Davies, and Wyatt (1968) determined that alveolar macrophages of the mouse have a turnover time of 7 days.

Bertalanffy (1964) reported that most alveolar macrophages are extruded from the respiratory tract into the sputum, and in this manner remove the bulk of inhaled particulate material from the lung. Some alveolar macrophages enter lymph channels, as evidenced by the gross darkening of hilar lymph nodes with accumulated dust particles, especially in older city-dwellers.

The migration of liver macrophages into the lung and their elimination via the respiratory tract has been suggested by the work of Nicol and Cordingly (1966), who found that intravenously injected carbon was

stored in Kupffer cells of the rat for several weeks. At 3 to 6 weeks, carbon-laden macrophages, presumably from the liver, appeared in the lungs. Carbon-laden cells in the liver decreased in number over a period of several months, during which time these cells appeared in the lungs. Similar findings were described following trypan blue administration (Nicol and Cordingly, 1967). Nicol and Cordingly suggested that, under normal conditions, some alveolar macrophages may be derived from Kupffer cells and other tissue macrophages. These findings also suggested that macrophages may have a life span of many months. Alternatively, it is possible that the particles were released from the macrophages which originally ingested them, to reach other macrophages. Evidence favoring these possibilities has also been presented by Singer *et al.* (1967) who suggested that this offers a mechanism for recirculation of antigen.

It is difficult, for several reasons, to entertain the concept that in the normal animal an appreciable percent of alveolar macrophages represent migrated Kupffer cells, unless one assumes that such cells modulate rapidly in the new environment. First of all, the morphology of the alveolar macrophage is distinct from that of the Kupffer cell. Second, the aerobic metabolism and enzyme content of alveolar macrophages vary considerably from those of all other macrophages. Finally, results of the *in vitro* studies of Bennett (1966, 1967) indicate that all macrophages can be placed in three groups on the basis of various properties including cultural characteristics, and that alveolar macrophages and Kupffer cells belong to distinct physiological groups. Alveolar macrophages belong to the group characterized by a relatively high mitotic rate and the ability to attach and spread readily on glass. The second group, to which peritoneal macrophages and blood monocytes belong, attach and spread readily on glass, but have a low mitotic rate. The third group, consisting of fixed macrophages from the bone marrow, spleen, and liver, attach and spread slowly on glass but have a relatively high rate of mitosis. Thus Kupffer cells are characterized by slow attachment and spreading on glass, whereas alveolar macrophages attach and spread rapidly. Other notable dissimilarities between alveolar macrophages and Kupffer cells are differences in metabolic patterns. On the whole, the evidence does not favor the concept that appreciable numbers of Kupffer cells migrate to the lungs.

As previously mentioned, $T_6$ cell transfer studies in mice have indicated that, whereas two thirds of alveolar macrophages arise from transfused marrow cells, the remaining one third is derived from existing pulmonary tissue (Pinkett, Cowdrey, and Nowell, 1966). Additional evidence that substantial numbers of these cells normally arise from division of existing alveolar macrophages has been reviewed by Bertalanffy (1964).

In their studies on brain macrophages, Kosunen, Waksman, and Samuelsson (1963) found that blood-borne cells differentiate into histiocytes

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in the lesions of experimental allergic encephalomyelitis. By tritiated-thymidine labeling, Konigsmark and Sidman (1963) showed that circulating leukocytes were a major source of macrophages at the site of a stab wound in the brain. However, during the first 2 days of wound healing some macrophages were derived from cells already present in the brain. It seems likely that under ordinary circumstances division of preexisting brain macrophages is sufficient to account for the maintenance of normal numbers of these cells.

### D. *The Controversy Concerning Lymphocyte-to-Macrophage Transformation*

The extensive literature pro and con on the transformation of lymphocytes into macrophages has been reviewed in great detail by Rebuck and Crowley (1955). As early as 1888, Metchnikoff noted that hematogenous lymphocytes migrate into tuberculous lesions of rodents with experimental tuberculosis. Lymphocytes in the lesions were alleged to differentiate into macrophages, epithelioid cells, and giant cells. Numerous workers have repeated these claims, as documented in the review quoted above.

Celloidin chambers implanted in tissues were employed by Maximow (1903) to study inflammation, healing, and scar formation. From the results of a series of observations, extending over the period from 5 hours to 8 months after the initiation of inflammation, Maximow concluded that macrophages are formed by transformation of blood-borne lymphocytes as well as by division of sessile histiocytes. These observations were originally challenged on the basis that lymphocytes are nonmotile and hence are unable to migrate into inflamed regions, a thesis that is no longer tenable.

Cappell (1929b) described the characteristics of cells of the omentum and mesentery, as observed by the use of vital staining. Macrophages were aggregated around vessels. Many cells resembling lymphocytes were believed to be reserves of relatively undifferentiated cells. Other cells were presumed to be intermediate between these "lymphocytes" and mature macrophages. The *taches laiteuses,* or "milk spots," are found throughout the omentum and mesentery. They are not associated with blood vessels, but are usually connected with fine lymphatic channels. Following RES stimulation, lymphocyte-like cells from the milk spots were alleged to differentiate into typical mature macrophages (Cappell, 1930). Later EM observations by Carr (1967) are in agreement with this concept.

Subsequent to the development of satisfactory cell culture techniques, Bloom (1928) reported that cells of thoracic duct lymph, predominately small lymphocytes, transform *in vitro* to monocytes, then to typical macrophages, and finally to "fibroblast-like" cells. Similar experiments using lymphocytes from other sources have been repeated by many investigators, and although some have agreed with the interpretation of Bloom (1928,

1938b), others have argued that macrophages and fibroblasts contaminate the thoracic duct lymph and that they eventually overgrow and replace dying lymphocytes. These experiments have been reviewed by Trowell (1965). Rebuck and Crowley (1955) presented data, obtained with the skin-window technique, which supports the theory that lymphocytes can transform to macrophages. They reported that during acute inflammation resting tissue histiocytes divide and give rise to an increased number of macrophages; however, the greatest share of the increase appeared to stem from intense proliferation of either blood monocytes or lymphocytes, or both.

Although the concept of lymphocyte-to-macrophage transformation is still open to question, recent reports of both *in vitro* and *in vivo* experiments lend it strong support.

Gough, Elves, and Israëls (1965) found that virtually pure preparations of blood lymphocytes did not transform into macrophages during culture *in vitro*. However, when a small proportion of neutrophils was mixed with the purified preparation of lymphocytes, much lymphocyte-to-macrophage transformation occurred. These results strongly suggest that the lymphocyte-to-macrophage transformation, presumed to occur *in vivo,* depends on homeostatic control by neutrophils or their degradation products.

Howard (1964) performed experiments *in vivo,* which strongly support the concept that lymphocytes can give rise to macrophages under certain conditions of intense RES stimulation, such as a graft-versus-host reaction. Parental thoracic duct lymphocytes with a chromosome marker were transfused into $F_1$ hybrids. Later, examination of Kupffer cells of the recipient showed that large numbers bore the marker and hence were derived from donor cells.

In agreement with other workers (Volkman and Gowans, 1965b), Howard, Boak, and Christie (1966) and Boak *et al.* (1968) reported that under normal conditions most macrophages in division are of bone marrow origin. However, in syngeneic radiation chimeras produced with thoracic duct cells, it was shown that during RES stimulation brought about by administration of *Corynebacterium parvum* vaccine, approximately two thirds of the liver macrophages in division were derived from chromosome-marked donor cells. Although the many nondividing cells could not be identified as to origin, the results showed decisively that thoracic duct cells, which are chiefly small lymphocytes, can give rise to macrophages during conditions of intense RES stimulation.

It would appear that the lymphocyte-to-macrophage controversy rests, at least in part, on a problem of cell heterogeneity and nomenclature. The term "small lymphocyte" is used to designate a small (6 to 8 $\mu$) cell with a dense network of nuclear chromatin, surrounded by a thin rim of cytoplasm. These cells are normally found in blood, lymph, and lymphoid tissues. However, the morphological definition, small lymphocyte, has no implications with respect to heritage or ultimate fate, but simply designates an inactive resting cell with most of its genome repressed. It seems probable that small lymphocytes merely represent resting cells of heterogeneous origins and potentialities (Berman, 1966; Volkman, 1966) and that they can respond to different stimuli by differentiating to various cell types. Although there is good evidence that some of these cells recirculate for months or years (Everett and Tyler, 1967) it is known that some can respond to certain stimuli with derepression and differentiation (Gowans and McGregor, 1965). For example, many, but not all, lymphocytes respond to phytohemagglutinin (PHA) with blast transformation. Their nuclei become activated and histones are acetylated (Pogo, Allfrey, and Mirsky, 1966). Nucleoli form, which probably serve as the site of ribosomal synthesis, and greatly increased numbers of ribosomes cause increased basophilia of the cytoplasm. Such blast cells can in turn divide and give rise to small lymphocytes.



Elves (1967) presented data which suggest that at least two populations of lymphocytes exist: one which can be stimulated to undergo blast transformation, and another which has the capacity to differentiate into macrophages in the presence of PMNs. As previously stated, it is presumed that the transformation of lymphocytes to macrophages is initiated by products of dying PMNs. This could readily explain the sequence of cellular changes in inflammatory exudates.

The existence of more than one population of lymphocytes was also suggested by the data of Berman and Stulberg (1962), who reported that human buffy coat cultures after 15 to 25 days *in vitro* consist almost entirely of macrophages, giant cells, and small lymphocytes. Presumably many of the macrophages in the cultures arose from small lymphocytes. When PHA was added to the 15- to 25-day-old cultures, the remaining lymphocytes underwent blastogenesis. These results indicate that in the presence of PMNs some of the lymphocytes in blood are capable of transforming into macrophages, and that other lymphocytes lack this capacity but are able to respond to PHA with blast transformation.

Even if some small lymphocytes have the capacity to differentiate into macrophages following appropriate stimuli, there is no reason to believe that all lymphocytes have this capacity. Since macrophages evidently do not form antibodies, it is also reasonable to suppose that any lymphocyte progenitor of macrophages would likewise be incapable of forming antibodies. Better techniques for cell identification ultimately may permit more precise classification of small lymphocytes than is possible with morphological criteria alone. For example, if a population of cells exists with the morphology of lymphocytes and the potential of differentiating

into macrophages, its members may already possess certain characteristics of macrophages such as cytophilic antibody receptors on their surfaces. This would permit the distinction of these cells from lymphocytes with other potentialities, such as hematopoietic capabilities or the capacity to respond to antigens with blast transformation.

To recapitulate, the controversy about a possible lymphocyte-to-macrophage transformation remains open. The strength of evidence indicates that some, but certainly not all, cells with the morphology of small lymphocytes can respond *in vitro* to the presence of PMNs by transforming into macrophages. The *in vivo* circumstances in which such a transformation is claimed to occur invariably involve the presence of PMNs (see Chapter 5).

*SUMMARY:* Evidence is cited in support of the bone marrow origin of most of the macrophages normally found in lung, peritoneal exudate, and local inflammatory lesions. There is general agreement that monocytes migrate from the circulation and mature into typical tissue and exudative macrophages. Other potential sources of macrophages include mobilization and proliferation of existing tissue macrophages, migration of Kupffer cells into the lung, the transformation of lymphocytes to macrophages, and the remote possibility that macrophages may arise by differentiation of specialized endothelium. The controversy concerning lymphocyte-to-macrophage transformation is discussed. Most of the available data are consistent with the concept that some lymphocytes can transform into macrophages under the homeostatic control of PMNs or their degradation products.

# Chapter 5 | Macrophages and Cellular Homeostasis

THE PROBLEM of determining the factors responsible for cell differentiation and homeostasis in complex organisms is currently one of the most fascinating aspects of biological research. Macrophages function in the homeostasis of several cell types in higher animals, while other cells influence macrophage proliferation and differentiation.

## A. *General Theories Concerning Homeostasis*

Recently several interesting hypotheses relating to the overall problem of homeostasis have been set forth. Burwell (1963) and Burch and Burwell (1965) postulated that "tissue coding factors" (TCF) released from tissues reach regional lymph nodes where they may be processed by macrophages before being presented to lymphoid cells. They proposed that, by reacting with specific cell-bound receptor molecules on the surfaces of mitotically competent lymphoid cells, TCF enables these cells to regulate tissue growth throughout the mammalian organism. According to another theory, formulated by Heinmets (1968), exoenzymes on cell membranes of macrophages or other cells act on substrates on the surfaces of adjacent cells. It is proposed that the products of substrate degradation are carried into the exoenzyme-bearing cells where they can act as operon activators to control synthetic processes. Strong experimental evidence to support these hypotheses has not been presented.

One of the best examples of the *modus operandi* of cell differentiation and homeostasis in adult mammals is the development of erythropoietic and lymphoid tissue. The existence of undifferentiated stem cells as progenitors of certain cell types is well known, although their morphology has not been established. A bone marrow cell morphologically similar to the lymphocyte may serve as the stem cell for erythroid and lymphoid tissue (Yoffey *et al.*, 1961). It is probable that the bone marrow stem cell is multipotent (Trentin and co-workers, 1967). Virolainen and Defendi (1968) presented findings which suggest that a single bone marrow stem cell can give rise to cells of the erythrocytic, lymphocytic,

A growth regulator of singular interest has been described by Bullough and Laurence (1967) and Bullough *et al.* (1967), who extracted an antimitotic substance from the epidermis of the mouse, man, and even codfish which acts on epidermal cells of the same and other species. This substance, called epidermal chalone, is tissue-specific but acts across wide species barriers, and appears to be a low molecular weight glycoprotein with basic properties. Tissue-specific chalones have been extracted from liver, kidney, and granulocytes, and it is reasonable to assume that similar mitosis-regulating substances exist for other cell types, including the macrophage.

It has been postulated by Bullough that during the healing of skin wounds the concentration of mitosis-inhibiting chalone within epidermal cells is reduced owing to fluid uptake by the cells, with a consequent increase in epidermal mitosis. Following replacement of destroyed epidermal cells, mitosis would cease because the level of chalones again becomes normal. It is assumed that the lower the chalone concentration, the more active is the mitosis operon and the more rapid the onset of synthesis of enzymes essential to mitosis.

These general theories of homeostasis have not been tested experimentally in the macrophage system; however, certain lipids and hormones appear to play important roles in macrophage homeostasis. There is also evidence to suggest that interactions between macrophages and other cells play an important part in maintaining homeostasis of the interacting cells.

## B. *Factors Influencing Macrophage Proliferation and Differentiation*

Substances which are known to influence macrophage differentiation and proliferation are discussed below.

### 1. Lipids

The abundant evidence that lipids affect the phagocytic capacity of macrophages will be discussed elsewhere. Certain lipids also influence macrophage proliferation. Stanley (1949; 1950) extracted a chloroform-soluble lipid called monocytosis-producing agent (MPA) from *Listeria monocytogenes,* an organism so named because of the characteristic monocytosis that it produces in mammalian hosts. Stanley showed that MPA can produce monocytosis in animals. Lipids from tubercle bacilli and other mycobacteria also elicit monocytosis and granulomatous responses (reviewed by Canetti, 1955). The chemical nature of these monocytogenic lipids has not been determined.

### 2. Endotoxins

Endotoxins exert an influence on macrophages, but the manner in which this influence is mediated is uncertain. The results of endotoxin treatment are so extremely diverse that it is difficult to determine the mechanisms of action leading to any one effect (see Chapter 7, Section F).



Windle *et al.* (1950) studied the effects of a *Pseudomonas* endotoxin on the cells and tissues of a variety of laboratory animals. They reported that the most consistent alteration observed following endotoxin treatment was hyperplasia and sometimes metaplasia of the lymphoid and myeloid organs. Small repeated doses of endotoxin given over a short period of time led to increased phagocytosis. More intensive treatment over longer periods of time resulted in the accumulation of large numbers of macrophages and lymphocytes in spleen, lymph nodes, and bone marrow. Thus, although the mechanism of action is unknown, it would appear that endotoxin administration can lead to extensive proliferation of various types of cells, including macrophages.

A later report of Windle *et al.* (1954) may also be significant. Although spinal cord transection is almost universally irreparable there has been a report of limited restoration of function in a patient infected with gram-negative bacilli. It was postulated that endotoxin from gram-negative organisms might inhibit scarring and permit nerve regeneration in the cord. To test this hypothesis, Windle and co-workers subjected adult cats with upper lumbar cord transection to endotoxin treatment. They reported a temporary limited restoration of nerve function in some of the animals. Eventually, the formation of scars led to loss of function of the regenerated cord. These results suggest that macrophages and fibroblasts exert a homeostatic influence on each other and that, when macrophage function is impaired, fibroblast proliferation, healing, and scarring are inhibited. However, it is also possible that some other effect of endotoxin may have led to these interesting results.

In general, endotoxins play an ill-defined role in macrophage homeostasis. They destroy macrophages under certain conditions, but can also lead to macrophage proliferation; dosage and timing of endotoxin administration are of primary importance. Through their action on macrophages, endotoxins could affect homeostasis of other cell types as well. Whether indirect effects of endotoxin, such as hormone alterations, contribute to their overall activity to any extent is not known.

### 3. Monocytogenic Hormone

Willoughby, Coote, and Spector (1967) reported that the injection of Freund's complete adjuvant directly into lymph nodes is followed by a marked and prolonged monocytosis (three to ten times the normal number of circulating monocytes) lasting for 6 weeks or longer. They postulated that a monocytogenic hormone, produced by the stimulated lymphoid tissue, probably acts on bone marrow, since the active factor could be transferred with serum. These authors suggested that this monocytogenic hormone causes proliferation of macrophages during states of delayed sensitivity.

*in vivo* is innocuous (Curran and Rowsell, 1958). The relation of crystalline form to cytotoxicity of silica particles is of interest. Recent *in vitro* experiments using electron microscopy and enzyme histochemical procedures have disclosed the probable mechanism of macrophage destruction by silica (Allison, Harington, and Birbeck, 1966; Comolli, 1967). Evidently phagocytized silica particles act on phagolysosomal membranes and allow leakage of hydrolytic enzymes into the cytoplasm, and eventually into the medium, resulting in cell death. The selectivity of crystalline silica for macrophage membranes suggests that the crystals may interact with some specific chemical configuration peculiar to macrophage membranes. Upon lysis of the cell, the silica is liberated and is soon phagocytized by other macrophages. Cycling of the silica, which cannot be metabolized, results in a prolonged period of macrophage destruction. After a time, usually months or years, the cycle ends when fibrosis finally walls off the offending particles in granulomatous lesions.

Fibroblasts are present in silicotic lesions from their inception and rapidly increase in numbers. Collagen production is marked and as much as 40% of the total protein of the silicotic nodule is collagen (Pernis, 1955). The necrosis of macrophages which could be observed in tissue sections prior to fibroblast proliferation strongly suggested that some factor released from dying mature macrophages induces fibroblast proliferation and collagen synthesis. Confirmation of this concept has come from experiments *in vitro* which have shown that a factor released from dying rat peritoneal macrophages after silica ingestion causes increased fibrogenesis by chick embryo fibroblasts (Heppleston and Styles, 1967).

With respect to the role of the macrophage in the homeostasis of fibroblasts, it is of interest that there have been reports of reticulum cell sarcomas apparently caused by long-term RES stimulation. Gillman, Gillman, and Gilbert (1949) noted that reticulum cell sarcomas develop in the livers of rats following prolonged treatment with trypan blue. The work of Simpson (1952) confirmed this observation. Gillman and co-workers (1949) found that cysts and tumors were frequently formed in the liver, apparently by rapidly proliferating malignant histiocytes, and once formed they remained for many months. The absence of fibrosis was very remarkable in such livers. A probable explanation to account for this phenomenon is that immature neoplastic macrophages lack the capacity to synthesize the inducer of fibrosis.

A macrophage growth factor (MGF) produced by fibroblasts has been characterized by Virolainen and Defendi (1967), who found that culture medium removed from 3-day cultures of L-cells or mouse embryo fibroblasts serves to stimulate the proliferation of mouse macrophages *in vitro*. Medium collected from syngeneic or allogeneic, but not xenogeneic, fibroblasts was effective. It was possible to maintain macrophage cultures for



several cell divisions, provided MGF was added regularly. The factor was stable at 65° C for 1 hour but lost activity when heated to 100° C for 10 minutes. Activity was fully maintained during storage for 6 months at 4° C, but when held at 37° C for 6 months there was some loss of activity. It was not dialyzable, either before or after trypsin treatment, and it was resistant to a wide variety of hydrolytic enzymes. Activity was not destroyed by treatment with ether or with ultraviolet light for 30 minutes. It is possible that MGF is identical or closely related to the macrophage inducer described by Ichikawa, Pluznik, and Sachs (1966, 1967) and discussed below.

#### 4. Polymorphonuclear Neutrophil and Macrophage

Homeostasis of normal mouse macrophages and granulocytes has been studied in an *in vitro* system by Pluznik and Sachs (1966) and Ichikawa, Pluznik, and Sachs (1966, 1967). It was found that cultured fibroblasts and some other types of cells release a substance(s) which can induce the formation of colonies of macrophages or granulocytes from single cells of embryo liver or adult spleen. The inducer substance was nondialyzable and stable at 37° C for at least a week. It lost only 30% of its activity after heating at 56° C for 30 minutes, but lost all activity after heating at 90° C for 30 minutes. Subsequent experiments revealed that the ability of the substance to induce macrophage colony formation from progenitor cells is inhibited in the presence of irradiated macrophage feeder layers. This was shown to be due to a dialyzable inhibitor substance produced by the macrophages (Ichikawa *et al.*, 1967). Although the inducer substance was required for formation of colonies of either macrophages or granulocytes, the inhibitor substance formed by irradiated macrophages inhibited macrophage colony growth only. It was proposed that the system represents a mechanism for homeostatic control of growth and development of macrophages and granulocytes; the inducer for both types of cells being produced by fibroblasts, and the inhibitor for macrophages being produced by mature macrophages.

There is evidence that granulocytes contain a factor(s) which causes cells resembling small lymphocytes to transform into macrophages. Gough, Elves, and Israëls (1965) found that blood lymphocytes alone did not transform into macrophages *in vitro;* however, if granulocytes were added to purified lymphocytes, extensive transformation occurred.

Other evidence in support of PMN stimulation of macrophage differentiation has come from the work of Jones (1966). When mixed cultures of genetically dissimilar blood leukocytes are prepared, many macrophages are present at 3 days and many blast cells are seen at 6 to 9 days. On the other hand, when most of the PMNs are removed from the blood before mixed cultures are prepared, they contain relatively small numbers of

medium contains 20% newborn calf serum rather than 1% serum. Macrophages of the rat, but not the mouse, were able to synthesize certain serum proteins in a synthetic medium containing 0.1 to 0.2 μg/ml of hydrocortisone in lieu of serum; higher concentrations (1 μg/ml) of hydrocortisone completely inhibited synthesis. It was postulated that cortisol, which is naturally bound to albumin in the serum, is slowly released and acts as a membrane stabilizer or in some other way to profoundly affect metabolism.

Macrophages have not been grown in completely synthetic media without cortisone. Even when the medium contains serum, macrophages may have limited metabolic potential. As a rule, macrophage proliferation is sparse or absent in ordinary culture media (Jacoby, 1965; Bennett, 1966); however, it can be induced in several ways. One procedure is to add macrophage growth factor to the culture. This factor, described by Virolainen and Defendi (1967), is present in medium collected from cultures of syngeneic or allogeneic (but not xenogeneic) fibroblasts. Proliferation can also be induced with appropriate amounts of endotoxin (Forbes, 1965), or by means of an antigen-antibody reaction (Forbes and Mackaness, 1963; Rowley and Leuchtenberger, 1964; Forbes, 1966). Macrophages from sensitized animals can respond *in vitro* to specific antigen with mitosis and proliferation, evidently by virtue of cytophilic antibodies which combine with the antigen at the cell surface.

## B. *Energy Sources*

### 1. Peritoneal Macrophages

The *in vitro* studies of Harris and Barclay (1955) showed that rabbit peritoneal macrophages are facultative anaerobes. In the presence of oxygen, they had a constant $O_2$ uptake which did not depend on the oxygen tension of the medium. Most of the glucose used was converted to lactic acid, even when $O_2$ was present. Increased glycolysis supplied cellular energy requirements under anaerobic conditions. The conclusion drawn was that the metabolism of peritoneal macrophages depends largely on energy obtained by glycolysis under either aerobic or anaerobic conditions. Similar conclusions were reached by Oren *et al.* (1963), who found that peritoneal macrophages of the guinea pig depend on glycolysis as the principal source of energy for phagocytosis.

Energy for pinocytosis may be derived in other ways. Cohn (1966) demonstrated that the high degree of pinocytosis exhibited by mouse peritoneal macrophages cultured in 50% newborn calf serum can be markedly reduced by a number of metabolic inhibitors. The most effective inhibition of pinocytosis was achieved with cyanide, antimycin A, or anaerobiosis, thus indicating that these cells depend largely on mitochondrial activity for energy to support extensive pinocytosis.

Thus, peritoneal macrophages are facultative anaerobes which utilize glycolysis as a major metabolic pathway. They are also capable of obtaining energy via aerobic metabolism in certain circumstances.



### 2. Alveolar Macrophages

It is generally agreed that alveolar macrophages are facultative aerobes. Oren *et al.* (1963) compared the metabolism of alveolar macrophages, peritoneal macrophages, and peritoneal PMNs of the guinea pig. They showed that resting alveolar macrophages have a much higher level of oxygen uptake than does either of the other two cell types. Furthermore, inhibitors of oxidative phosphorylation suppressed phagocytosis by alveolar macrophages, indicating that these cells depend on aerobic oxidative metabolism for the energy used in phagocytosis (see Chapter 7, Section C).

The metabolism of alveolar macrophages of rabbits has been investigated by Ouchi, Selvaraj, and Sbarra (1965). In agreement with the work just discussed, the results of these investigators indicated that noninduced alveolar macrophages utilize aerobic metabolism to supply the energy required for phagocytosis. Inhibitors of the tricarboxylic acid (TCA) cycle did not inhibit phagocytosis, even though it was shown that they enter the cells, indicating that the TCA cycle is not a major source of energy for phagocytosis by these alveolar macrophages. It was subsequently shown by Myrvik and Evans (1967a, b) that, following challenge of sensitized rabbits with BCG, there is a large increase in hexose monophosphate shunt metabolism by macrophages.

Thus it would appear that the energy requirements of alveolar macrophages are normally met by oxidative metabolism. The production of energy required for phagocytosis will be further discussed in Chapter 7.

## C. *Synthesis of Enzymes*

From the time of Metchnikoff it has been recognized that macrophages contain cytoplasmic granules which stain with neutral red, and that activated macrophages contain more of these granules than do nonactivated macrophages. Cappell (1929a, b, c) studied the principles and techniques of neutral red staining. He noted that neutral red is basic and stains the granules of macrophages in a characteristic manner. By use of the more sophisticated techniques of histochemistry and electron microscopy, North (1966b) showed that acid phosphatase is abundant in the lysosomes of macrophages and that, following fusion of phagosomes with lysosomes, the enzyme is discharged into phagolysosomes. That the enzymes in macrophage lysosomes are actively synthesized by the cell is apparent from the studies of Cohn and Benson (1965b), in which metabolic inhibitors and radioactive labels were used.

Macrophages not only contain large quantities of acid hydrolases enclosed within lysosomes, but also show characteristic patterns of enzyme activity (see review by Cohn, 1965). Indeed, Braunstein, Freiman, and

Gall (1958) suggested that the characteristic pattern of enzyme activity might prove to be as reliable an indicator for distinguishing macrophages from other cells in certain mixed cell populations as the silver stain described by Marshall (1956).

The enzyme loads of macrophages can vary considerably, depending on the regional source of the cells, the stimulus to their proliferation or maturation, and other factors. Because the cell populations studied frequently have been from either peritoneum, lung, blood, or other tissues, it is possible to discuss some of the evidence illustrating the enzyme pattern of each of these regional populations of macrophages.

### 1. Peritoneal Macrophages

Hydrolytic enzymes of the peritoneal macrophages of rabbits were assayed by Dannenberg and Bennett (1964). Lysosomes were ruptured by freezing and thawing the cells, and two proteases were characterized in the resulting lysate. The first, with a pH optimum of 4.0, is similar to the pepsin-like proteinase I of the lung; the second, with a pH optimum between 5.0 and 5.8, resembles chymotrypsin. Esterases that hydrolyze methyl butyrate and β-naphthyl acetate at pH 7.0 to 8.0 were present, as well as a lipase with a pH optimum of 6.1, which has many of the characteristics of a lipoprotein lipase previously described. Rabbit peritoneal macrophages have also been shown to contain cholesterol esterase (Day, 1960a; Day and Gould-Hurst, 1963).

Histochemical studies (Kessel *et al.*, 1963) indicate that the macrophages of several species contain the following enzymes: succinic dehydrogenase, alkaline and acid phosphatases, esterases, aminopeptidase, lipase, DPN and TPN diaphorase, and β-glucuronidase.

Sonically disrupted peritoneal macrophages from normal rats and guinea pigs and from BCG-immunized guinea pigs were analyzed for enzyme content by Colwell, Hess, and Tavaststjerna (1963). The following enzyme activities were found: alkaline phosphatase in rat but not in guinea pig peritoneal macrophages; acid phosphatase in guinea pig and rat peritoneal macrophages; β-glucuronidase in both; lysozyme in both, but more in peritoneal macrophages from rats; esterase (tributyrinase) in peritoneal macrophages from guinea pigs but not rats; and lipase in both.

The lysozyme content of peritoneal macrophages from normal outbred rabbits is much lower than that of their alveolar macrophages (Myrvik, Leake, and Fariss, 1961b). Macrophages from normal rabbits of strains inbred for either resistance or susceptibility to tuberculosis show no substantial difference in lysozyme content; however the alveolar macrophages of normal rabbits contain about seven times as much lysozyme as their peritoneal macrophages (Carson and Dannenberg, 1965).

Following infection, the enzyme content of macrophages increases.



Carson and Dannenberg (1965) reported lysozyme levels 1.4 times normal in peritoneal macrophages collected from tuberculous rabbits. Saito and Suter (1965) found significant increases in acid phosphatase, β-glucuronidase, and cathepsin, in peritoneal macrophages from mice infected with BCG. Similar results were obtained by Heise, Myrvik, and Leake (1965) with alveolar macrophages of the rabbit.

Bennett (1966) compared the *in vitro* growth characteristics of relatively purified populations of mouse macrophages from the peritoneum, lung, and other sites. He found that the lysosomal acid phosphatase activity of peritoneal macrophages increases after 24 hours *in vitro*. Cohn and Benson (1965c) evaluated the effects of varied cultural conditions on hydrolase activity of mouse peritoneal macrophages. Little or no increase in enzyme levels occurred when small amounts of newborn calf serum were added to the culture medium; when the concentration of serum in the medium was increased, acid phosphatase, cathepsin, and β-glucuronidase were formed more rapidly and in larger amounts. Evidently, increased enzyme production was related to improved cellular function which resulted from the increased pinocytosis induced by high levels of serum.

North (1966a) demonstrated a nucleoside phosphatase, apparently ATPase, on the membrane of guinea pig peritoneal macrophages. It was postulated that the activity of this enzyme supplies energy for phagocytosis and membrane movement.

Many other enzymes have been demonstrated in the peritoneal macrophages of various species; some of these are listed in the table presented on pages 48 and 49 of this chapter.

### 2. Alveolar Macrophages

By virtue of their location within alveoli, lung macrophages are of prime importance in protecting the respiratory tract against microbial invasion. They are extremely rich in enzymes, some of which are known to be involved in intracellular destruction of bacteria. Myrvik, Leake, and Fariss (1961a) described a method for obtaining large quantities of relatively pure suspensions of alveolar macrophages, which has been very useful for *in vitro* studies. With this technique, approximately 0.1 to 0.2 ml of packed alveolar macrophages can be washed from the lungs of a normal rabbit. Following vaccination and subsequent challenge with tubercle bacilli the yield is tremendously increased to 2.5 to 8.0 ml packed cells (Myrvik, Leake, and Oshima, 1962).

Myrvik, Leake, and Fariss (1961b) measured lysozyme in extracts of alveolar macrophages obtained by freezing and thawing the cells. The lysozyme level in alveolar macrophages was 3400 μg/ml of packed cells; in contrast, 540 μg of lysozyme were present in each milliliter of packed peritoneal cells having a similar protein content. The authors postulated

## D. *Synthesis of Lipids*

It has long been known that macrophages are active in lipid synthesis. For example, Lumb (1954) noted that macrophages consistently participate in the disposal of excess lipids; Byers (1960) reviewed many of the findings which implicate macrophages as being active in lipid synthesis. Included among these findings are evidence for (1) uptake and both hydrolysis and esterification of cholesterol, (2) release of accumulations of cholesterol and vitamin A esters from reticuloendothelial cells after the injection of colloids, (3) uptake of chylomicrons from the serum by cells of the RES in a manner analogous to the uptake of colloids, and (4) a relation between macrophage storage of fats, macrophage proliferation, and the lipidoses (e.g. Hand-Schüller-Christian, Niemann-Pick, Hurler's and Gaucher's diseases).

Antonini (1967) postulated that reduction of the phagocytic functions of the RES in senescence could contribute to the reduced rate of cholesterol metabolism during aging.

Day (1967) reviewed much of the literature on lipid metabolism by macrophages in relation to atherosclerosis. There is much data indicating that macrophages play a major role in both the degradation and synthetic utilization of cholesterol and other lipids.

Macrophages within lymph nodes of the rat readily ingest and retain cholesterol for at least 4 months (French and Morris, 1960). By using either $H^3$, $C^{14}$, or $P^{32}$ to label different lipids, Day, Gould-Hurst, Steinborner, and Wahlqvist (1965) showed that, when cholesterol, triglyceride, and phospholipid are ingested simultaneously by lymph node macrophages, cholesterol is removed more slowly than the triglyceride and phospholipid. After the uptake of cholesterol, other lipids accumulate within the lymph nodes, presumably as the result of macrophage metabolism (Day, 1960b). It is possible that cholesterol ester may be utilized by lymph node macrophages in the subsequent synthesis of lipoproteins (Day, Fidge, Gould-Hurst, and Wilkinson, 1965; Day, 1967). Triglycerides are hydrolyzed within lymph node macrophages and the resultant fatty acids are incorporated largely into phospholipid (Day *et al.*, 1966).

Peritoneal and alveolar macrophages may also be active in lipid metabolism. Isolated peritoneal macrophages of the rabbit can incorporate labeled acetate into cholesterol (Day and Fidge, 1964) and can degrade cholesterol. Day and French (1959) and Day (1960a) showed that homogenates of peritoneal macrophages have both synthetic and hydrolytic cholesterol esterase activity. Day and Gould-Hurst (1963) demonstrated partial esterification of $C^{14}$-labeled cholesterol by homogenates of peritoneal macrophages; the addition of lecithin to the reaction mixture inhibited esterification and facilitated the hydrolysis of cholesterol. Alveolar macrophages have also been shown to contain cholesterol esterase (Day, 1967) and lipase (Cohn and Weiner, 1963; Elsbach, 1965).



Fatty acids and triglycerides are taken up and oxidized by rabbit peritoneal macrophages (Day, 1960c; 1961). By the use of electron microscopy, Casley-Smith and Day (1966) showed that triglyceride emulsions are readily phagocytized by peritoneal macrophages and become localized within large phagosomes. The subsequent appearance of a clear space between the electron-dense triglyceride and the phagosome membrane suggests that the lipid is degraded within the phagosome.

Increased phospholipid synthesis has been reported to accompany high phagocytic activity by peritoneal exudate cells, predominately PMNs (Karnovsky and Wallach, 1961), and probably reflects the formation of many phospholipid phagosomal membranes. Obviously, phagocytic activity by macrophages also demands the formation of new phospholipid membranes. Increased phospholipid synthesis is accompanied by an increase in oxygen uptake and glucose metabolism through the hexose monophosphate shunt (Day, 1967).

It is only remotely possible that the cells that produce lung surfactant are related to alveolar macrophages. Most investigators agree that great alveolar cells produce surfactant. While it has been suggested that these cells can give rise to alveolar macrophages, this suggestion is not widely accepted. Great alveolar cells, also known as type II cells, septal cells, or granular pneumocytes, are the more numerous of the cells which constitute the alveolar wall. By use of the electron microscope and cytochemical techniques, Sorokin (1966) showed that great alveolar cells synthesize and secrete various materials, and that lysosome-like cytosomes of these cells contain considerable amounts of lipids, especially phospholipids. Buckingham *et al.* (1966) also reported that great alveolar cells synthesize phospholipids, as shown by the incorporation of tritiated acetate or palmitate. Radioautographs of lung revealed that the labeled compounds accumulate within the cytoplasm of the large alveolar cells. The results of Vatter *et al.* (1968) are in agreement with the concept that type II cells synthesize surfactant and, in addition, showed that the esterase activity of the lung is most prominent in the great alveolar cells. Goldfischer, Kikkawa, and Hoffman (1968) observed that great alveolar cells contain hydrolases and synthesize surfactant; however, these authors do not favor the concept that alveolar macrophages are derived from great alveolar cells. Thus, although it is probable that the great alveolar cell is responsible for the synthesis of surfactant, present evidence provides no substantial support for the concept that it is a precursor of macrophages, and the possible genealogic relationship between this cell and the alveolar macrophage remains undetermined (see also review by Day, 1967).

## E. *Synthesis of Interferon*

It is logical to expect that macrophages play an important role in antiviral defense, especially of the respiratory tract. Recent studies have conclusively demonstrated the production of interferon by macrophages and have established the importance of this cell in defense against viral infections.

Compelling evidence implicating the macrophage as a source of interferon was presented by Kono and Ho (1965), who showed that tissues rich in macrophages, such as spleen and liver, form large quantities of interferon within 24 hours *in vitro*. In contrast, tissues which contain few macrophages, e.g. kidney and brain, formed much smaller quantities of interferon, which were not detectable until after 24 hours. Kono and Ho also measured interferon in the serum of animals given virus 18 hours after a dose of thorium dioxide (Thorotrast) sufficient to cause RES blockade. They noted that in such animals a consistent depression of serum interferon levels was evident at 3 hours after virus inoculation; however, by 7 hours no effect of the thorium dioxide treatment was apparent. It was concluded that reticuloendothelial tissues respond more rapidly to stimuli for interferon production than do other tissues. Moreover, treatment of mice with agents known to cause stimulation of the RES results in the appearance of interferon in the serum, as has been shown by Stinebring and Youngner (1964).

Conclusive evidence that alveolar macrophages can produce interferon has come from the work of Acton and Myrvik (1966). They showed that rabbit alveolar macrophages, inoculated *in vitro* with parainfluenza-3 virus, produce a viral inhibitor which is evidently interferon. The substance is nondialyzable, is stable at pH 4.0, and does not sediment at 100,000 x g. It protects cells of animals of the same species against infection by viruses unrelated to the original virus which induced interferon production. Incubation of normal rabbit alveolar macrophages with this material for 18 hours before challenging the cells with rabbit pox virus protected the cells against destruction by the second virus. Thus, it is clear that alveolar macrophages can produce interferon.

Peritoneal macrophages of rabbits have also been shown to produce interferon (Smith and Wagner, 1967a). These cells synthesized interferon as efficiently as rabbit kidney (RK) cells. By 2 hours after exposure of the macrophages to virus, interferon was detected in the medium and peak titers were found by 4 to 6 hours. Actinomycin blocked interferon production by RK cells if given before 60 to 120 minutes after virus infection and by macrophages only if given prior to 30 to 60 minutes after infection. Smith and Wagner suggested that this may indicate that messenger RNAs responsible for interferon production are transcribed faster and earlier in macrophages than in RK cells.



Smith and Wagner (1967a,b) also showed that bacterial endotoxins and viruses elicit interferon production by rabbit peritoneal macrophages at the same rate. However, the total amount of interferon produced after endotoxin was only 1% or less of that produced in response to virus. The physical properties of the macrophage-produced interferons were examined; the two principal kinds had molecular weights (MWs) of 37,000 and 45,000. In rabbit sera, most interferons have approximate MWs of 51,000 and $>134{,}000$. The data suggest that interferons produced by peritoneal macrophages are not identical with the majority of serum interferons synthesized in response to viral infection.

## F. *Synthesis of Serum Proteins*

Stecher and Thorbecke (1967a) studied the *in vitro* incorporation of $C^{14}$-labeled amino acids into serum proteins by various kinds of cells. By use of radioautographic and immunoelectrophoretic techniques they identified labeled serum proteins in cell-free culture fluids. The types of adult rat cells included in the study were thoracic duct cells, peripheral blood leukocytes, and peritoneal exudate cells. The peritoneal cells were separated into fractions rich in either mast cells, eosinophils, or macrophages. It was found that peritoneal macrophages, isolated on glass, synthesized much more $\beta_{1C}$ globulin (C′ 3) and transferrin than did any of the other cell types. Both peritoneal and alveolar macrophages from mice, guinea pigs, and rabbits invariably produced $\beta_{1C}$ globulin *in vitro*, whereas primate macrophages synthesized $\beta_{1E}$ globulin (C′ 4) as well. The synthesis of components of C′ is evidently a common property of macrophages of many mammalian species.

## G. *Relation of Metabolic Activities to Cellular Functions*

The synthetic activities of macrophages, reviewed above, are diverse. As discussed in Chapter 3, it was originally thought that in lower animals the principal function of macrophages was to provide food for the animal by digesting foreign material. During evolution, other functions have become more prominent, a fact reflected, for example, by the capacity of macrophages to synthesize interferon and components of C′, and to degrade foreign and effete autologous cells.

When erythrocytes age and become nonfunctional they are usually destroyed by macrophages (see Chapter 8). Bulmer (1964) described esterase activities in the ovarian macrophages of rats, which he postulated may contribute to the disposal of engulfed erythrocytes, because such macrophages also contain ferric iron, presumably derived from hemoglobin.

Macrophages may normally engulf and degrade effete plasma cells, fibroblasts, and lymphocytes. Swartzendruber (1964) presented electron micrographs which clearly show whole and fragmented plasma cells within

tingible-body macrophages of the spleen. *In vitro* studies have shown that effete fibroblasts are disposed of by macrophages (Jacoby, 1965). In special circumstances, e.g. following administration of antilymphocyte serum, lymphocytes that have been injured, killed or opsonized are often observed within macrophages and appear to be in the process of being digested (Pearsall and Weiser, unpublished observation).

After phagocytosis, the lysosomes of macrophages commonly increase both in size and in the intensity of staining for acid phosphatase. The fusion of lysosomes and phagosomes gives rise to large phagolysosomes which react strongly to the test for acid phosphatase, coincident with the disappearance of phagocytized material. Other enzymes, such as esterases, are also demonstrable in the lysosomes early after phagocytosis, and in the phagolysosomes later. It is virtually certain that the activity of these enzymes contributes to the degradation of ingested materials.

There appears to be a relationship between the increased enzyme content of macrophages and cellular immunity. For example, Saito and Suter (1965) and Heise, Myrvik, and Leake (1965) stressed the adaptive capacities of macrophages to respond to stimuli such as BCG infection with an increase in lysosomal enzymes. Similarly, Mizunoe and Dannenberg (1965) suggested that increased levels of hydrolases formed by macrophages *in vivo* in response to tubercle bacilli are probably associated with a high degree of cellular immunity (see Chapter 12).

Carrageenan-induced granulomas in the rat have been used by Monis, Weinberg, and Spector (1968) to study the induction of specific enzymes in macrophages. Carrageenan is a polysaccharide, extracted from Irish moss, composed of a mixture of $\alpha$ and $\beta$ isomers of sulfated D-polygalactose. Following a single subcutaneous dose of the polysaccharide, granulomatous changes occur. By 7 days there is a marked increase in the number of macrophages in the area, which appears to result from the migration and maturation of blood monocytes and the proliferation of local tissue macrophages. Galactosidases are demonstrable in macrophages which respond to the galactoside-containing carrageenan. By contrast, carrageenan does not contain glucosides and glucosidases are not induced by this substance. This interesting work clearly indicates that a specific induction of macrophage lysosomal enzymes can occur in response to an ingested substrate.

Another point of interest in the work of Monis *et al.* (1968) is that the substance which induced enzyme synthesis is a polysaccharide. Although it is frequently stated that macrophages have little or no capacity to degrade polysaccharides, this is not always true. For example, they often contain lysozyme (muramidase), which hydrolyzes polysaccharide linkages in the aminopolysaccharide, muramic acid. The concept that macrophages cannot degrade polysaccharides probably arose from the observation that



substances such as certain pneumococcal polysaccharides are degraded by macrophages poorly or not at all, which may account for the persistence of these polysaccharides in tissues over long periods of time (Kaplan, Coons, and Deane, 1950). It should be appreciated that the ability of macrophages to hydrolyze a given polysaccharide or any other substance, e.g. synthetic polypeptides (Sela, 1966), depends on their genetic potential and on the nature of the chemical linkages in the substance.

The observation of Schwab and Ohanian (1967) that the cell walls of group A streptococci are uniquely resistant to degradation by macrophages is of singular interest. This resistance appears to be correlated with resistance to lysozyme, presumably because the group-specific polysaccharide masks the mucopeptide of the cell wall and protects it from hydrolysis by lysozyme. In consequence, group A streptococcal cell wall materials can persist in tissues and cause chronic irritation.

Enzymic degradation of lipids within macrophages has been clearly demonstrated in some instances (see Section D, above); in other cases it is strongly suggested by morphological appearances. Jacoby (1965) described photographic observations on the disappearance of ingested fats in the living chick macrophage. Several dozen fat granules and droplets, engulfed by one macrophage within an hour, were largely digested when the cell started to divide 3 hours later. Similarly, Tompkins (1946) observed that phagocytized cholesterol disappears from connective tissue macrophages over a period of 5 to 10 days after subcutaneous injection of the substance.

Thus, it is evident that the large quantities of lysosomal hydrolytic enzymes demonstrable within macrophages following phagocytosis are directly concerned in digestion of phagocytized materials, both autologous and foreign. The phagocytosis and processing of potential antigens by macrophages is a topic of particular importance, which will be discussed in Chapter 9, Section A2.

*SUMMARY:* In general, macrophages exhibit a high degree of metabolic activity. Their metabolic requirements *in vitro* have not been well defined. Usually serum is required for their cultivation, and an undefined growth factor obtained from syngeneic or allogeneic fibroblast cultures can stimulate metabolism and proliferation *in vitro*.

Macrophages contain the enzymes needed for a variety of energy-yielding metabolic pathways. Whereas peritoneal macrophages obtain energy principally through glycolysis, alveolar macrophages rely largely on oxidative metabolism for their energy. The hexose monophosphate shunt is a major source of energy for the increased metabolism of alveolar macrophages during periods of cellular activity.

In addition to the enzymes concerned with energy production, macrophages synthesize a wide variety of lysosomal hydrolytic enzymes which

bodies, are readily ingested during pinocytosis. Alveolar macrophages may contain antibodies (Hunt and Myrvik, 1964) which probably have been pinocytized and concentrated from their environment in the lung (Moore and Schoenberg, 1964). Thus, antibodies are sometimes found in alveolar macrophages even though these cells apparently lack the capacity to synthesize them (Hunt and Myrvik, 1964).

Policard and Bessis (1958) described another process related to phagocytosis and pinocytosis which they termed "rhopheocytosis." In this process of micropinocytosis, large molecules attach to the cytoplasmic membrane and enter the cell within vesicles about 500Å in diameter. Chapman-Andresen (1962), in a comprehensive discussion of the various means of entry of materials into cells, concluded that rhopheocytosis and pinocytosis are variations of the same process. Although they differ with respect to the size and solubility of materials ingested, rhopheocytosis, pinocytosis, and phagocytosis are similar in many of their other characteristics.

A unique mode of uptake of lipids by macrophages termed "appositional phagocytosis" was described by Carr (1962). In this process, certain lipids are ingested without the formation of membrane-bound vesicles. Electron microscopy revealed that the cell membrane is interrupted at areas of contact with high concentrations of lipid and that fat is taken directly into the cytoplasm. A similar transfer of materials by apposition of macrophages with PMNs was described by Rebuck, Whitehouse, and Noonan (1967). During induced inflammatory reactions, observed by use of the skin-window technique, it was found that glycogen is transferred from PMNs to macrophages following intimate apposition of the cell membranes.

In certain circumstances, cells may form "autophagic vacuoles," in which part of the cytoplasm of the phagocyte itself becomes sequestered and digested (Fedorko, Hirsch, and Cohn, 1968).

## B. *Mechanisms of Phagocytosis*

When particles adhere to the surfaces of macrophages and other phagocytes, either by being trapped against a suitable surface (i.e. surface phagocytosis), by electrostatic attraction, or by specific attachment to receptors or to cytophilic antibody, they are usually phagocytized. Although many kinds of cells can phagocytize, PMNs and macrophages are by far the most actively phagocytic. Cohn (1965) has reviewed much of the literature concerning phagocytosis by macrophages.

During early stages of inflammation, phagocytosis is attributable almost entirely to PMNs, probably for the simple reason that these cells are abundant in the circulation and most readily invade the area of injury. The PMNs are blood-borne as functionally mature cells and are attracted to the invading microorganisms or their products through positive chemotaxis.



Later in the inflammatory process, the major phagocytic cell type present is the macrophage. This cell type may either be blood-borne in the immature form, the monocyte, or may arise from the differentiation and proliferation of cells at the site of inflammation, as discussed in Chapter 4. In either case, maturation continues at the site.

Macrophages, as well as PMNs, can exhibit positive chemotaxis toward bacteria and other particles (e.g. Jacoby, 1944; Harris, 1953; Harris, 1954; Jacoby, 1965). Peritoneal macrophages resemble PMNs in their chemotactic response. Both types of cells advance fairly directly toward particles, such as bacteria or starch grains; however, macrophages move more slowly than PMNs. Oddly enough, tissue degradation products are not chemotactic *in vitro* (Harris, 1953).

A chemotactic factor for rabbit peritoneal macrophages has been found in lysates of rabbit neutrophils, and may be related to the cationic peptides of PMN lysosomes (Ward, 1968). Rabbit alveolar macrophages respond poorly to this and to the other chemotactic agents tested.

Particles which adhere to macrophages are engulfed by active pseudopodia or by invagination of the plasma membrane which pulls the invading particle into the cell. Fusion of the cell membrane around the engulfed particle completes the process known as endocytosis. The vesicle so formed, called a phagosome, is limited by a unit membrane identical with the plasma membrane from which it originated.

It is possible to dissociate phagocytosis into two phases: attachment and ingestion. Rabinovitch (1967) described experiments in which mouse peritoneal macrophages and aldehyde-fixed horse erythrocytes were used to quantitate the two phases. He found that the number of treated erythrocytes which attached to macrophages increased in a linear fashion in accordance with the number of red cells present. Although both phases were temperature-dependent, the first phase, attachment, did not require added serum or divalent cations, as did the second phase, ingestion. Vaughan and Boyden (1964) also demonstrated that serum is not required for attachment of effete erythrocytes to macrophages. They found that red cells stored for a period of time either in Hanks' balanced salt solution (HBSS) or in autologous serum adhered to the same extent; both adhered much more readily to macrophages than did fresh erythrocytes suspended in HBSS or in serum. In these experiments the authors did not measure the second phase of phagocytosis, but only adherence to macrophages. Two phases of microphagocytosis (pinocytosis) were discussed by Wiener (1967).

Metzger and Casarett (1967) studied the effects of divalent cations on *in vitro* phagocytosis by the peritoneal macrophages of rats. It was found that Ca ions are necessary for optimum phagocytosis; however, Mg ions apparently did not contribute to the process, in their system.

# Chapter 8
# Functions of Macrophages

MACROPHAGES have many important functions. They act as scavengers to dispose of foreign matter and effete autologous cells; most ingested substances are degraded and many of the products are utilized in body economy. In addition, macrophages play an important role in immunological events, and have a number of other specialized functions.

## A. *Scavenger Activities*

Some of the mechanisms involved in the clearance of foreign and effete material from the circulation have been discussed previously. The foreign material commonly disposed of by macrophages includes bacteria and many kinds of inhaled or injected substances, both particulate and soluble.

### 1. Cytophilic Opsonins as an Aid in Scavenger Activities

Enhancement of the phagocytic activities of macrophages by specific opsonins has been well documented (see Chapter 7, Section D). Many opsonins act by coating particles and changing their surfaces, either by altering surface charges or in other ways. In theory, such a mechanism might require extensive coating of surfaces by antibody and would prepare the particles for engulfment by all types of phagocytes. However, many opsonins are cytophilic for macrophages (Berken and Benacerraf, 1966). A portion of the cytophilic antibody molecule, distinct from its antigen-combining sites, can bind weakly but specifically with receptors on the macrophage membrane (see Chapter 10). Specific attachment of particles to the phagocyte surface occurs if the cytophilic antibody is first attached to the macrophage and then combines with antigen, or if antigen and antibody combine before cytophilic attachment is achieved. In either case, little cytophilic antibody may be required for the attachment of particles and their subsequent engulfment by the macrophage. When the particles are cells with few antigenic sites on their surfaces, complete engulfment may not occur; instead, phagocytic action may be limited to "membrane phagocytosis" in which small bits of the membrane of opsonized cells are phagocytized by macrophages. Usually, the close association between opsonized particle and macrophage membrane afforded by the cytophilic

attachment greatly facilitates phagocytosis. In addition, the influx of macrophages into sites of scavenger activity, such as areas of infection, provides a means for carrying opsonins attached to cytophilic receptors directly to the point where they are needed. Cytophilic antibodies on macrophages throughout the body contribute to a surveillance mechanism which functions to utilize small amounts of antibody most effectively.

Although many types of cells have limited phagocytic capabilities, specific opsonins appear to promote the uptake of particles only by strongly phagocytic cells such as macrophages and PMNs (North, 1968). Perhaps these are the only cells with receptors for cytophilic opsonins, either free or combined with antigen.

The contribution of cytophilic antibodies to the destruction of effete autologous erythrocytes by macrophages is not defined. Apparently opsonins are not required for the adherence to macrophages of RBCs altered by storage (Vaughan and Boyden, 1964). However, the second phase of phagocytosis, during which ingestion of adhering particles occurs, may require opsonic action. Jenkin and Karthigasu (1962) suggested that opsonins are necessary for the removal of effete RBCs by macrophages. Since the first phase of phagocytosis, adherence, does not require energy or divalent cations, whereas the second phase does, it is probable that opsonins are essential for only the ingestion phase of phagocytosis of effete erythrocytes, but facilitate both phases.

### 2. Disposal of Tissue Debris and Effete Cells

One of the major functions of macrophages is to dispose of tissue debris and effete cells. Macrophages, and not PMNs, are responsible for the removal of autochthonous materials. Vaughan (1965) observed that this activity is not attributable to serum antibodies. This does not rule out the possibility that cytophilic antibodies on macrophages can recognize materials which have become "foreign" because of antigenic changes during aging. Although some extraneous materials may be nonmetabolizable, ingested cellular constituents are usually readily metabolized. Thus the conservation and reutilization of cellular components, e.g. iron compounds, are facilitated by macrophage activities.

The role of the macrophage in iron metabolism is well established. Ingested iron compounds are absorbed by mucosal cells of the intestine and the iron is incorporated into ferritin. In the presence of reducing agents the iron is freed from the ferritin molecules and is bound to the $\beta_1$ globulin of the serum, transferrin. Each molecule of this protein has two binding sites for iron (Wintrobe, 1961). The iron is tightly bound by transferrin and delivered directly to erythroblasts in the bone marrow or to other cells concerned with iron metabolism (Bessis, 1963). Transferrin binds specifically to receptors on the surfaces of certain cells and may deliver



one atom of iron to the erythroblast and retain the other one, eventually storing it in the liver or elsewhere. Thus, transferrin is responsible for the distribution of iron throughout the body (Fletcher and Huehns, 1968). When iron is injected subcutaneously, its ingestion and the subsequent synthesis of ferritin in the macrophage cytoplasm can be studied by electron microscopy. Muir and Golberg (1961) described the pinocytic ingestion of iron-dextran by macrophages, the subsequent synthesis of apoferritin, and the incorporation of iron into ferritin molecules. Normally, excess iron is carried to the liver, where it is stored as ferritin (Thompson, 1961). Numerous ferritin granules are also seen in the cytoplasm of free alveolar macrophages (Karrer, 1960).

Although many substances are cleared from the blood stream largely by Kupffer cells of the liver, macrophages in the spleen and bone marrow account for most of the removal of effete nonsensitized erythrocytes. Within the macrophage, part of the hemoglobin is transformed to bilirubin which is eliminated via the liver, and iron and globin are retained and utilized (Dacie, 1960).

Macrophages which transport and store iron are not found in the blood of normal human subjects; however, Yam and co-workers (1968) found circulating iron-containing macrophages in a considerable percentage of patients with iron-storage diseases. Such cells were found rarely in patients with extreme iron-loading. In hemochromatosis, macrophages contain smaller granules of iron than in transfusion siderosis.

Erythrophagocytosis by macrophages can be seen in tissues from healthy individuals, but Essner (1960) has reviewed the evidence suggesting that phagocytosis does not occur often enough to account for the normal rate of destruction of RBCs. Of more importance is the process of fragmentation of erythrocytes, during which portions of the cells are pinched off and are subsequently ingested by macrophages. Doan and Sabin (1926) observed fragmentation of rabbit erythrocytes. Their interpretation was that poikilocytes represent the first stage of fragmentation and that microcytes result from loss of part of the red cell. Fragmentation was found to be constant in normal animals, and was greatly increased in various types of anemias.

The adherence of effete RBCs to macrophages, which can occur even in the absence of opsonins (Vaughan and Boyden, 1964), would allow effective membrane phagocytosis to occur. Therefore, the processes of fragmentation and membrane phagocytosis may account for much of the disposal of nonsensitized, as well as antibody-sensitized, erythrocytes (Rous, 1923; Weed and Reed, 1966).

Thus, macrophages not only serve as scavengers in the normal destruction of effete erythrocytes, but also function to conserve the iron present in these cells by their metabolic, transport, and storage activities.

There is abundant evidence that effete cells other than erythrocytes are phagocytized and metabolized by macrophages. For example, plasma cells and plasma cell fragments have been identified within tingible-body macrophages of the spleen (Swartzendruber, 1964). Myelin bodies and other materials frequently seen in the cytoplasm of macrophages provide additional evidence that effete cells are being disposed of by these phagocytes.

The many macrophages in the thymus undoubtedly serve to remove debris resulting from the rapid turnover of cells in this organ. Kostowiecki (1963) has reviewed the literature on thymic macrophages.

It is well established that macrophages play a major role in the resorption of tissue following inflammation, and in certain physiological circumstances. For example, Mayberry (1964) and Helminen and Ericsson (1968a, b, c) described the activities of macrophages in post-secretory mammary involution, and Deno (1937) reported an interesting study of their activities during postpartum involution of the mouse uterus. Whereas PMNs were the cells most active in removing debris during the first 2 days of the puerperium, macrophages subsequently assumed the major role of phagocytizing and digesting effete cells and cell remnants. The macrophages became filled with hemosiderin derived from erythrocyte digestion, and aggregated to form brown areas grossly visible on the dorsal surface of the uterus.

The reutilization of resorbed materials, as well as foreign substances, is accomplished by macrophages. Ehrenreich and Cohn (1968a, b) followed the fate of ingested proteins within mouse peritoneal macrophages. After their pinocytosis, radioactively labeled proteins were digested to the level of amino acids, or possibly small peptides and amino acids. It was postulated that pinocytosis by macrophages may make a considerable contribution toward the turnover of serum proteins, the conservation of stores of iron, cellular nutrition, and other metabolic events.

### 3. Action of Macrophages on Sensitized Erythrocytes

The fate of RBCs which have been sensitized by exposure to specific antibodies, either *in vitro* or *in vivo*, differs in several respects from that of nonsensitized effete cells. Whereas red cells are normally removed largely by macrophages in the spleen and bone marrow, sensitized erythrocytes are frequently eliminated from the circulation within the liver and other organs. Sabin and Doan (1926) and Doan and Sabin (1926) studied RBC destruction in tuberculous rabbits. Macrophages loaded with red cell fragments were found in the lungs, as well as in the spleen and bone marrow. In some cases the macrophages from these rabbits had erythrocytes adhering to them.

A similar red cell-macrophage adherence is shown in the electron micrographs of LoBuglio, Cotran, and Jandl (1967). These investigators reported that macrophages rapidly remove sensitized erythrocytes from the blood stream and destroy them by the unique process of "membrane phagocytosis." This process, which is also described by many others including Policard and Bessis (1953), Weed and Reed (1966), and Croft *et al.* (1968), depends on the presence of anti-RBC antibodies which, following their union with erythrocytes, become cytophilic for macrophages and lead to the formation of rosettes. When macrophages and erythrocytes carrying such antibodies come in contact, the macrophages seemingly are able to pull off and phagocytize fragments of the red cells, leaving osmotically fragile erythrocytes which become spherocytic and undergo fragmentation. The antibodies in man known to be responsible for membrane phagocytosis are functionally incomplete IgG molecules. They do not fix C′ or induce phagocytosis of whole erythrocytes, but after interaction with red cells they combine specifically with cytophilic receptors present on monocytes and mature macrophages, but not on granulocytes (Huber and Fudenberg, 1968).



In the mouse, a similar fragmentation of antibody-sensitized erythrocytes adhering to macrophages has been described (Lay and Nussenzweig, 1968). Results of this study indicate that macrophages of the mouse have surface receptors for complexes of antigen, xenogeneic antibody, and the first four components of C′. The combination of receptors on the macrophage with such complexes depended on the presence of divalent cations. Whereas, in this system, macrophage cytophilic receptors for 7S antibodies were resistant to trypsin treatment, the receptors for C′ components were destroyed by trypsin. The existence of receptors on macrophage surfaces for antigen:antibody:C′ complexes offers an additional mechanism for the efficient removal of sensitized erythrocytes and other materials from the body.

It is clearly evident that in autologous and allogeneic *in vivo* systems the nature of the antibody which sensitizes erythrocytes is of primary importance in determining the mode of the disposal of the erythrocytes. Of equal importance is the number of erythrocytes capable of being sensitized ("target" RBCs) as well as the relative number of RBCs present which do not bear the antigens specific for the antibodies used ("nontarget" RBCs). For example, if a small intravenous dose of Rh-positive RBCs coated with anti-D antibody is injected into a nonimmunized Rh-negative adult, membrane phagocytosis occurs and the cells are rapidly sequestered largely in the spleen (Jandl, Jones and Castle, 1957; Crome and Mollison, 1964). Since D antigenic determinants are sparse on the red cells, it is possible that the antibody coating of such cells is sufficient to permit their adherence to macrophages but is insufficient to permit phagocytosis. Stuart (1967) has proposed that the number of antibody molecules on the surface of

erythrocytes may determine whether they merely adhere to phagocytes or are phagocytized. By contrast to the above experiment with incomplete IgG antibody, when a small number of Rh-positive RBCs is administered to an Rh-negative adult possessing complete IgM anti-D antibody, the RBCs are cleared less readily from the circulation and are sequestered primarily in the liver. Alternatively, in the ABO system where the antibodies are nonlytic complete agglutinins, if large numbers of incompatible cells are injected (as in a mismatched blood transfusion) the aggregates of agglutinated cells are removed from the circulation largely by macrophages of both the liver and lungs (Jandl, Jones, and Castle, 1957; Jandl and Tomlinson, 1958).

Thus, the nature of the antibodies and the degree of antibody-coating of RBCs are important in determining the site of the sequestration of RBCs (Jandl and Kaplan, 1960). Heavily coated RBCs tend to sequestrate in the liver, and lightly coated RBCs in the spleen.

#### 4. Wound Healing

Macrophages are conspicuous in healing wounds and areas of tissue organization. They appear at the site of injury during the first few days, increase both in number and in maturity during following days, and decrease in number as fibrosis ensues. The emigration of blood monocytes into areas of organization, and their subsequent transition to mature macrophages, have been well documented by Ebert and Florey (1939). Other sources of the macrophages found in areas of healing are discussed in Chapter 4. Regardless of their source, macrophages function in the first stages of healing by invading the clot and by ingesting and digesting RBCs, fibrin, and cellular debris.

The sequence of cellular changes observed during wound healing illustrates the role of the macrophage in cell homeostasis, as discussed in Chapter 5. Granulocytes which first invade an injured area die and release cellular components which presumably facilitate the differentiation or the maturation of macrophages at the site. Ross (1964) found many extracellular granules, which appeared to be PMN granules, in wounds between the first and third day. He noted that macrophages were the predominating cell type in wounds at this time and that some of them contained bodies which appeared to be granules derived from PMNs. Macrophages also phagocytize whole dead PMNs. Substances are released from dying macrophages which stimulate fibroblast activity, resulting in fibrosis. During normal wound healing, fibrosis is minimal. However, in conditions in which excessive numbers of macrophages accumulate and die over a long period of time, fibrosis is excessive, e.g. in granulomatous diseases such as tuberculosis, sarcoidosis, silicosis, and histoplasmosis (Schowengerdt, Suyemoto, and Main, 1969).



### B. *Regional Activities*

Macrophages in various regions of the body may have characteristic functions.

#### 1. Peritoneum

The peritoneal cavity is normally sterile and the relatively small numbers of macrophages which can be collected from the healthy peritoneum appear to be relatively immature and inactive. Histological examination reveals depots of immature macrophages in milk spots of the omentum (Cappell, 1929b). During abnormal conditions, caused, for example, by bacterial infection or the introduction of particulates, large numbers of macrophages appear in the peritoneum. Many of these cells are mobilized from the omentum, others arise by division of existing macrophages (Aronson and Elberg, 1962), and still others may be derived from blood-borne immature precursors, as discussed in Chapter 4. Macrophages become highly activated following stimulation, i.e. they synthesize many lysosomes and frequently give evidence of extensive phagocytic activity. Extraneous material is rapidly cleared from the peritoneum by the phagocytic activities of macrophages and is usually digested intracellularly.

During the rejection of ascites tumor allografts (see Chapter 12) there is evidence that macrophages can kill tumor cells by a contact mechanism independent of phagocytosis, which results in the lysis of both macrophage and tumor cell. Later, other macrophages phagocytize and remove the cellular debris which remains (Baker *et al.*, 1962).

#### 2. Lung

The environment in the lung differs greatly from that of the peritoneum. Not only is the milieu different, but also the stimuli encountered by alveolar macrophages are stronger and more numerous. Inhaled gases and particulates, including microbes, serve as strong stimuli to activate lung macrophages. Thus, it is not surprising that alveolar macrophages are normally more activated than peritoneal macrophages, even in germ-free animals (Leake and Heise, 1967). Although bacteria are not present in the germ-free environment, inhaled dusts and gases can serve as stimuli for macrophage activation.

Macrophages are vitally important in policing and clearing the lung of extraneous material. Green and Kass (1964) demonstrated that bacteria, labeled with $P^{32}$ and given to mice in an acrosol, are quickly removed from the lung, largely by alveolar macrophages. Their data suggest that the action of the mucociliary stream serves to clear the lung of phagocytes containing ingested material. The ability of mice to clear bacteria from the respiratory tract is greatly inhibited by acute renal failure; Goldstein and Green (1966) postulated that this inhibition is caused by the bio-

chemical changes known to occur subsequent to renal failure, which could adversely affect alveolar macrophage function. This might account for the observation that pulmonary infection is a frequent complication of renal failure.

A unifying hypothesis to explain the mechanisms of disposal of inhaled particles was presented by Heppleston (1963). From data obtained in experiments in which rats were exposed to hematite or silica dusts and examined at various time intervals, Heppleston drew the following conclusions. Nonmetabolizable particles, ingested by alveolar macrophages, are liberated upon death of the cell, and are rephagocytized by other alveolar macrophages. Movement of the alveolar fluid film toward sites of lymphatic drainage aids in the export of phagocytes containing ingested particles. After heavy exposure to dust, many particles remain in the alveoli for long periods of time. Dust foci enlarge and macrophage proliferation and disintegration continue; in the case of silica dust, fibrosis ensues. It is suggested that dusts enter the lymphatic system, either free or within macrophages, and localize in the hilar nodes.

Alveolar macrophages also contribute to immunity against viruses, by the production of interferon (see Chapter 6) and in other ways, discussed by Mims (1964a).

Increased numbers of macrophages, and a phenomenon known as "macrophage congregation" (Sherwin *et al.*, 1968), are seen in lungs exposed to $NO_2$, an important component of polluted air. "Macrophage congregation" is defined as ". . . three or more 'spread' macrophages on a single epithelial cell." The extent of macrophage congregation was much greater in guinea pig alveolar cells exposed to 10 ppm $NO_2$ than in non-exposed cells. The significance of macrophage congregation is not clearly defined; however, it seems to reflect lung tissue damage. Cell selectivity was observed, in that some epithelial cells had many macrophages spread on their surfaces, while adjacent cells had none. Evidently damaged lung epithelial cells specifically attract alveolar macrophages, which congregate on their surfaces.

The observation that cigarette smoke depresses the *in vitro* antibacterial activity of rabbit alveolar macrophages (Green and Carolin, 1967) may be related to the effects of $NO_2$ or other gases in the cigarette smoke, since the active component of the smoke was soluble in water.

The lipid-containing alveolar macrophages studied by Bertalanffy (1964) differ in several ways from those without lipid inclusions. For example, their life span of 3 weeks within the lung is approximately three times that of nonlipid-containing alveolar macrophages, which are extruded from the lung within a week after mitosis. It was suggested that the lipid-containing macrophages remove cholesterol and other lipids from the blood while in intimate contact with lung capillaries, while alveolar macrophages without lipid inclusions function chiefly in the removal of particulate matter.



Macrophages throughout the body probably play a major role in maintaining the normal equilibrium of serum lipids (Bertalanffy, 1964; Day, 1967). There is also a remote possibility that alveolar macrophages may function in the production of lung surfactant, necessary for the integrity of this organ (see Chapter 6).

### 3. Central Nervous System

Normally the central nervous system (CNS) is not exposed to foreign material because the blood-brain barrier functions to effectively exclude the entrance of even many soluble substances into this system. When this barrier is breached, because of inflammation, trauma, or other causes, macrophages of the CNS soon become activated. As described in Chapter 2, microglial cells are the inactive stellate macrophages normally seen in the CNS. Following stimulation, they transform into typical ameboid, phagocytically active macrophages containing many lysosomes (Hosokawa and Mannen, 1963). These activated macrophages remove and digest materials in a manner analogous to that shown by peritoneal or alveolar macrophages.

Experimental allergic encephalomyelitis (EAE) can be produced in animals by injecting brain tissue or certain brain components incorporated in Freund's complete adjuvant. This disease resembles the human demyelinating disease, multiple sclerosis. Macrophages in the brain may contribute to the pathogenesis of EAE, as discussed in Chapter 12.

### 4. Lymphoid Tissue

Reticular cells of lymphoid tissue have been reported to have a nurse function for lymphocytes (see review by Trowell, 1965). It is postulated that lymphocytes with little cytoplasm and few mitochondria are so inactive that they have difficulty in generating enough energy to meet their requirements, and consequently must be supplied nutrients by nurse cells. Many investigators have observed contact and in some instances apparent continuity between lymphocytes and macrophages through cell processes. Trowell (1965) reviewed the evidence suggesting that macrophages transfer ATP or other cellular constituents to lymphocytes.

Macrophages in lymphoid tissue figure prominently in the removal of foreign material and its digestion into antigenic fragments (see Chapter 9). Macrophage-cytophilic antibodies are retained for long periods of time on dendritic reticular cells in lymph nodes, where they may promote the immune response by trapping and retaining antigen. Another interpretation of the observed spatial relations between lymphocytes and macrophages of lymphoid tissue is that RNA or RNA-antigen complexes are passed to lymphocytes by macrophages which have processed the antigen.

Tingible-body macrophages in lymph nodes and spleen also remove effete cells, such as plasma cells, which may be numerous during and following an active immunological response.

#### 5. Testes

Carr, Clegg, and Meek (1968) summarized the literature on the Sertoli cell of the testes as a macrophage. Their electron micrographs clearly establish that Sertoli cells are typical macrophages, which remove extraneous materials and dead cells in seminiferous tubules. The possibility that they may function as nurse cells for spermatozoa is discussed by Trowell (1965).

#### 6. Skin

Another type of macrophage postulated to have a nurse function is the melanophage of the skin (Trowell, 1965; Niebauer, 1968). Cells of this type apparently supply melanin to melanocytes in the skin.

#### 7. Bone

Macrophages probably function in bone metabolism by serving as precursors of osteoclasts. Jee and Noland (1963) reported that charcoal, injected into the femurs of growing rabbits, was phagocytized by macrophages over the first 10 days. At 15 to 30 days a large number of charcoal-laden osteoclasts appeared, apparently formed by the fusion of macrophages. Jee and Nolan presented their data in support of the theory of Hancox (1949); namely, that phagocytic histiocytes can fuse to form osteoclasts. Data in conflict with this theory have been reported by Tonna (1963), and the question must remain open at present.

### C. *Additional Functions of Macrophages*

#### 1. Detoxification

Macrophages can detoxify certain substances. For example, highly toxic but slightly soluble granules of arsenic trisulfide, when administered intraperitoneally to laboratory animals, are taken up by macrophages and degraded into nontoxic compounds which are excreted in the urine. The same amount of arsenic trisulfide, however, is fatal to the recipients if it is placed in sacs within the peritoneum where it is protected from phagocytosis and detoxification by macrophages but instead can be released in a soluble toxic form (Metchnikoff, 1905).

The powerful exotoxin of diphtheria kills cultured spleen, kidney, or peritoneal macrophages from nonimmunized guinea pigs within 24 hours (Frolova and Sokolova, 1964). In contrast, peritoneal and splenic macrophages from immune animals are highly resistant to the toxin and remain viable and mobile in its presence. Attempts were made to determine whether cytophilic antibodies could account for these results. Nonimmune guinea pig macrophages were exposed to horse serum diphtheria antitoxin, washed, and tested with dilutions of toxin. Since no protection was afforded by this procedure, it was concluded that cytophilic antibodies do not contribute to the observed immunity against diphtheria toxin. However, the cells



were from guinea pigs and the serum antibodies were of horse origin, hence it is possible that cytophilic antibodies were present but not demonstrable because the cytophilic receptors of guinea pigs and horses differ. It would be of interest to reinvestigate this question in the light of recent advances in the understanding of cytophilic antibody activity (see Chapter 10).

Rutenburg, Schweinburg, and Fine (1960) determined that macrophages can detoxify endotoxins from *E. coli in vitro*. Ravin *et al.* (1960) showed that bacterial endotoxins are absorbed from the intestinal tract of normal rabbits and of rabbits in the state of hemorrhagic shock. Such absorbed endotoxins remain in the serum of shocked animals. Schweinburg and Fine (1960) demonstrated that fatal endotoxemia develops because the RES is unable to destroy absorbed endotoxins during conditions of shock induced in several ways. Wiznitzer *et al.* (1960) reported that the normal RES removes and rapidly inactivates circulating endotoxin; however, the RES damaged by blockade or by reversible hemorrhagic shock can remove only a small amount of circulating toxin and is able to detoxify very little of the removed material. In addition, there is evidence that the development of tolerance to the toxic effects of endotoxins depends on enhanced activity of the RES (Freedman, 1960).

Ballantyne (1967) postulated that esterase activity of RES cells may contribute to the detoxification of endotoxins and of potentially toxic lipids produced during metabolism associated with extensive cellular mitosis. Macrophages may also indirectly influence the detoxification of barbiturates (DiCarlo *et al.*, 1965; Barnes and Wooles, 1968).

It has been postulated that iron in macrophages plays a role in the detoxifying activities of these cells. Janoff (1964) suggested that iron may act in two ways, first to activate lysosomal enzymes and second to neutralize toxins. The acid pH and accumulation of reducing substances within phagolysosomes would favor release of iron for both of these functions.

#### 2. Inactivation of Thromboplastin

Blood thromboplastin prepared *in vitro* and administered to an intact animal of the sames species (rat) is cleared from the circulation by the RES (Spaet *et al.*, 1961). It has also been reported that rabbit peritoneal macrophages can inactivate rabbit blood thromboplastin in an *in vitro* system, while alveolar macrophages have no effect (Arakawa and Spaet, 1963). It was concluded that the laboratory-prepared thromboplastin used was treated as foreign particulate material by the RES. No evidence was presented concerning similar activity under normal physiological conditions.

The RES has been implicated in the *in vivo* production of a hypercoagulable state in dogs (Rabiner and Friedman, 1968). This state is defined as an increased tendency of the blood to clot intravascularly because of

alteration of its constituents. This alteration can be created experimentally by infusing autologous hemolyzed RBCs (hemolysate). Depression of RES function by pretreatment with carbon or by splenectomy accentuated the hypercoagulable state. It was postulated that the augmentative effect of RES depression on the state of hypercoagulability induced with hemolysate could be related to lack of clearance of liberated coagulant particles.

*SUMMARY:* Macrophages, frequently aided by the activity of opsonins, act as scavengers to dispose of foreign and effete cells and other materials. They can degrade and reutilize many of the ingested substances, thereby contributing to the conservation of stores of iron, turnover of serum proteins, nutrition of cells, and resorption of tissue. Wound healing depends, in part, on the scavenger activities of macrophages; in addition, macrophages may influence the homeostasis of fibroblasts and thus indirectly affect the healing process and fibrosis.

Within the peritoneum, macrophages are normally inactive but can be stimulated to proliferate and to become highly activated. Such macrophages readily clear the peritoneum of extraneous material. Alveolar macrophages are normally more activated than peritoneal macrophages and function in clearing the lung of inhaled particles, producing interferon, and other activities. Macrophages in other locations throughout the body may have specialized functions unique to their location.

Other functions of macrophages include: detoxification of certain simple chemicals, exotoxins, and endotoxins; possibly the regulation of clotting under certain special conditions; and immunological activities (discussed in other chapters).

# Chapter 9

# The Role of Macrophages in the Antibody Response

THE IMPORTANCE of macrophages in the antibody response has been recognized for virtually as long as the response has been studied. Although originally it was postulated that macrophages might be the site of antibody synthesis (see review by McMaster, 1953), present evidence indicates that they do not produce antibodies (Storb and Weiser, 1968). However, they contribute to the antibody response in several important ways. Macrophages trap, process, and store antigens. In addition, they can present specific information to antibody-forming cells in the form of a fragment of antigen coupled to ribonucleic acid (RNA), or possibly in other ways. These functions have been studied extensively, both *in vivo* and *in vitro*.

## A. *Contributions of Macrophages to the Antibody Response in Vivo*

Both the initiation and extent of the antibody response are influenced greatly by the route of entrance of the antigen into the body and its subsequent fate. The fate of antigen varies depending on numerous factors, including its dose and solubility, and previous exposure of the host to the antigen.

The fate of radioactively labeled bovine gamma globulin (BGG) injected i.v. into rabbits has been traced by Dixon *et al.* (1953) and serves as a classical example of the disposition of a soluble antigen. The injected BGG initially equilibrates between vascular and extravascular fluids and is not concentrated or retained in appreciable amounts by any tissue; nevertheless, enough is taken up to stimulate antibody production. By approximately the fourth day, when specific antibodies appear, the labeled BGG rapidly complexes with antibody and is eliminated from the circulation, so that by about day 7 the antigen is cleared completely and free serum antibodies are demonstrable. If the antigen is again introduced while circulating antibodies are still present, it is cleared within 1 to 2 days. Even though antibodies may no longer be measurable in previously sensitized animals, reintroduction of the antigen results in an anamnestic response, and antigen is cleared in an accelerated manner within 4 days. When antigen

and antibody combine, the complexes are rapidly removed and catabolized by phagocytic cells of the RES.

Particulate antigens are cleared from the circulation by cells of the RES, as described in Chapter 7. Their fate depends on a number of factors, such as the size of the particles and the presence of either natural or immune antibodies.

Following their primary injection, some antigens are retained over long periods within cells of the RES, but others are catabolized quickly and are no longer demonstrable within cells (Dixon *et al.*, 1953; Ehrenreich and Cohn, 1968a).

### 1. Antigen Trapping

Nossal, Ada, and Austin (1964a, b) have used the flagella of certain *Salmonella* species and their soluble subunits, flagellin, as antigens. Since both are easily labeled and highly antigenic, they are ideally suited for studies on the fate of either particulate or soluble antigen. Nossal, Ada, and Austin (1964b) demonstrated that particulate flagella are first trapped by macrophages which line the medullary sinuses of lymph nodes draining the injection site. Soon thereafter, antigen appears in primary lymphoid follicles in the cortex of the nodes. By combining electron microscopy with radioautography, Ada *et al.* (1967) showed that a primary dose of flagella is taken up by typical macrophages of the lymph node medulla. Within the cell, antigen, either free or in pinosomes, becomes surrounded by tiny lysosomal vesicles and is completely enclosed within lysosomal structures by 30 minutes after injection. These lysosomes fuse to give rise to large pinolysosomes which persist in the medullary macrophages for 6 weeks or longer. Although PMNs are present in the nodes they do not participate to an appreciable extent in antigen uptake. In cortex of the lymph nodes, trapped antigen is adsorbed to fine dendritic processes of reticular cells, which appear to be nonphagocytic macrophages; here it may be retained for as long as 2 weeks following its primary administration. This association allows close contact between lymphoid cells and antigen on the surface of macrophages.

Ada *et al.* (1967) described a third type of cell, the tingible-body macrophage, which is capable of trapping flagellar antigens, especially during the secondary response. This cell retains label within myelin inclusions.

When soluble flagellin is used instead of intact flagella, the antigen is seen throughout the node soon after its primary subcutaneous introduction. One to 2 days later, it becomes localized in a manner similar to that observed with flagella within an hour after injection (Ada *et al.*, 1967).

The fate of soluble antigen bearing a radioactive label has been followed by Szakal and Hanna (1968), using electron microscopic radioautography. Szakal and Hanna noted that the antigen became localized on highly convoluted infoldings of splenic reticular cells (macrophages) in lymphoid follicles during the primary response in the mouse spleen. Villous extensions of ribosome-rich "immunoblasts" were observed in close association with antigen on macrophage surfaces. Demonstrable antigen decreased from 20 to 30 days after administration, and by 30 days was no longer detectable.



The localization of antigen within lymphoid follicles has been observed by many other investigators (see review by McDevitt, 1968). This pattern of antigen trapping appears to result from the reaction of antigen with cytophilic antibodies on the surfaces of dendritic macrophages. The amount of antigen captured increases as antibody is formed; passively transferred antibodies also cause a marked follicular localization of antigen (Lang and Ada, 1967). There is also evidence that the Fc portion of the IgG antibody molecule is largely responsible for the reaction of IgG with the macrophage membrane, as would be expected for cytophilic antibodies (Ada *et al.*, 1967).

### 2. Antigen Processing

As described above, macrophages ingest and sequester soluble antigen within pinolysosomes. Particles such as SRBCs, a complex of different antigens, are also ingested and processed by macrophages. The question of whether macrophage intervention contributes or is necessary to antibody formation has been approached experimentally. It is, of course, possible that macrophages which degrade erythrocytes, bacteria, or other particulate antigens are simply fulfilling their role as scavengers and play no part in antibody formation. That this is not the case has been demonstrated by many studies which have shown that inhibition of RES function reduces the extent of the antibody response, and, conversely, that stimulation of RES function leads to increased antibody production (e.g. Thorbecke and Benacerraf, 1962; Stuart and Davidson, 1964; Franzl and McMaster, 1968a, b). Thus, it is well established that macrophage activity facilitates antibody production by retaining and processing antigen, as well as in other possible ways.

The processing of a bacterial antigen, streptococcal M protein, by macrophages was studied by Gill and Cole (1965). It was postulated that particles containing a complex of antigens are phagocytized and processed into small antigenic moieties. Other streptococcal antigens, the group-specific polysaccharide of Group A, may also be processed by macrophages; an enzyme for the hydrolysis of this substance has been described in rabbit alveolar macrophages (Ayoub and McCarty, 1968).

The ability of macrophages to process antigen for antibody production may not be expressed in newborn animals. Braun and Lasky (1967) reported that intraperitoneal (i.p.) injection of adult peritoneal macro-

cellular immunity may be operative against such tumors. Much of the pertinent literature has been reviewed by Alexander and Fairley (1963), Smith (1968), and Hellström and Hellström (1969).

Tumor immunity is primarily a cellular immunity which operates in much the same manner as allograft immunity. Because tumors grow rapidly it has been proposed that they outgrow or overwhelm the immune forces of the host and consequently flourish even in the face of an active immune response.

It is probable that tumors oppose the immune forces of the host by the interaction of several mechanisms (see review of Hellström and Hellström, 1969). Among numerous possibilities are the following: (1) the tumor cell may produce a coating of sialomucin, or other substances, which protects against immune forces; (2) the massive amounts of antigen produced by the rapidly growing tumor may incite a state of immunological paralysis or tolerance; and (3) enhancing antibodies which interfere with cellular immunity may be synthesized by the host.

Lymphocytes play the major effector role in cellular immunity to autochthonous tumors, but it is possible that macrophages also perform effector functions. There is evidence suggesting that macrophages are important in the effector phase of immunity against certain chemically induced tumors; Old, Boyse, Bennett, and Lilly (1963) demonstrated that macrophage-rich preparations of peritoneal cells from animals immunized with a tumor could transfer specific immunity to syngeneic recipients. An

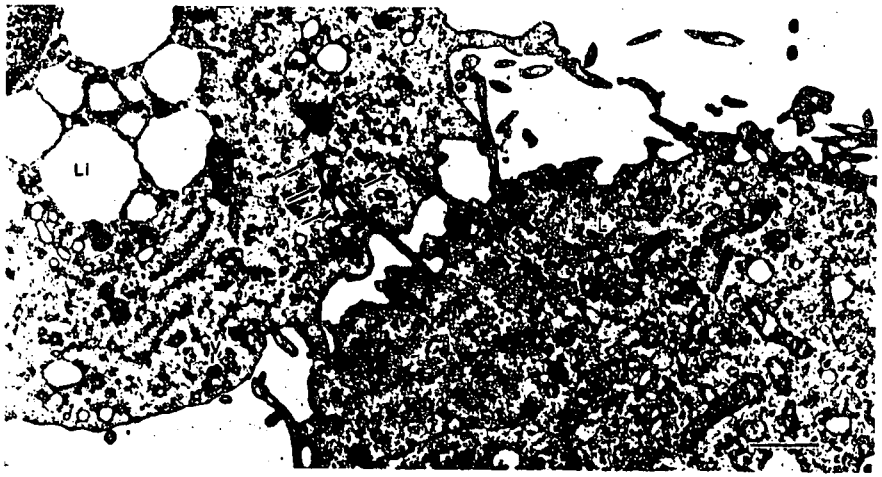


FIGURE 12-2. "Membrane phagocytosis" in immune macrophage-target cell interaction. Surface projections of an L cell (L) extend into invaginations of the lipid (Li)-containing macrophage (M) and appear to be undergoing phagocytosis (↗). A virus particle (V), from the L cell, has apparently been phagocytized. Magnification: 8,700. (From Chambers and Weiser: Cancer Res. 29:312, 1969.)



observation made many years ago by Murphy (1926), and subsequently verified by others (see review by Alexander and Fairley, 1963), was that nonspecific stimulation of macrophages by RES-stimulating agents frequently results in increased resistance to autochthonous and other types of tumors.

### 3. Autoimmune Diseases

As suggested earlier, a cellular immunological response against altered or occult antigens of self appears to be the chief basis of autoimmune diseases. MacKay and Burnet (1963), Turk (1967), and Miescher and Müller-Eberhard (1968) have discussed many aspects of autoimmune disease in detail.

Waksman (1960) showed that during the development of experimental autoimmune orchitis, lymphocytes and macrophages infiltrate seminiferous tubules and appear to destroy spermatocytes.

In 15 of several hundred patients with male infertility, large numbers of macrophages were demonstrated in the semen (Phadke and Phadke, 1961). The origin of these cells is uncertain; however, they were actively phagocytizing and disposing of spermatozoa. Autoantibodies were demonstrated in a few of these patients, but their role in the pathogenesis of the disease was not evident. Autoantibodies cytophilic for macrophages may have contributed to the process; however, this possibility was not investigated.

The histopathological picture in experimental allergic encephalomyelitis is similar to that in other autoimmune diseases, with lymphocytes and macrophages infiltrating the target area (Waksman, 1959; Patterson, 1968). The histopathology of these autoimmune diseases is similar to that described for skin allograft rejection (see review by Rose, 1965).

The initiation of autoimmunity appears to depend primarily on lymphocyte activities. Although macrophages are conspicuous in the lesions of many autoimmune diseases, their exact role in these circumstances is uncertain. They undoubtedly serve as scavenger cells, but, in addition, may function as immune effector cells.

### 4. Theoretical Aspects of Anti-tissue Cellular Immunity

It is reasonably certain that both immune lymphocytes and macrophages, carrying antibodies on their surfaces, can act as effector cells in anti-tissue cellular immunity. These surface antibodies, which cause specific adherence of immune cells to target cells, are synthesized by the lymphocytes which carry them but are adsorbed as cytophilic antibodies by macrophages.

The effector activities of immune cells may depend, not only on surface antibodies, but also on other properties of the cells. Immune macrophages adhere to target cells by virtue of a specific reaction between macrophage-cytophilic antibodies and target antigens; however, cell destruction results

Activated macrophages are essential for the expression of antimicrobial cellular immunity because the parasites are destroyed or inhibited within these cells. Macrophages may be activated by both specific and nonspecific mechanisms. The role of cytophilic antibodies in cellular immunity and the possible relation of delayed sensitivity to cellular immunity are considered. Although delayed sensitivity is not essential for cellular immunity, it could contribute; it is postulated that lymphocytes may act as effector cells or may synthesize antibodies that are cytophilic for macrophages, and in both ways may contribute to cellular immunity. In addition, during delayed sensitivity reactions, lymphocytes synthesize and release substances which may cause the chemotaxis of macrophages, their immobilization at the site of antigen, and perhaps their activation. Overall, lymphocytes and macrophages cooperate in an effective cellular immune response.

# Chapter 13

# Macrophages in Disease

THE PARTICIPATION of macrophages in cellular immunity against a number of chronic infectious diseases has been discussed in Chapter 12. Macrophages also play a prominent role in the pathogenesis and course of other diseases.

Perhaps the most readily recognized abnormality involving macrophages is monocytosis, i.e. an increase in the proportion, or occasionally in the absolute number, of monocytes in the blood. Normally, monocytes constitute 3 to 8% of the leukocytes of peripheral blood, but the percentage of monocytes may be as high as 90% during certain infections and neoplastic states. Bacterial infections which commonly result in monocytosis include tuberculosis, brucellosis, typhoid fever, and subacute bacterial endocarditis (SBE). Infections with certain protozoa and rickettsiae also lead to monocytosis.

## A. *Infectious Diseases*

In addition to their essential role in the pathogenesis of granulomatous diseases, macrophages can play a prominent role in other infectious diseases. For example, substantial numbers of mature macrophages are sometimes observed in the peripheral blood of patients with SBE. Daland *et al.* (1956) reviewed this subject and reported a number of cases. Macrophages are more easily demonstrable in ear lobe blood, because the ear lobe is a dependent low-temperature area where circulating cells tend to accumulate. More than 10% of the leukocytes in the first drop of blood from an ear lobe puncture of patients with SBE may be macrophages. Greenberg (1964) reported a patient in whom mature macrophages constituted 32% of the leukocytes in the first drop of ear blood collected after puncture; ear lobe blood of this patient contained 8 to 40 times as many macrophages as venous blood. There was a good correlation between the response of the patient to antibiotic therapy and decreasing macrophage counts.

The tendency for circulating macrophages to accumulate in the ear lobe may also account for the reported success in demonstrating acid-fast bacilli (presumably *M. leprae*) in ear lobe blood (S. H. Han, personal communication) of patients with lepromatous leprosy.

The increased susceptibility of diabetic patients to infection may be related to a failure of the usual mechanism for transfer of glycogen from PMNs to macrophages (Rebuck, Whitehouse, and Noonan, 1967). Normally, glycogen is transferred from neutrophils to macrophages and apparently is utilized as an energy source for macrophage activities. Although the transfer of glycogen to macrophages was substantial in inflammatory foci of normal subjects, it was greatly suppressed in patients with severe diabetes, particularly during post-acidotic periods.

### B. *Metabolic Diseases*

The role of macrophages in the pathogenesis of atherosclerosis has been reviewed by Day (1967). The deposits of lipid found in the intima of atherosclerotic arteries result, at least in part, from macrophage activity. The composition of these lipids indicates that they are not simply deposited by filtration, but instead are metabolic products, probably derived from macrophages in the arterial wall. Fat-laden macrophages (foam cells) are abundant in early atherosclerotic lesions, and play an active role in the metabolism of lipids in the lesions.

Other abnormalities of lipid metabolism are also associated with unusual changes in macrophages. Histiocytosis X is a generic term used to designate a group of diseases of unknown etiology in which xanthomatous granulomas are the distinguishing characteristic. Eosinophilic granuloma, Letterer-Siwe disease, and Schüller-Christian disease are included in the general category of Histiocytosis X (Avioli, Lasersohn, and Lopresti, 1963). These diseases progress through four stages: (1) a phase of macrophage proliferation and accumulation of eosinophils, (2) a vascular-granulomatous phase, during which macrophages and eosinophils persist and small collections of lipid-laden macrophages form, (3) a diffuse xanthomatous phase, during which many foam cells accumulate, and (4) in chronic cases, a final phase of healing and fibrosis.

An unusual rodlike tubular body has been found in macrophages of patients with Histiocytosis X (deMan, 1968). Although these bodies are probably related to the disease process, their origin and nature are unknown. It is postulated that they may represent either nonspecific cytoplasmic products which react in some way during the disease, storage of some organic products such as lipids, or some infective agent such as a virus.

Gaucher's disease is characterized by lipid accumulations within greatly enlarged macrophages; however, the glycolipids found in these (Gaucher) cells are glucocerebrosides containing equimolar amounts of sphingosine, fatty acid, and glucose (Moore, 1967). The disease is a rare familial autosomal recessive disorder, apparently caused by deficiency of an enzyme necessary for the degradation of certain glycolipids.



Another example of a hereditary lipidosis is the hypercholesterolemia that is transmitted as an autosomal dominant trait. In this disease, nodular masses of macrophages containing lipids, called xanthomas, are found in the skin and tendons (Walter and Israel, 1963).

### C. *Intercellular Imbalances*

Pulmonary diseases characterized by unusual macrophage activity are numerous, and usually involve an imbalance in the normal interrelationship of cells in the lung. They include diffuse interstitial pulmonary fibrosis (Ebert, 1967), endogenous lipoid pneumonia (Maier, 1967), idiopathic pulmonary hemosiderosis (Christie, 1967a), and the pneumoconioses (Christie, 1967b). Fibrosing alveolitis, which precedes pulmonary fibrosis, is commonly associated with hyperfunction of macrophages. The relation between macrophages and fibroblasts has been discussed in Chapter 5. It is clear that destruction of mature macrophages is associated with fibrosis.

A desquamative interstitial pneumonia has been described; in this disease masses of large PAS-positive cells accumulate in the alveolar spaces, accompanied by an infiltration of lymphocytes and plasma cells (Liebow, Steer, and Billingsley, 1965). A model closely approximating this disease has been produced in rabbits by i.v. injection of Freund's complete adjuvant (Deodhar and Bhagwat, 1967). It was proposed that both the human disease and the animal model represent a hypersensitivity reaction in the lungs. Both respond well to treatment with steroid hormones.

A rapidly fatal form of silicosis has been described by Buechner and Ansari (1969), who reported four cases of acute silico-proteinosis in sandblasters. In each case, the patient had been exposed to large quantities of extremely small particles of silica over the relatively short period of 3 to 6 years. After onset of symptoms the average survival was only 7.5 months before the patients died with an acute alveolar proteinosis. It is possible that this form of acute silicosis results from a prolonged massive destruction of alveolar macrophages by large quantities of inhaled minute particles of silica; that this involves a compensatory proliferation with huge numbers of alveolar macrophage being continuously formed and rapidly destroyed; and that the destroyed macrophages release the proteinaceous material which fills the alveoli. It is probable that the lack of granulomatous foci typical of chronic silicosis can be attributed to the continuous and rapid proliferation of immature macrophages which are overwhelmed by the large quantity of silica and destroyed before granuloma formation can take place.

Abnormal activities of macrophages and certain macrophage-like cells occur in rheumatic diseases. The lining cells of synovial membranes are classified as Type A, macrophage-like phagocytic cells, and Type B, fibroblast-like cells with a rich endoplasmic reticulum. Electron microscopy of

synovia from patients with rheumatoid arthritis reveals proliferation of Type A cells and the presence of many inclusions, containing a variety of ingested materials, within Type A cells (Norton and Ziff, 1966; Wyllie, Haust, and More, 1966). Lysosomal enzymes derived from PMNs, synovial lining cells, macrophages, and perhaps other types of cells are substantially increased above normal levels in rheumatoid synovial fluid (Weissman, 1966).

Certain anti-inflammatory agents, useful in the treatment of rheumatoid arthritis and other rheumatic diseases, are known to affect lysosomal stability and activity. Fedorko, Hirsch, and Cohn (1968) reported that colloidal gold is a membrane stabilizer. Persellin and Ziff (1966) demonstrated that gold salts, which are often used in treatment of rheumatic diseases, become concentrated in the lysosomes of guinea pig macrophages. It was further shown that gold salts inhibited the activities of lysosomal enzymes within these cells.

These and similar observations suggest that lysosomal enzymes of PMNs and macrophages participate in the pathogenesis of rheumatoid arthritis, gout, and pseudogout. The relative roles of various cell types in the pathogenesis of these diseases deserve intensive study.

## D. *Macrophages and Neoplasms*

The literature on histiocytic neoplasms was reviewed by Braunsteiner and Gall (1962), who described the characteristics of various human neoplasms of reticuloendothelial tissue, and determined the enzyme content of both normal and neoplastic monocytes.

Neoplasms which cause monocytosis include monocytic leukemia, Hodgkin's disease, and the reticuloendothelioses (Leavell and Thorup, 1960). Dargeon (1966) made a clinical survey of the reticuloendothelioses of childhood.

Salm (1962) has proposed that the presence of collections of lipid-containing macrophages in endometrial lesions is strongly indicative that the lesions are malignant. Such macrophage collections were found in 10.8% (14 of 129) of patients having endometrial polyps, and in 7.5% (8 of 107) of patients with endometrial adenocarcinoma. Other investigators have also found collections of macrophages in association with adenocarcinoma of the uterus in approximately 10% of the patients reported (Harris, 1958; Krone and Littig, 1959; Scully and Richardson, 1961).

## E. *Possible Contributions of Macrophages to the Pathogenesis of Disease States*

Salky, Mills, and DiLuzio (1965) measured the clearance of a test lipid from the blood stream of patients with various diseases characterized by immunological abnormalities. Enhanced RES activity was demonstrated in patients with either rheumatoid arthritis, rheumatic fever, or certain other diseases. The intravascular half-times of injected particulates were on the order of 4.0 minutes as compared with normal values of about 7.5 minutes. Because macrophages can contribute to antibody production by virtue of their phagocytic and digestive activities (see Chapter 9), it was proposed that the enhanced RES activity observed in the above-named diseases contributes to the pathogenesis of these diseases.



Böhme (1965) observed an increase in phagocytosis by the RES following the introduction of antigen into experimental animals. He suggested that the RES functions in the development of certain experimental autoimmune diseases by removing and modifying the antigens.

Macrophages produce an endogenous pyrogen which may be responsible for fever in diseases in which they are prominent in the lesions (Hahn *et al.*, 1967). It was shown that peritoneal macrophages can produce a pyrogen with characteristics similar to those of the pyrogen produced by PMNs.

*SUMMARY:* Increased numbers of circulating immature macrophages are found during the course of a number of infectious diseases and neoplasms. This monocytosis probably represents a migration of newly formed macrophages to areas of inflammation. Mature macrophages are sometimes present in the peripheral blood of patients with subacute bacterial endocarditis, especially in the blood from the ear lobe.

Macrophage activity is important in atherosclerosis and other abnormalities of lipid metabolism. Granulomatous and fibrotic diseases of the lung also entail a high degree of macrophage activity. Rheumatic diseases are characterized by increased release of lysosomal enzymes from macrophages and other cells in the joints; lysosomal stabilizers are useful in therapy.

Macrophages may function in the pathogenesis of certain diseases. They may modify antigens in autoimmune states, and they can produce endogenous pyrogen which may contribute to the pathogenesis of many diseases.