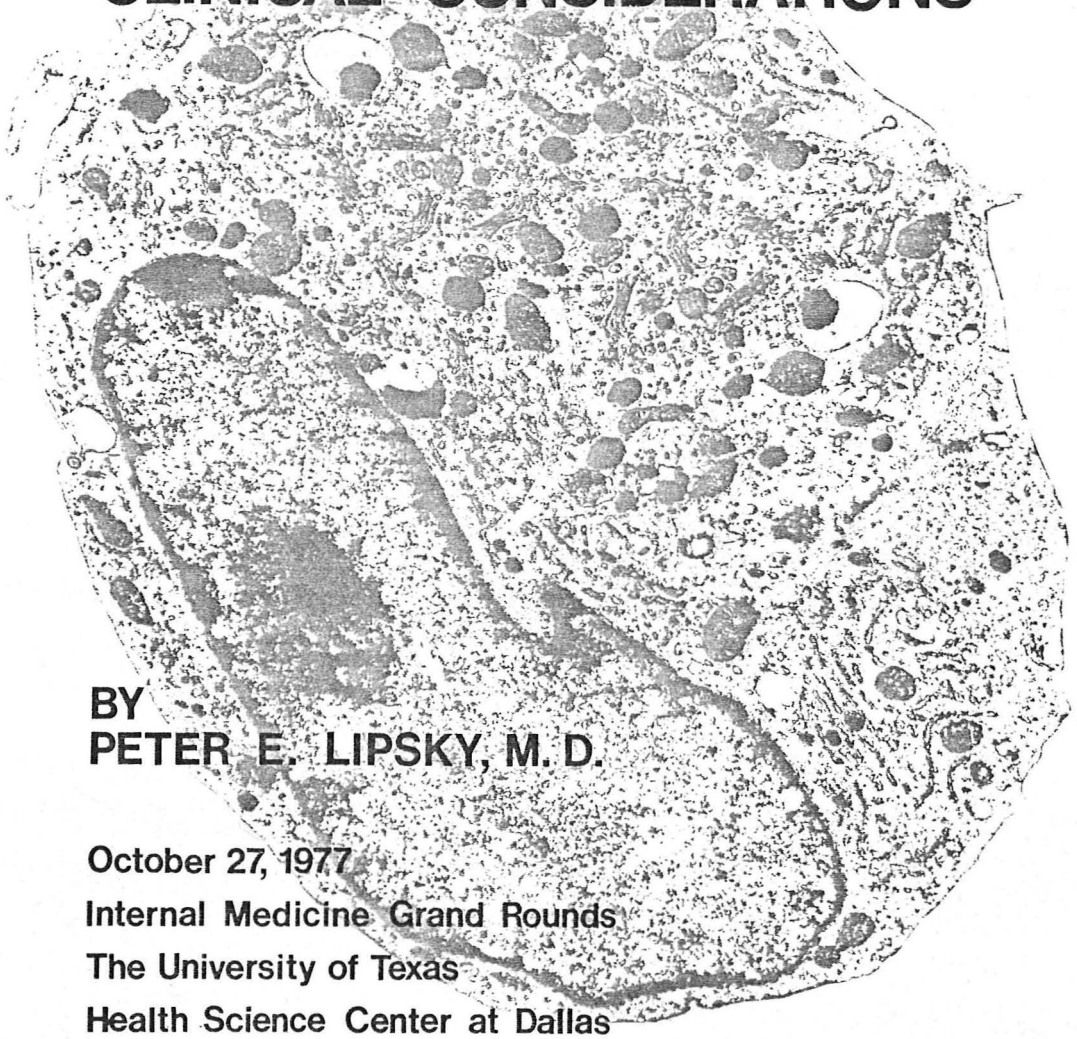
THE MONONUCLEAR PHAGOCYTE SYSTEM: PHYSIOLOGICAL AND CLINICAL CONSIDERATIONS



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INTRODUCTION

The concept that mononuclear phagocytes play a central role in host defense derives from the work of the Russian biologist Elie Metchnikoff. In 1883, while living in Messina, Italy and studying digestive processes of invertebrates, Metchnikoff postulated the Theory of the Phagocytes (1, 2). He recalls the critical observation as follows (3):

> "One day when the whole family had gone to a circus to see some extraordinary performing apes, I remained alone with my microscope, observing the life in the mobile cells of a transparent star-fish larva, when a new thought suddenly flashed across my brain. It struck me that similar cells might serve in the defense of the organism against intruders. Feeling that there was in this something of surpassing interest, I felt so excited that I began striding up and down the room and even went to the seashore in order to collect my thoughts.
>
> I said to myself that, if my supposition was true, a splinter introduced into the body of a star-fish larva, devoid of blood vessels or of a nervous system, should soon be surrounded by mobile cells as is to be observed in a man who runs a splinter into his finger. This was no sooner said than done.
>
> There was a small garden to our dwelling, in which we had a few days previously organized a "Christmas tree" for the children on a little tangerine tree; I fetched from it a few rose thorns and introduced them at once under the skin of some beautiful star-fish larvae as transparent as water.... Very early the next morning I ascertained that it had fully succeeded.
>
> That experiment formed the basis of the phagocyte theory, to the development of which I devoted the next twenty-five years of my life."

Metchnikoff continued his work and described the function and distribution of phagocytes not only in invertebrates but also in the liver, spleen, lymph nodes and in the central nervous system of vertebrates, including man (1, 2). He later grouped the

free and fixed large mononuclear cells together and called them "macrophages" to distinguish them from the leukocytes of the circulating blood which he called "microphages".

On the basis of his observations, Metchnikoff formulated the cellular theory of immunity which held that the phagocytes were solely responsible for defense against foreign materials and microorganisms.

An appreciation of the originality of this hypothesis requires an understanding of the theories of host defense which were prevalent at that time. The presence of ameboid mononuclear cells had been observed at inflammatory sites by von Recklinghausen in 1863 (4). It also had been recognized for many years that microorganisms commonly were found within phagocytes at such inflammatory sites. However, it was not appreciated that phagocytes could play a beneficial role by killing the infecting organisms. Rather, it was felt that they had the detrimental effect of disseminating microorganisms throughout the body during infections.

The importance of inflammatory reactions in host defense also was not understood. It was generally felt that inflammation was a morbid process which caused tissue injury and was not beneficial or protective to the host. Immunity was felt to result solely from the action of specific and nonspecific humoral factors, later identified as antibody and complement, which had recently been discovered (5).

Metchnikoff's thesis brought him into conflict with the leading biologists of the day, especially those advocating humoral theories of immunity such as Koch, Erlich, von Behring and Bordet, and bitter debates were carried on. Metchnikoff was derided for attributing "psychic perception" to the phagocytes. The disagreement between Metchnikoff and the humoralists was resolved somewhat by the observation of Sir Almroth Wright in 1903 (6) that serum factors made bacteria more susceptible to phagocytosis. As described by the Shavian physician, Sir Colenso Ridgeon (7), "The phagocytes won't eat the microbes unless the microbes are nicely buttered for them. Well, the patient manufactures the butter for himself all right; ....Opsonin is what you butter the disease germs with to make your white corpuscles eat them".

Recognition of the importance of phagocytes in host defense, as well as a truce in the debate between those advocating the cellular theory of immunity and those favoring the humoral theory, came in 1908 when Metchnikoff and Paul Ehrlich, one of the founders of the humoral theory of immunity, were jointly awarded the Nobel Prize in Physiology and Medicine.

The concept of the macrophage system as defined by Metchnikoff was expanded by Aschoff, who introduced the term "reticulo-endothelial system" to cover the entire range of cells which possessed endocytic capacity as judged by the ability to take up vital dyes (8). The term "reticulo-endothelial system" was derived from the fact that the cells forming the system were thought to be involved in the formation of the "reticulum" of the lymph nodes and spleen, or were those cells lining blood or lymph sinusoids.

The concept of the reticuloendothelial system (RES), however, has been called into question since it defines a system of cells linked only by their ability to take up vital dyes *in vivo* (9). Certain cells are excluded inappropriately from this system, such as blood monocytes which do not take up vital dyes efficiently. Moreover, the term reticulo-endothelial system is unfortunate since neither reticulum cells (10) nor endothelial cells (8) are part of the system.

During the past 2 decades there has been a vast increase in knowledge about mononuclear phagocytes. Current understanding has led to the concept of the Mononuclear Phagocyte System (11). This system is composed of cells which are widely distributed throughout the body (Table I).

TABLE I

THE MONONUCLEAR PHAGOCYTE SYSTEM (9)

Cell Type	Location
Monoblast	Bone marrow
Promonocyte	Bone marrow
Monocyte	Bone marrow Peripheral blood
Macrophage	Tissues Connective tissue (histiocytes) Liver (Kupffer cells) Lung (alveolar macrophages) Lymph nodes (free and fixed macrophages) Spleen (macrophages) Bone marrow (macrophages) Serous cavities (pleural and peritoneal macrophages) Bone (osteoclasts) Nervous system (microglial cells) Synovium (Type A cells) Inflammatory sites (macrophages)

They are grouped on the basis of a number of characteristics. First, mononuclear phagocytes share certain morphologic characteristics, although this is somewhat dependent on the organ or tissue in which they reside. Second, they are avidly phagocytic. Rabinovitch (12) has termed these cells "professional phagocytes" to distinguish them from a number of other cells which may develop modest phagocytic capacity under certain defined conditions. Finally, there is considerable evidence that most, if not all, mononuclear phagocytes are derived from a common precursor in the bone marrow, the monoblast. For these reasons, mononuclear phagocytes have been grouped into a morphologically, functionally and lineally related family of cells. It must be remembered, however, that this system is really a model upon which to base future research. As new information is obtained, changes in this concept may become necessary. As pointed out by Carr (13),

> "We should use the term Reticuloendothelial System (RES) with respect and affection as of an aged relative, 'old and grey and full of sleep' who has served the world well but now nods by the fireside; we should use the term Mononuclear Phagocyte System as of a lover in whose company there is delight, though not necessarily the consolation and companionship of a lifetime."

Throughout the years, a number of descriptive terms have been coined to identify cell types found in various lymphoid organs and other tissues (Table II).

TABLE II

TERMS USED TO DENOTE CELLS OF THE RETICULOENDOTHELIAL SYSTEM

Sinus lining cells	Dendritic macrophages
Lymphatic endothelial cells	Phagocytic reticular cells
Reticulo-endothelial cells	Non-phagocytic reticular cells
Retothelial cells	Fibroblast-like reticular cells
Sinus reticular cells	Fixed macrophages
Reticulum cells	Tingible-body macrophages
Primitive reticular cells	Interdigitating cells
Dendritic cells	Metalophil cells
Dendritic reticular cells	Resting wandering cells

The exact relationship between many of these cell types and the mononuclear phagocyte system is not completely clear. Some of these cells, such as reticulum cells (10), dendritic cells (14) and dendritic reticular cells (15), clearly are not a part of the Mononuclear Phagocyte System. Use of many of the terms in Table II should probably be avoided until the functional significance of the various cell types is better understood.

Investigation of the structure and function of the Mononuclear Phagocyte System has continued at a rapid pace. For example, during 1975 and 1976, an average of 54 articles per month were published on various aspects of macrophage physiology. While early work focused on the role of macrophages as nonspecific scavenger cells, it has become apparent that these cells are intimately involved in a number of other physiological processes as detailed in Table III.

TABLE III

FUNCTIONS OF MONONUCLEAR PHAGOCYTES

1) Effector cells in chronic inflammatory responses.
2) Defense against certain microorganisms.
3) Removal of damaged, senescent or dead cells and cellular debris.
4) Induction, regulation and expression of immunity.
5) Defense against the development and spread of neoplastic cells.

MORPHOLOGY

Peripheral blood monocytes and the macrophages found in the various organs and tissues of the body are the cells of the Mononuclear Phagocyte System found outside the marrow. The blood monocyte is a large round cell with a diameter of 10-18 μm. In Wright-stained preparations, monocytes have an abundant grayish-blue cytoplasm containing small azurophilic granules. These granules are primary lysosomes containing stored hydrolytic enzymes (16). The nucleus is large, centrally located, reniform or horseshoe-shaped and contains a network of fine lacy chromatin.

In thin sections studied under the electron microscope, the monocyte has a characteristic fine structure (16-18). There is a well developed Golgi apparatus, numerous lysosomal granules and mitochondria evenly distributed throughout the cytoplasm. The nucleus is eccentric, kidney-shaped with moderately condensed chromatin. Pseudopodia extend from the cell surface and there is evidence of endocytic activity.

In the tissues, monocytes develop into tissue macrophages (16, 19-21). Macrophages are morphologically heterogeneous, as might be expected from their widespread tissue distribution. Their size varies from 10-80 μm in diameter. They contain one or more oval or indented, often eccentrically located, nuclei and may have prominent nucleoli. Their cytoplasm is more abundant than that of the monocyte, and contains numerous dense granules, endocytic vacuoles and mitochrondria, usually clustered near the centrosphere. The cytoplasm also contains ribosomes, polyribosomes, microfilaments, microtubules, varying amounts of endoplasmic reticulum and a variety of vacuoles containing the remains of engulfed material. The Golgi apparatus, which is usually situated near the nuclear indentation, is particularly prominent in mature macrophages and is composed of elongated cisternae and small membrane-bound vesicles.

The dense granules of the centrosphere are lysosomes (20). Primary lysosomes are membrane-bound structures which contain a variety of hydrolytic enzymes. They bud from the Golgi apparatus and may fuse with phagocytic or autophagic vacuoles to form digestive bodies or secondary lysosomes.

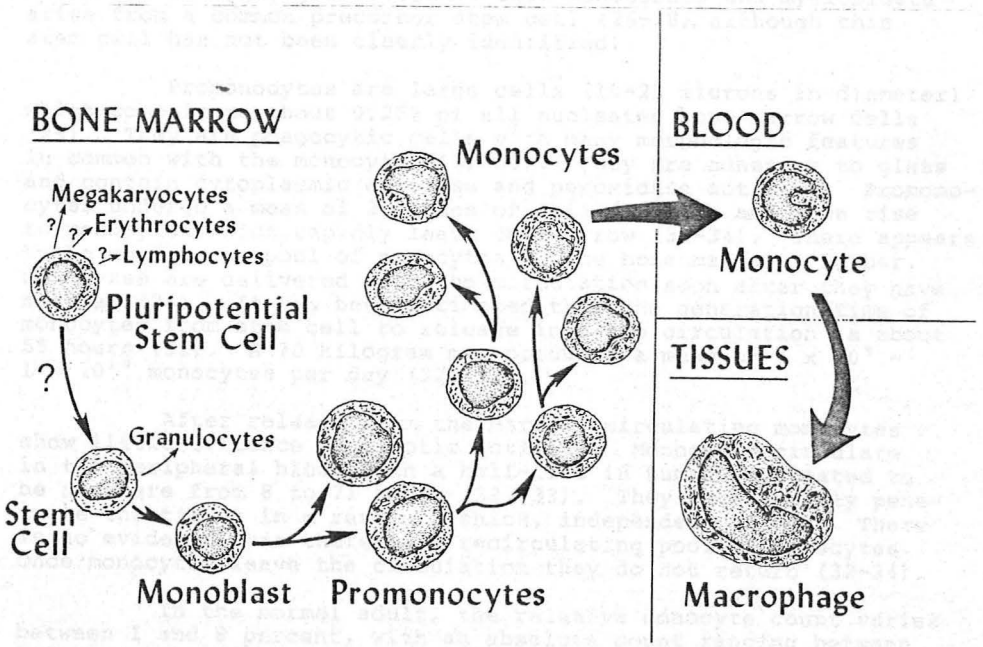
The presence of cytoplasmic inclusions depends upon the previous phagocytic history of the cell under study. These inclusions represent the end-product of phagocytosis or autophagic activity. Macrophages may exhibit unusual appearances because of their content of iron pigments, melanin, or foreign pigment materials. They may also contain fat, cholesterol, sebaceous material, keratin, thyroid colloid, or cellular or microbial debris within their vacuoles.

The cellular organization of the macrophage is provided by the system of microtubules which extend throughout the cytoplasm providing a rigid framework (22).

The living macrophage is an actively motile cell, often extending and retracting pseudopods. The periphery of the macrophage, which is the site of endocytosis, undergoes continuous undulating movement and membrane ruffling. This activity of the macrophage membrane is accomplished by the action of the contractile microfilaments (22).

LIFE HISTORY

The bone marrow is the source of the circulating monocytes and indirectly the origin of most, if not all, tissue macrophages.



The most immature cell of the mononuclear phagocyte system that has been fully characterized is the monoblast of the bone marrow (23). The monoblast is a round cell with a diameter of 10-12 microns. The monoblast contains the cytoplasmic enzymes characteristic of mononuclear phagocytes, including peroxidase and nonspecific esterase, and it synthesizes lysozyme. The monoblast is pinocytically and phagocytically active and possesses both IgG and complement receptors. In these respects, the monoblast has the features typical of mononuclear phagocytes although they are less well developed than those found associated with more mature mononuclear phagocytes.

Monoblasts are rapidly dividing cells which undergo a mean of one round of cell division and differentiate into promonocytes (16, 24, 25). It remains unclear whether monoblasts themselves differentiate from a more primitive pluripotential stem cell which may also give rise to neutrophiles and erythrocytes. There is, however, good evidence that monoblasts and myeloblasts arise from a common precursor stem cell (26-28), although this stem cell has not been clearly identified.

Promonocytes are large cells (10-25 microns in diameter) which constitute about 0.25% of all nucleated bone marrow cells (29). They are phagocytic cells with many morphologic features in common with the monocyte (16, 30). They are adherent to glass and contain cytoplasmic esterase and peroxidase activity. Promonocytes undergo a mean of 2 cycles of cell division and give rise to monocytes which rapidly leave the marrow (31-34). There appears to be no reserve pool of monocytes in the bone marrow. Rather, monocytes are delivered into the circulation soon after they have matured (34). It has been estimated that the generation time of monocytes from stem cell to release into the circulation is about 55 hours (32). A 70 kilogram man produces a mean of 1×10^9 - 1×10^{10} monocytes per day (32, 33).

After release from the marrow, circulating monocytes show little evidence of mitotic activity. Monocytes circulate in the peripheral blood with a half-life in humans estimated to be anywhere from 8 to 71 hours (32, 33). They subsequently penetrate the tissue in a random fashion, independent of age. There is no evidence that there is a recirculating pool of monocytes. Once monocytes leave the circulation they do not return (32-34).

In the normal adult, the relative monocyte count varies between 1 and 8 percent, with an absolute count ranging between 280 and 550 cells/mm^3. The total blood monocyte pool is comprised of a circulating and a marginated pool. The marginated pool consists of monocytes adhering to or rolling along the endothelial cells of blood vessels (35) and constitutes up to 75% of the total blood monocyte pool (31-33).

The monocytes are actively motile and can migrate between the endothelial cells of capillaries. This occurs throughout all capillary beds, where monocytes penetrate the basement membrane and take up residence in the tissues (24). In addition, they may adhere to the walls of sinusoids in liver and spleen and become more active phagocytes. Monocytes manifest more active endocytosis, motility and membrane ruffling than their immediate precursors, the promonocytes (20).

Once in the tissues the monocyte matures into a more functionally active cell, the tissue macrophage. It was first demonstrated by Lewis in 1925 (36) that in tissue culture, monocytes transform into macrophages, epithelioid cells and multinucleated giant cells. It was noted that circulating mononuclear phagocytes from a number of species of vertebrates were capable of differentiating into "macrophages similar to those found in connective tissues, liver, spleen and lymph nodes, and epithelioid cells and giant cells precisely like those found in tuberculous lesions" (37).

The tissue macrophages divide only infrequently (22, 38). They may have an extremely long life span in the tissues, often surviving for months or even years (39-42). Large numbers of macrophages are found, outlining the distribution of all blood vessels in the connective tissue. They are particularly prominent in the lung, liver, spleen, and bone marrow. Under conditions of local inflammation, large numbers of monocytes accumulate and become macrophages. During intense local inflammatory responses, such as occurs as part of a delayed hypersensitivity reaction, local proliferation of immature macrophages may be observed (43, 44). In chronic inflammation, macrophages may form tight clusters or granulomata. Under these conditions their endocytic activities become less prominent, many mitochondria develop, and they take on the characteristics of "epithelioid cells" (21, 45). In some cases macrophages may fuse to form multinucleated "giant cells" (21).

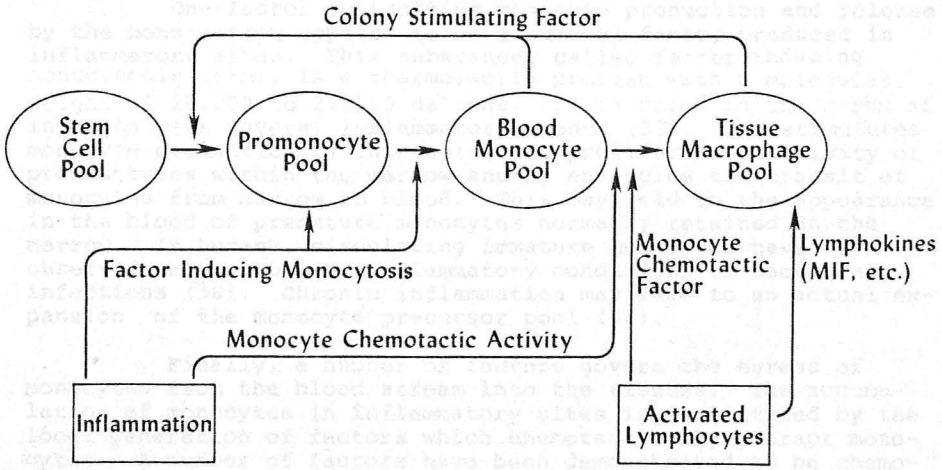
Granulomatous inflammation can be divided into high turnover and low turnover granulomata (46). High turnover granulomata, which occur in response to infections with a number of intracellular organisms such as Mycobacterium tuberculosis, exhibit a high level of recruitment of circulating monocytes and local proliferation of young macrophages. Low turnover granulomata, such as are observed in response to a number of inert particles which cannot be easily eliminated, are characterized by a negligible rate of monocyte recruitment. The macrophages in these lesions are extremely long-lived, sometimes living for many years, and cell division is uncommon.

While it is accepted that the ultimate progenitor of the various tissue macrophages resides in the bone marrow, the identity of the immediate precursor of some of these cells remains controversial. It has generally been held that tissue macrophages are direct descendants of blood monocytes. Recent experiments, however, have suggested that some resident macrophage populations, such as Kupffer cells and peritoneal macrophages, may renew themselves by local cell division and that an influx of monocytes is only important during inflammation (47). Alveolar

macrophages, however, appear to derive largely from circulating monocytes. This may be related to their location and consequent chronic exposure to the external environment.

The mononuclear phagocyte system is controlled by a complex regulatory system which ensures an adequate supply of macrophages to the tissues under normal as well as pathological conditions.

REGULATION OF THE MONONUCLEAR PHAGOCYTE SYSTEM



One control mechanism involves the action of colony stimulating factor (CSF), which can be detected in the serum and urine of animals and man (48-51). This factor, a glycoprotein of about 45,000 daltons (52), stimulates the maturation of bone marrow stem cells *in vitro* in extremely low concentrations. There are several lines of evidence indicating that colony stimulating factor is also involved in the *in vivo* regulation of monocytopoiesis and granulocytopoiesis. CSF is thought to stimulate the committed stem cells and thus to augment their rate of differentiation into monocytopoietic and granulocytopoietic precursor cells (53).

Monocytes and macrophages themselves actively secrete CSF (54, 55) and thus appear to be capable of regulating the rate of production of their own precursors from the marrow.

Animals rapidly mobilize monocytes from the bone marrow in response to inflammatory stimuli of various sorts (34, 56). Two mechanisms appear to be operative in this mobilization. The first involves the release of premature monocytes from the marrow and the second a shortening of the promonocyte cell cycle time by about 50% with a consequent doubling of the marrow output of monocytes.

One factor controlling monocyte production and release by the bone marrow appears to be a humoral factor produced in inflammatory sites. This substance, called factor inducing monocytosis (FIM), is a thermolabile protein with a molecular weight of 18,000 to 24,500 daltons. It is found in the serum of mice injected with several inflammatory agents (57). FIM stimulates monocyte production by increasing the proliferative activity of promonocytes within the marrow and by enhancing the transit of monocytes from marrow to blood. This may lead to the appearance in the blood of premature monocytes normally retained in the marrow. In humans, circulating immature monocytes have been observed in a number of inflammatory conditions or bacterial infections (58). Chronic inflammation may lead to an actual expansion of the monocyte precursor pool (56).

Finally, a number of factors govern the egress of monocytes from the blood stream into the tissues. The accumulation of monocytes in inflammatory sites is facilitated by the local generation of factors which chemotactically attract monocytes. A number of factors have been demonstrated to be chemotactic for monocytes, including bacterial products (59), factors generated as a result of complement activation such as C5a (60,61), and other products likely to be present at sites of inflammation including kallikrein, plasminogen activator (62), and basic proteins released from lysosomal granules of neutrophils (59). Moreover, appropriately activated thymus-derived or bone marrow-derived lymphocytes also release monocyte chemotactic factors (63-65). Other lymphokines, such as macrophage migration inhibitory factor (MIF), may contribute to the localization of recently arrived monocytes (66, 67).

A great variety of pathologic conditions (68, 69) result in an increase in the number of blood monocytes (Table IV).

TABLE IV

CAUSES OF MONOCYTOSIS (68, 69)

Infectious and Parasitic Diseases:

a) Bacterial:

Tuberculosis	Brucellosis
Typhoid fever	Subacute bacterial endocarditis
Syphilis	Post acute infection

b) Rickettsial:

Rocky Mountain spotted fever
Typhus

c) Parasitic:

Malaria
Trypanosomiasis
Leishmaniasis

Neoplastic Diseases:

Myelomonocytic leukemia	Multiple myeloma
Hodgkin's disease	Nonhematological malignancy
Lymphomas	

Hematologic Diseases:

Polycythemia vera	Hemolytic anemia
Post splenectomy	Neutropenia
Myeloid metaplasia	

Connective Tissue Diseases:

Rheumatic fever
Systemic lupus erythematosus
Rheumatoid arthritis

Chronic Inflammatory Diseases:

Ulcerative colitis
Crohn's disease

Miscellaneous:

Sarcoidosis	Cirrhosis
Drug reactions	Idiopathic

Monocytosis is normal in the neonate and may persist for several weeks after birth. The infectious diseases causing monocytosis are usually chronic processes produced by intracellular micro-organisms and parasites. Other causes of monocytosis include a variety of neoplastic conditions, responses to hematopoietic cell destruction, and a number of chronic inflammatory conditions of uncertain etiology.

A number of clinical conditions (69) may be associated with expansion of the Mononuclear Phagocyte System (Table V)

TABLE V

CONDITIONS CAUSING HYPERPLASIA OF THE MONONUCLEAR PHAGOCYTE SYSTEM (69)

I. Inflammatory:

a) Intracellular parasites -

1. Bacterial - Tuberculosis, Brucellosis, Salmonellosis, Leprosy, Syphilis

2. Fungal - Histoplasmosis, Crytococcosis

3. Parasitic - Malaria, Leishmaniasis, Trypanosomiasis, Toxoplasmosis, Schistosomiasis

b) Idiopathic -

1. Sarcoidosis

II. Chronic Endogenous Stimulation:

1) Chronic hemolytic anemia

2) Storage diseases -

a) Gaucher's disease

b) Neimann Pick disease

III. Neoplastic:

1) Monocytic leukemia

2) Malignant Histiocytosis

3) Hodgkin's disease

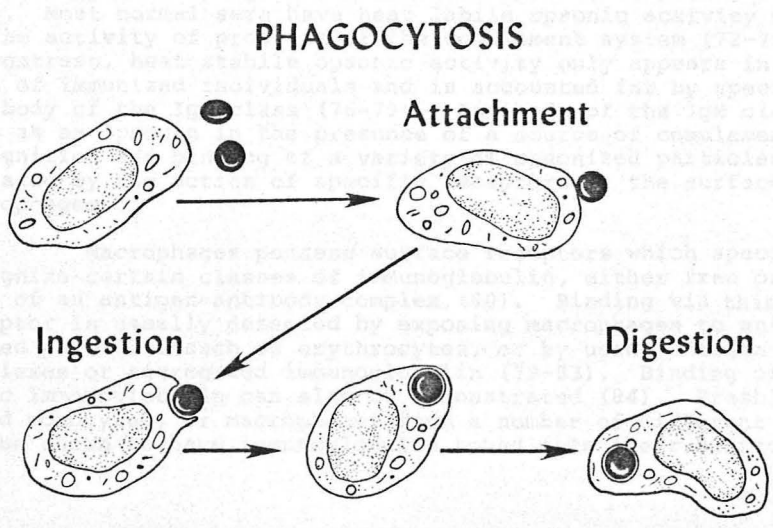
Hyperplasia may result from exposure to a number of chronic stimuli or neoplastic transformation of cells of the Mononuclear Phagocyte System. Clinically, hyperplasia is often manifested by enlargement of organs rich in mononuclear phagocytes, such as liver, spleen and lymph nodes.

CELL BIOLOGY

I. Endocytosis

A. Phagocytosis

The cells of the Mononuclear Phagocyte System share a number of biological characteristics which facilitate their role in host defense. The first, and perhaps more important function to be described was phagocytosis, or the capacity to ingest particles. The sequence of events leading to the interiorization of a particle by a macrophage involves initial attachment of the particle to the macrophage membrane, followed by the formation of microprogections of macrophage plasma membrane which surround the particle, and finally fusion of the membrane with budding of the particle-containing vacuole into the cytoplasm. In order to better analyze this complex series of events, phagocytosis has been separated into two separate phases (12, 70). The first involves recognition of the particle by the macrophage and attachment of the particle to the macrophage cell membrane. The second step involves the actual ingestion of the particle.



1) Attachment

Attachment of a particle to the macrophage plasma membrane is a necessary first step in phagocytosis (12). The net adhesive forces between the macrophage and a particle determine the ease with which this step of phagocytosis is accomplished. Particle attachment can be influenced by electrostatic forces, forces between hydrophobic and hydrophilic surfaces and other chemical interactions. Therefore, the surface properties of a particle which determine whether it will be bound by phagocytes are quite complex (71). Of importance, however, is the observation that many microorganisms and other particles are totally ignored by macrophages or other phagocytes. This is likely to be an important factor contributing to the pathogenicity of these organisms. Many of the organisms which are not normally recognized by macrophages, however, may be bound and ingested in the presence of fresh normal or immune serum (6). This augmented phagocytosis resulting from the action of serum factors is referred to as opsonization.

Opsonins are serum constituents which bind to particles and make them more likely to be bound and, therefore, endocytosed by phagocytes. The presence of opsonins on the surface of a particle is an important factor governing the fate of that particle. Particles coated with opsonins are avidly bound by macrophages, while the same particles may be neither bound nor ingested by macrophages in the absence of opsonins.

Serum opsonins classically have been divided by their sensitivity to heating into heat stabile and heat labile components. Most normal sera have heat labile opsonic activity owing to the activity of proteins of the complement system (72-75). By contrast, heat stabile opsonic activity only appears in the sera of immunized individuals and is accounted for by specific antibody of the IgG class (76-79). Antibody of the IgM class only acts as an opsonin in the presence of a source of complement (12). Recognition and binding of a variety of opsonized particles is mediated by the action of specific receptors on the surface of macrophages.

Macrophages possess surface receptors which specifically recognize certain classes of immunoglobulin, either free or as part of an antigen-antibody complex (80). Binding via this receptor is usually detected by exposing macrophages to antibody coated particles such as erythrocytes, or by using antigen-antibody complexes or aggregated immunoglobulin (79-83). Binding of monomeric immunoglobulin can also be demonstrated (84). Freshly prepared monocytes, or macrophages from a number of different species, can be shown to have immunoglobulin bound onto their surfaces (81).

This macrophage receptor shows a marked specificity for IgG (79-84), although there are a few studies which suggest that under some circumstances IgM may also be bound (85, 86). There is no evidence to indicate that serum or secretory IgA is bound by a macrophage receptor (87). Moreover, in species where subclasses of IgG have been defined, not all subclasses have binding activity. In man, the receptor binds IgG1 and IgG3, but not IgG2 or IgG4 (88-90). A similar situation occurs in the mouse where IgG2a and IgG2b both interact with the receptor, but IgG1 does not (84).

The macrophage receptors specifically recognize the Fc region of the IgG antibody molecule (81,88). The Fc region of IgG is composed of two pairs of regions or domains, the amino-terminal $C\gamma 2$ domains and the carboxy-terminal $C\gamma 3$ domains. The region recognized by the macrophage Fc receptor has been localized to the $C\gamma 3$ domain (91). By contrast, complement fixation appears to be mediated by the $C\gamma 2$ domain.

Recently, evidence has been presented to indicate that macrophages may possess two distinct Fc receptors (84). One receptor appears to mediate the binding of IgG-antigen complexes and IgG opsonized particles, while the second binds soluble, monomeric IgG which has not been complexed to antigen or aggregated. These receptors can be distinguished by their differential sensitivity to proteolytic digestion with the former being trypsin resistant and the latter trypsin sensitive.

The chemical nature of the macrophage Fc receptor is unknown, and there is little information about its synthesis or turnover. The receptor appears to be relatively stable in that it is maintained during prolonged *in vitro* incubation. Macrophages possess between 1×10^5 to 2×10^6 Fc receptors per cell (83, 84, 92). It has been estimated that about 0.2% of the macrophage surface would be occupied if all the Fc receptors bound IgG. Activated macrophages develop more Fc receptors concomitant with their increase in surface area (84).

Binding of IgG by macrophages does not require the expenditure of metabolic energy, occurring to an equivalent degree at 4°C and 37°C (80, 84).

Fc receptors are found on a variety of different cell types besides macrophages. Thus, Fc receptors have been demonstrated on polymorphonuclear leukocytes, K-cells, B lymphocytes, T lymphocytes, mast cells, basophils, platelets and cells of human placental membranes (80). It is not known, however, whether the Fc receptors on these cells are structurally similar to those found on macrophages.

The major biological role for Fc receptors would appear to involve facilitating phagocytosis. Although antibody is not an obligatory requirement for endocytosis of many particles, its presence greatly enhances this process. The macrophage Fc receptor is also likely to play a central role in a number of other biological functions of macrophages, including antibody dependent cell-mediated cytotoxicity (93), macrophage "arming" in tumor immunity (94), and the triggering of macrophage enzyme release (95).

Macrophages can also bind antigen-antibody-complement complexes (96, 97) by means of a surface receptor which recognizes the third component of complement (C3). Either of two fragments of the C3 molecule, C3b or C3d, can mediate the binding to the macrophage surface receptor (98). By contrast, polymorphonuclear leukocytes which also have a C3 receptor, are able to bind only C3b and not C3d opsonized particles. The C3 receptor of macrophages has different characteristics than the Fc receptor in that binding is temperature dependent and is abolished when the macrophage is exposed to trypsin (96). Moreover, the C3 receptor is lost when macrophages are cultivated *in vitro* for prolonged periods (99). Both unactivated and activated macrophages possess C3 receptors. C3 receptors are also found on B lymphocytes (100) and renal glomerular cells (101).

A nonspecific particle receptor has also been defined on the surface of macrophages which binds a number of particles such as glutaraldehyde treated erythrocytes or latex particles (12). Binding is independent of temperature and removed by exposure of the cells to trypsin. This receptor is not unique to macrophages in that it is also found on certain cultured fibroblasts.

2) Ingestion

The normal consequence of binding of a particle to the macrophage membrane is the initiation of ingestion. The cytoplasm of the macrophage extends to form pseudopodia, which spread to surround the particle. The pseudopodia fuse on the distal side of the particle resulting in the formation of a phagocytic vesicle or phagosome. The lining of this vacuole is composed of inverted plasma membrane. The vesicle buds off from the cell periphery and migrates into the cytoplasm.

While particle attachment occurs in the absence of an expenditure of metabolic energy, ingestion is highly temperature dependent and requires metabolic energy (102). The source of metabolic energy varies with different populations of macrophages. Thus, phagocytosis by peritoneal macrophages is dependent on glycolysis, occurs under anaerobic conditions, and is unaffected

by inhibitors of respiration and oxidative phosphorylation (102, 103). On the other hand, phagocytosis by alveolar macrophages depends upon energy derived from respiratory pathways and is inhibited at low oxygen tensions (102-104).

The mechanism underlying the extension of pseudopods and engulfment of particles is thought to involve the action of cytoplasmic contractile units of actin and myosin (105). Thus, dense areas of microfilaments are seen by electron microscopy in the cytoplasm of macrophages undergoing endocytosis (22) and inhibitors of microfilament function, such as cytochalasin B, inhibit phagocytosis (106).

While macrophage-particle adherence is a necessary first step, it is not always sufficient to trigger endocytosis. For example, the plant lectin concanavalin A will mediate the attachment of a number of particles to macrophage surfaces without initiating internalization (107). Opsonins themselves vary in their ability to trigger endocytosis. Thus, IgG through its Fc fragment directly stimulates particle ingestion, while C3 primarily mediates the binding of the particle to the macrophage and is an inefficient inducer of phagocytosis (98, 108). However, there appears to be synergy between the two opsonins in that particle bound C3 can markedly reduce the amount of IgG necessary to induce phagocytosis (98).

Binding of a particle to the macrophage surface, even after opsonization with IgG antibody, does not always trigger phagocytosis. Recent evidence indicates that binding per se does not trigger ingestion. Rather, for IgG opsonized particles a "zipper-like" mechanism seems to be involved (109, 110). Phagocytosis of a particle requires initial attachment of the particle to a macrophage membrane receptor followed by sequential attachment of sites on the particle to macrophage receptors not involved in the original attachment. In this way, the macrophage membrane is guided around the particle.

The stimulus to internalize a particle is specific and not general (111). Thus, phagocytosis of one particle does not trigger ingestion of other particles attached to the macrophage surface. Rather, the endocytic stimulus is confined to the segment of the cell's plasma membrane immediately adjacent to the particle being ingested.

After ingesting a large number of particles, macrophages retract their pseudopods, round up and cease pinocytic and phagocytic activity for a number of hours (112, 113). Macrophages,

unlike polymorphonuclear leukocytes, have the capacity to synthesize new surface membrane. The amount of new membrane synthesized is related to the degree of initial membrane internalized. After new plasma membrane and receptors are resynthesized, the macrophages may again commence phagocytic activity.

B. Pinocytosis

Pinocytosis refers to the uptake of extracellular fluids by cells. Macrophages engage in two forms of pinocytosis (114). Macropinocytosis refers to ptake of fluid into relatively large vesicles (0.05-2μM in diameter) which are easily visible with light microscopy. This process is observed in macrophages, as well as many other types of cells including fibroblasts, tumor cells, thyroid epithelium and kidney tubule cells. Micropinocytosis is characterized by the formation of extremely small vesicles (70-100 ηM in diameter) which are visible only with the electron microscope. This type of pinocytosis is also found in endothelial cells, as well as nerve cell terminals.

The undulating membrane of the macrophage pseudopods is the site of pinocytic vesicle formation. The vesicles form at the cell surface and begin to migrate centrally toward the perinuclear area. During this migration they commonly fuse with each other to form larger vacuoles. The movement of pinocytic vesicles toward the center of the cell appears to involve the action of the microtubules. Agents such as colchicine or vinblastine, which inhibit microtubular assembly, have no effect on the formation of pinocytic vesicles, but interfere with their migration toward the center of the cell causing them to accumulate randomly throughout the cytoplasm (115).

Macropinocytosis is an energy requiring process (116). Thus, lowering the ambient temperature to 4°C significantly inhibits pinocytosis. Studies carried out with metabolic inhibitors indicate that the energy for macropinocytosis derives from aerobic respiration and oxidative phosphorylation. By contrast, micropinocytosis apparently does not require metabolic energy (114).

Several agents have the capacity to stimulate pinocytosis *in vitro*. Thus, a number of serum proteins such as albumin, other proteins, polysaccharides and nucleic acids which exist as anions at physiological pH can stimulate pinocytosis. Uncharged or cationic materials usually are ineffective (117).

The uptake of extracellular solutes by macrophages depends to a large extent on the binding of the compound to the plasma membrane (70). If a material has no binding to the plasma membrane, it will be taken up as a simple solute. This process is referred to as fluid phase pinocytosis. Compounds which bind to the plasma membrane are concentrated at this site and, thus, are

interiorized to a greater extent. This process is referred to as adsorptive pinocytosis. An example of adsorptive pinocytosis is the uptake of soluble immune complexes. These are bound by the macrophage Fc receptor and, thus, are interiorized at a markedly accentuated rate compared to that of the soluble protein alone.

II. Intracellular Digestion

The fusion of endocytic vacuoles with primary or secondary lysosomes initiates the process of intracellular digestion which results in the degradation of internalized microorganisms or other ingested materials (118-120). Intracellular vacuoles are able to exchange their contents by means of membrane fusions (70). Endocytic vacuoles of both pinocytic and phagocytic origin fuse with each other and with both primary and secondary lysosomes. This leads to a constant exchange of digestive enzymes and materials from the extracellular environment. Membrane fusions in the macrophage are selective, involving membranes derived from the cell surface, Golgi and lysosomes, but excluding endoplasmic reticulum and mitochondria. The molecular mechanisms involved in these membrane fusions are unclear.

Intracellular digestion is an enzymatic process utilizing preformed lysosomal hydrolases. Acid hydrolases are synthesized in the endoplasmic reticulum, incorporated into vacuoles in the Golgi and stored within the cell in primary and secondary lysosomes (16, 20). When lysosomes fuse with endocytic or autophagic vacuoles, hydrolytic enzymes are released into these structures initiating enzymatic degradation of the contained material. Endocytosis by macrophages of a number of materials leads to an increase in the level of intracellular lysosomal acid hydrolases (121-123). The induction of these hydrolytic enzymes is nonspecific in that ingestion of one digestible material results in the induction of many unrelated enzymes (122, 123). When macrophages are cultured *in vitro* under conditions in which endocytic activity is decreased, the existent lysosomes and their content of acid hydrolases gradually disappear from the cytoplasm (122). Both the quantity of endocytosed material and its rate of digestion control the level and persistence of lysosomal enzymes. Thus, ingestion of degradable erythrocytes results in a greater synthesis of lysosomal enzymes than does endocytosis of indigestible particles such as latex spheres (123).

Proteins are degraded within secondary lysosomes to amino acids and dipeptides which may then diffuse out of the cell into the extracellular space. Peptides with molecular weights

above 230 daltons are retained within lysosomes (117). Several polysaccharides such as dextran sulfate, and disaccharides such as sucrose, are not degraded in macrophage lysosomes and are retained for prolonged periods of time. Most monosaccharides can diffuse out of the lysosomes (124).

Materials which are endocytosed by macrophages usually do not regain access to the external environment in an undigested form unless the macrophage is killed. Following uptake, nondigestible materials such as colloidal gold or certain bacterial constituents are stored within secondary lysosomes of the macrophages where they remain for long periods of time. They are not subsequently shed into the external environment unless the macrophage is killed or damaged. Similarly, when digestible proteins are taken up, less than 2% of the intracellular material is subsequently released from the macrophage in undegraded form (115).

III. Microbicidal Activity

Mononuclear phagocytes kill ingested microorganism by a number of mechanisms. The action of acid pH, lysosomal enzymes and hydrogen peroxide is responsible for the bactericidal activity of many of these cells. There is a post phagocytic burst of metabolic activity (103) which results in the production of lactic acid and a fall in intraphagosome pH to 3-4 (125), which may inhibit the growth of certain microorganisms.

Mononuclear phagocytes also make use of a system which generates hydrogen peroxide to kill some microorganisms (126). This system is dependent on a source of oxygen as indicated by the observation that the bactericidal activity of human monocytes is impaired in the absence of oxygen (127); while in the presence of oxygen and an agent that stimulates H_2O_2 production, the killing of microorganisms is enhanced (128). The enzyme myeloperoxidase, which markedly potentiates the antimicrobial activity of H_2O_2 in the presence of halide ions (129), is present in peripheral blood monocytes but absent in most tissue macrophages (130). Thus, in blood monocytes the antimicrobial activity of H_2O_2 may be facilitated by myeloperoxidase but this does not appear to occur in tissue macrophages. Catalase, present in macrophages from a number of tissues, may substitute for myeloperoxidase as the catalyst of the H_2O_2 dependent microbicidal system in these cells (126).

Defense against a number of microorganisms, such as Mycobacterium tuberculosis, Histoplasma capsulatum, Toxoplasma gondii or Listeria monocytogenes, may not result from microbicidal activity at all but rather from the ability of macrophages to inhibit the multiplication of these organisms once they have been

ingested (131-133). Such organisms may slowly multiply within the macrophage vacuolar system or remain dormant, but viable, for months or years.

IV. Secretory Activity

Since the observations of Metchnikoff, macrophages have usually been thought of primarily as phagocytic cells whose function in host defense involves the ingestion and degradation of noxious materials. Notwithstanding the importance of the phagocytic function of macrophages, recent studies have suggested that some of the actions of these cells in inflammatory sites may be accomplished through the secretion of a wide variety of biologically active materials. These secreted products (Table VI) include hydrolytic enzymes active at acid or neutral pH with the capacity to digest tissue constituents, products which may be important in modulating the inflammatory response, factors which can influence the function of other cells in the immediate vicinity, as well as factors capable of regulating the immune response.

TABLE VI

SECRETORY PRODUCTS OF MONONUCLEAR PHAGOCYTES

I. Enzymes

a) Lysosomal hydrolases
b) Lysozyme
c) Neutral proteases
   - collagenase
   - plasminogen activator
   - elastase

II. Enzyme Inhibitors

a) α_2-macroglobulin

III. Biologically Active Factors

a) Prostaglandins
b) Complement components
c) Pyrogen
d) Factors stimulating bone resorption

IV. Factors Which Regulate Functions of Other Cells

a) Colony stimulating factor
b) Interferon

V. Factors Which Regulate the Immune Response

a) Stimulatory factors
b) Inhibitory factors

Information about the synthesis and secretion of biologically active materials from mononuclear phagocytes has been obtained by studying purified monocyte or macrophage populations under controlled conditions of *in vitro* incubation. This approach has allowed for detailed investigation both of the nature of the secreted materials, as well as an understanding of the mechanisms regulating secretion.

A. Enzymes

1) Lysosomal hydrolases

The lysosomal hydrolases of macrophages are primarily involved in intracellular digestion. However, macrophages may also be stimulated to release these enzymes into their external milieu in a selective manner (134). This phenomenon is not unique to macrophages in that polymorphonuclear leukocytes, fibroblasts and other cell types also can be induced to release lysosomal enzymes upon appropriate stimulation (135, 136). Phagocytosis of a number of different particulate materials such as latex particles or erythrocytes will induce the release of a small fraction of the macrophage's lysosomal enzymes (10-25%) over a brief period of time. However, when macrophages ingest certain other particles such as zymosan, asbestos or streptococcal cell walls, they respond by releasing a very large fraction of their lysosomal contents (up to 80%) into the extracellular environment (137). Release of lysosomal enzymes is selective in that there is no concomitant release of the cytoplasmic enzyme lactate dehydrogenase, an indicator of cell damage. It is of interest that the particles which cause selective lysosomal enzyme release from macrophages have been shown to lead to chronic inflammation when injected into experimental animals, and some such as asbestos have clearly been implicated as causative of illness in humans.

Lysosomal enzyme release may also be induced by interaction of macrophages with immune complexes formed at equivalence (138) or by the action of secreted factors from antigen or mitogen activated thymus derived lymphocytes (139).

2) Lysozyme (Muramidase)

Lysozyme is a cationic protein with a molecular weight of 14,000 daltons. It hydrolyses N-acetyl muramic β1,4 N-acetyl glucosamine linkages in bacterial cell walls leading to lysis of highly susceptible organisms, such as Micrococcus lysodeikticus (140).

Monocytes and macrophages from man and other species synthesize and secrete large amounts of lysozyme (141). Under *in vitro* culture conditions 85-90% of the total enzyme production is excreted, whereas only a small constant fraction remains intracellularly. Lysozyme represents the major secretory product of the macrophage and may account for up to 2.5% of the total protein synthesized by the cell. In patients with monocytic leukemias, high levels of lysozyme may be found in serum and urine (142, 143). Moreover, in certain chronic inflammatory diseases, such as Crohn's disease, elevated serum levels of lysozyme may be found (144).

During *in vitro* culture, macrophages produce a constant amounts of lysozyme for prolonged periods of time. The secretion of lysozyme appears to be independent of the activity of the cell. For example, endocytosis does not alter its rate of secretion. Moreover, no specific inducers of lysozyme production have been described (141).

3) Neutral Proteases

A number of neutral proteases, including enzymes which act on plasminogen, collagen, elastin and proteoglycans are actively synthesized and secreted by stimulated macrophages (145).

Plasminogen activator is a serine protease which converts plasminogen to plasmin. Secretion of such an enzyme may play an important role in inflammation not only because of the action of plasmin in fibrinolysis, but also because plasmin can generate active mediators by interaction with the coagulation, complement activation and kinin generation systems (146, 147). Plasminogen activator production is not unique to macrophages in that it is synthesized by a number of other cell types, including mouse embryo fibroblasts, after malignant transformation (148).

Collagenase is secreted by macrophages, as well as by neutrophils, fibroblasts, and rheumatoid synovial cells (149-151). The macrophage collagenase is similar in specificity to other collagenases, cleaving native collagen into fragments which can then unfold and be digested by other proteolytic enzymes. Macrophage collagenase appears to be secreted in latent form which can be activated by a number of proteolytic enzymes, including plasmin (152). Elastase is a serine esterase which degrades insoluble elastin. The macrophage elastase seems to have different specificity than that described for the pancreatic or neutrophil elastases (153).

The neutral proteases, in general, are inducible enzymes associated with macrophage activation and markedly stimulated by phagocytosis (145). These enzymes are synthesized de novo by macrophages in response to appropriate stimulation. Their production is abolished by inhibitors of protein synthesis. The bulk of the newly synthesized enzymes are secreted with up to 75% found in the extracellular culture medium. While it is clear that these enzymes are not released from lysosomes, the exact mechanisms involved in their secretion have not been elucidated.

Unstimulated macrophages synthesize negligible amounts of neutral proteases, but can be triggered to do so either by various stimuli causing macrophage activation or by phagocytic challenge (154). Collagenase and plasminogen activator secretion by macrophages has been reported to be enhanced by exposure of macrophages to factors secreted from activated thymus-derived lymphocytes (155, 156).

B. Enzyme Inhibitors

Cultured human monocytes secrete α_2-macroglobulin, a protease inhibitor known to inhibit lysosomal hydrolases, plasminogen activator, elastase and collagenase (157). The mechanism controlling synthesis and secretion of α_2-macroglobulin by monocytes is unknown.

C. Biologically Active Factors

Macrophages secrete a number of biologically active factors which may be important in regulating inflammatory responses. Macrophages secrete prostaglandins (158). This activity is not unique to macrophages, however, in that neutrophils (159), platelets (160) and rheumatoid synovial cells (161) also release prostaglandins. Macrophages also contain phospholipase A_2 which can generate arachidonic acid, the precursor of prostaglandins (162).

Mononuclear phagocytes also secrete a number of complement components. Thus, macrophages actively synthesize and release C3, C4, C2, and possibly C1 and factor B (163-166). Synthesis is abolished by inhibitors of protein synthesis and augmented when the macrophages are stimulated or when they ingest bacteria (167).

Exogenous pyrogens, such as bacterial endotoxin, induce the release of pyrogenic materials from both polymorphonuclear leukocytes and monocytes. These endogenous pyrogens are thought to act directly on the hypothalamus to produce fever. Human neutrophiles and monocytes synthesize two distinct endogenous pyrogenic molecules (168). Pyrogen is not spontaneously released by monocytes *in vitro*, but rather is stimulated either by exposure to endotoxin or phagocytic challenge (169).

Human monocytes secrete a factor(s) which stimulates the release of both mineral and matrix from bone (170). This factor acts directly and does not require the presence of osteoclasts. The activity may be important in the pathogenesis of the bone resorption which occurs in chronic inflammatory conditions such as rheumatoid arthritis.

D. Factors Which Regulate Functions of Other Cells

A number of macrophage products have been identified whose main function appears to involve the regulation of the activities of other cell types. Thus, monocytes or macrophages secrete a factor, colony stimulating factor (CSF), which can stimulate the differentiation of individual bone marrow stem cells into colonies of mature granulocytes or monocytes (54, 55). Exposure of monocytes to endotoxin results in an increased production of colony stimulating factor. Monocytes or macrophages are not unique in the production of colony stimulating factor in that stimulated lymphocytes also appear to produce CSF (171).

Interferon is an antiviral protein produced by a number of different cell types. Macrophages synthesize and secrete interferon (171, 172). Following infection with virus the rate of synthesis increases, although the interferon produced may be of a different molecular weight (173).

E. Factors Which Regulate The Immune Response

Macrophages secrete a number of factors which may have profound effects on the immune response (174). First, they secrete factors which may be important in maintaining the viability and functional integrity of lymphocytes (175, 176). They also produce a number of factors which may enhance or stimulate lymphocyte function. For example, macrophages produce a factor which may be directly mitogenic for thymus-derived lymphocytes or enhance their responsiveness to other mitogenic stimuli (177). Another factor may facilitate the differentiation of bone marrow derived lympho-

cytes into antibody forming cells under appropriate conditions (178, 179). Finally, factors have been described which facilitate the generation of appropriate helper T cells (180). Secretion of many of the molecules can be enhanced by phagocytic challenge, exposure to activated T cells or bacterial endotoxin (181). In a number of *in vitro* systems, macrophages have also been demonstrated to secrete factors, usually of low molecular weight, which inhibit lymphocyte proliferation (182, 183), as well as DNA synthesis by other cell types. At least one of these factors appears to be newly synthesized, thymidine (183-185).

FUNCTIONS OF THE MONONUCLEAR PHAGOCYTE SYSTEM

The cells of the Mononuclear Phagocyte System have a number of functions which are of importance in maintaining the integrity and homeostatic balance of the organism (Table III). They are essential participants in chronic inflammatory responses of diverse types where they not only act as scavenger cells to remove dead or damaged cells and cellular debris, but also are important in tissue repair and scar formation (186). Macrophages also play a critical role in the induction and regulation of the immune response (187), act as the main instrument of defense against a number of microorganisms (133) and may have an important function in defense against the development and spread of neoplastic cells (188). Finally, mononuclear phagocytes are involved in the removal of senescent, damaged or dying cells from the circulation. Table VII lists the characteristics of the Mononuclear Phagocyte System, which facilitate the accomplishment of these various functions.

TABLE VII

CHARACTERISTICS OF THE MONONUCLEAR PHAGOCYTE SYSTEM

1) Extensive tissue distribution.
2) Ability to migrate to areas of inflammation.
3) Potential to respond to challenge with foreign stimuli by increased production of active cells.
4) Capacity to ingest various materials.
5) Ability to augment endocytosis with serum opsonins.
6) Ability to degrade ingested materials.
7) Capacity to secrete biologically active materials.
8) Capacity to "process" antigen for the induction of an immune response.
9) Potential to be functionally activated in response to environmental factors.

I. Defense Against Infection

The mononuclear phagocyte system is the principal defense against infection by a number of microorganisms especially intracellular pathogens such as Mycobacteria, Salmonella, Listeria, Brucella, Histoplasma, Cryptococcus, Candida, Toxoplasma, Leishmania, Trypanosoma, and Plasmodia (76, 189)

Metchnikoff initially described the role of mononuclear phagocytes in host defense against microorganisms and clearly delineated the physiologic steps involved (1, 2). These included:

1) Production of adequate numbers of phagocytes.

2) Delivery of peripheral blood phagocytes to the tissues.

3) Establishment of contact between phagocytes and microorganisms.

4) Phagocytosis and killing of microorganisms.

A. Macrophage Activation

Metchnikoff realized that the mononuclear phagocytes were unable to deal with many pathogenic microorganisms and that acquired resistance to many infectious agents depended upon "the perfecting of the phagocytic and digestive powers of the leukocytes (2)."

The mechanism of acquired cellular resistance to various microorganisms, or macrophage activation, has been extensively investigated by Mackaness (190) who studied the response of mice to sublethal systemic infection with the intracellular pathogen Listeria monocytogenes. After injecting mice with Listeria, he observed that the organisms were rapidly cleared from the circulation and localized in the liver and spleen, where they replicated. There was a steady growth of Listeria for about 72 hours within these organs associated with an inflammatory response. Initially polymorphonuclear leukocytes predominated, but monocytes began to accumulate in the lesions after the second day. On the fourth day, there was a dramatic change as bacterial growth abruptly stopped and elimination of the bacteria from the animals ensued. During this phase of the infection, the lesions contained a predominance of mononuclear cells. It was further observed that macrophages at the site of the lesions became structurally altered at the time the organisms began to disappear from the tissues (191). The cells were larger and more metabolically active (192, 193), contained more lysosomal hydrolases (194), and appeared to take up (195) and kill (196) organisms more efficiently.

If experienced animals were reinfected (197), the initial phase of bacterial growth and the neutrophil response did not occur. Mononuclear cells infiltrated the lesions immediately, and killing of the organisms began promptly. The acquired capacity to kill the microorganisms was found to reside in the mononuclear phagocytes of the lesions (190, 198). Macrophages obtained from convalescent mice could be demonstrated to reflect the systemic state of resistance of the animals. When tested *in vitro*, every cell from experienced animals could kill Listeria, whereas macrophages from naive mice could not kill these organisms. On the contrary, organisms multiplied within the phagolysosomes of unexperienced mouse macrophages.

The macrophages obtained from animals recently recovered from a sublethal infection with Listeria, or a number of other intracellular pathogens, were found to have a number of structural and functional differences (190-199) from macrophages obtained from normal animals (Table VIII), and are referred to as "activated macrophages".

TABLE VIII

CHARACTERISTICS OF ACTIVATED MACROPHAGES

A. Structural

1) Larger.
2) More mitochondria and lysosomes.
3) More cytoplasmic vesicles.
4) Increased membrane ruffling.

B. Metabolic

1) Increased incorporation of glucosamine.
2) Increased hexose monophosphate shunt activity.
3) Increased respiratory rate.
4) Alterations in various enzymes:

   a) Increased content of acid hydrolases.
   b) Increased content of lactate dehydrogenase.
   c) Decrease in plasma membrane 5' nucleotidase.
   d) Increase in plasma membrane adenylate cyclase.
   e) Synthesis and secretion of neutral proteases.

C. Functional

1) Accentuated glass adherence.
2) Increased pinocytic rate.
3) Increased phagocytic capacity.
4) Augmented ability to kill certain microorganisms.
5) Augmented tumoricidal capacity.
6) Phagocytosis of C3 opsonized particles (206).

During the course of systemic infection with a number of intracellular parasites, activated macrophages are initially found at the time cell mediated immunity is first detectable (200-202). It was apparent that macrophage activation depended on the development of effective cell mediated immunity to the infecting microorganism. Once a state of cell mediated immunity is established, however, the concomitant enhanced macrophage resistance is nonspecific in that it is not solely directed toward the infecting microorganism. Thus, animals which had been challenged with one microorganism, such as BCG, developed the capacity to resist infection with other antigenically dissimilar microorganisms, such as Listeria or Salmonella (201).

The development of macrophage activation during infection with intracellular microorganisms is dependent on the activity of a particular population of antigen-primed nonrecirculating short-lived thymus-derived lymphocytes with a characteristic tendency to home to sites of inflammation (203). There are at least two mechanisms by which sensitized T lymphocytes collaborate with mononuclear phagocytes to cause increased resistance to intracellular parasites. First, sensitized lymphocytes exert a focusing effect on blood monocytes. These lymphocytes secrete monocyte chemotactic factor, thereby promoting the egress of monocytes from the circulation into the inflamed tissue (63, 64). The sensitized lymphocytes are also triggered to produce lymphokines in response to interaction with specific bacterial antigen. These lymphokines in turn appear to stimulate the monocyte-derived macrophages to develop the enhanced microbicidal capacity characteristic of activated macrophages (66, 67, 204).

A wide variety of *in vivo* or *in vitro* stimuli have been demonstrated to result in some of the changes characteristic of macrophage activation (205). For example, *in vivo* exposure to nonspecific irritants such as mineral oil, or *in vitro* exposure to immune complexes, complement components, ingestible materials of various sorts, or products of activated thymus-derived or bone marrow-derived lymphocytes, all have been reported to result in some of the changes of macrophage activation. The degree of activation, however, as judged by bactericidal capacity is usually less pronounced than that manifested by macrophages obtained from infected animals expressing cell mediated immunity.

Activated macrophages have greater bactericidal activity than unstimulated macrophages (190, 196, 198). Although the basis of the enhanced killing capacity of activated macrophages is not completely understood, it may relate either to their enhanced phagocytic capacity, their greater content of lysosomal

enzymes, or their increased metabolic activity resulting in more efficient generation of acid and H_2O_2.

B. Mechanisms by which Microorganisms Avoid Destruction in Mononuclear Phagocytes

Ingestion and intracellular killing of various microorganisms by cells of the Mononuclear Phagocyte System is of paramount importance in host resistance to these organisms. However, a number of microorganisms have developed mechanisms to avoid killing by mononuclear phagocytes (Table IX).

TABLE IX

MECHANISMS BY WHICH MICROORGANISMS AVOID DESTRUCTION WITHIN MONONUCLEAR PHAGOCYTES

Mechanism	Organism
Avoid Attachment	Pneumococci, Streptococci, Staphylococci, E. Coli, Crytococcus
Avoid Ingestion	Mycoplasma sp.
Escape from Phagocytic Vacuole	Vaccinia, Trypanosoma cruzi
Prevent Lysosomal Fusion	Toxoplasma gondii Mycobacterium tuberculosis
Resist Destruction in Lysosomal Environment	Mycobacterium lepraemurium Mycobacterium tuberculosis Listeria monocytogenes

Some organisms, especially those with capsules, avoid attachment to phagocytes entirely. For example, E. Coli and Cryptococcus possess polysaccharides in their cell walls and capsules respectively, which inhibit the attachment of these microorganisms to macrophages (207, 208). Specific antibody, however, can reverse this effect and cause attachment of many of these organisms to macrophages and, thus, facilitate phagocytosis and intracellular killing (209).

Some microorganisms can inhibit the ingestion phase of phagocytosis. For example, some Mycoplasma are bound by specific receptors on the surface of macrophages but avoid triggering the ingestion process (210). A protein in the cell wall of these organisms appears to be responsible for this inhibition of ingestion. The inhibition of phagocytosis requires living Mycoplasma. Dead organisms are rapidly ingested. Ingestion of living organisms can be triggered by the action of specific antibody of the IgG class.

Some organisms prevent destruction within macrophages by escaping from the phagosome before these vacuoles fuse with lysosomes. An example is provided by vaccinia virus (211, 212). The virus enters the macrophage within an endocytic vacuole. Interaction between the virion and the membrane of the vacuole causes dissolution of both the outer lipoprotein coat of the virus and the vacuolar membrane, and results in the release of the DNA-containing viral core into the cytoplasmic matrix. The surface properties of the virus are critical determinants of its capacity to escape the endocytic vacuole. In the presence of specific antibody the capacity of the virus to penetrate the phagosome and enter the cytoplasm is blocked. As a result, the virus is exposed to lysosomal enzymes and is destroyed. Like vaccinia, the trypomastigotes of Trypanosoma cruzi also escape from phagocytic vacuoles, enter the cytoplasm of the macrophage, and initiate replication (213).

Another mechanism whereby organisms evade the digestive system of macrophages is by inhibiting the fusion of lysosomes with the phagosomes containing the organisms. This has been shown to be the case for Toxoplasma gondii (214) and Mycobacterium tuberculosis (215). Toxoplasma, for example, are taken up into phagocytic vacuoles, prevent the fusion of lysosomes, and then replicate within the vacuoles of the macrophage. Inhibition of lysosome-phagosome fusion is only seen when living organism are ingested. When killed organisms are taken up by macrophages, lysosomal fusion and digestion take place normally. In the presence of specific antibody, the ability of living organisms to inhibit phagolysosome formation and thus avoid exposure to lysosomal enzymes is also reversed (216, 217).

Finally, several obligate intracellular organisms, such as species of Mycobacteria and Listeria, can resist the action of lysosomal enzymes and replicate within the lysosomes of macrophages (217, 218). Some of these organisms, such as Listeria, are killed more efficiently by activated macrophages (196, 201), but others such as M. tuberculosis are not (219).

C. Function of Mononuclear Phagocytes in Viral Infections

Macrophages play a central role in host defense against viral infections (212). The importance of macrophages in resistance to viral infections has been demonstrated in a number of systems. For example, adult mice are resistant to infection with herpes simplex virus, while newborn mice are highly susceptible to this agent (220). Host susceptibility to infection with this virus is related to the ability of the virus to replicate within the mononuclear phagocytes. Thus, herpes simplex virus is able to replicate in the macrophages of newborn mice, while unable to do so in adult macrophages. Maturation of the ability of murine macrophages to limit replication of virus occurs during the first three weeks of postnatal life. Newborn mice can be effectively protected against herpes simplex virus infection by adoptive transfer of adult macrophages (221).

Similarly, resistance to vaccinina virus infection resides within the mononuclear phagocytes. During infection of mice with vaccinia virus, macrophages act as nonpermissive host cells capable of removing the virus from a focus of infection and rendering it noninfectious (212).

Digestion within lysosomes is not the only way macrophages can inactivate viruses. Macrophages also produce interferon (172, 173), as indicated above. Moreover, macrophages infected with some agents such as vaccinia and herpes simplex do not kill the virus, but rather take it up and act as nonpermissive host cells, in that they do not permit viral replication (212, 222). In the case of vaccinia infection, the virus cannot direct the macrophage to synthesize an uncoating enzyme, and as a result viral DNA is trapped within the viral cores and replication does not occur (212). However, infection of macrophages with vaccinia virus results in death of the cell and discharge of noninfective viral particles into the extracellular milieu. These are subsequently ingested and degraded by other mononuclear phagocytes.

Anti-viral antibodies alter the intracellular fate of viruses in macrophages. Opsonized viral particles are usually taken up at an increased rate by macrophages, sequestered within lysosomes and degraded (212). Protection against certain viruses requires collaboration between mononuclear phagocytes and serum antibody. For example, newborn mice are highly susceptible to infection with Coxsackie B-3 virus, but can be rendered resistant

with adoptive transfer of both adult macrophages and specific antibody. Transfer of either antibody or macrophages alone is much less effective at protecting newborn mice (223). Some viruses, however, such as dengue virus, may require the presence of antibody to facilitate uptake into macrophages. Such antibody-mediated uptake may then lead to productive infection of these cells (224).

Activated thymus-derived lymphocytes are also important in mediating host defense to infection with various viruses (226, 227). This resistance may involve direct cytotoxic activity of the T cells themselves, or production of factors which lead to localization and activation of mononuclear phagocytes. It is not known, however, whether activated macrophages are more resistant to the intracellular growth of viruses.

Many viruses can productively infect macrophages. For example, ectromelia in mice (228) and vesicular stomatitis virus in humans (229) cause productive infections of the host's mononuclear phagocytes, causing lysis of these cells and clinically apparent disease. A number of other viruses, such as lymphocytic choriomeningitis virus (230) and lactic dehydrogenase virus (231), are able to replicate within macrophages despite the presence of circulating anti-viral antibody. This results in productive infections with chronic viremias and immune complex deposition (232).

II. The Role of Mononuclear Phagocytes in Immune Responsiveness

The cells of the immune system can be broadly divided into two major classifications. Bone marrow-derived lymphocytes, or B cells, are cells whose progeny secrete antibody. B cells possess immunoglobulin molecules as an integral part of their surface membranes. These immunoglobulin molecules act as receptors for specific antigen. After stimulation with antigen, B cells differentiate into immunoglobulin secreting cells which secrete antibody of the same specificity as that originally found associated with the B cell surface membrane. Thymus-derived lymphocytes, or T cells, do not secrete antibody after stimulation with antigen. Rather, they are responsible for the various manifestations of classic delayed hypersensitivity reactions. Moreover, T cells play a central role in the regulation of the activities of both B cells and other T cells. Thus, a subpopulation of "helper" T cells is necessary for the generation of an antibody response to many antigens. Other subpopulations of "suppressor" T cells can exert negative influences on the activities of both T and B lymphocytes.

Mononuclear phagocytes, likewise, play a critical role in the induction, regulation and expression of both cellular and humoral immune responses. Although macrophages play an important

TABLE X

FUNCTIONS OF MONONUCLEAR PHAGOCYTES IN THE IMMUNE RESPONSE

I. *Induction of the Immune Response*

- a) Antigen uptake.
- b) Interactions with immunocompetent lymphocytes.
  - 1) Activation of T lymphocytes.
  - 2) Cooperation between T lymphocytes and B lymphocytes.

II. *Regulation of the Immune Response*

- a) Inhibition
- b) Augmentation

III. *Expression of the Immune Response*

- a) Chemotaxis
- b) Endocytosis and intracellular digestion
- c) Secretory activity
- d) Bactericidal capacity
- e) Cytotoxic capacity
- f) "Activation"

role in the responsiveness of both thymus-derived and bone marrow-derived lymphocytes, they differ from these cells in that they do not themselves possess immunologic specificity but rather act as nonspecific accessory cells.

Experiments utilizing a number of different models have indicated that induction of both cellular and humoral immune responses is dependent on the active participation of mononuclear phagocytes. One important function of macrophages in the initiation of an immune response involves the uptake of antigen (187). Other phagocytically or pinocytically active cells can endocytize

various potentially antigenic materials, but cells of the mononuclear phagocyte system have the unique capacity not only to take up these antigens but also to maintain them in such a configuration as to facilitate antigen recognition by various lymphocyte populations (233).

A number of *in vivo* observations have suggested that uptake of antigen by mononuclear phagocytes is an important step in the induction of an immune response. First, it has been noted that administration of antigen to an animal results in its uptake by macrophages in lymphoid organs where it tends to persist for prolonged periods of time (234-237). Secondly, immunization studies indicated that priming animals with aggregated antigens which were readily taken up by macrophages resulted in a more intense immune response than immunization with soluble or deaggregated material which was less extensively taken up by macrophages (238-241). The importance of antigen uptake by macrophages was further emphasized by studies in which agents, such as colloidal carbon, were administered to animals to overwhelm the endocytic capacity of the Mononuclear Phagocyte System. Such treatment was also found to interfere with antigen uptake by macrophages and tended to depress the development of both antibody production and cellular immune responses (187). Finally, the relationship between the uptake of antigen by macrophages and the subsequent immunogenicity of that antigen was evaluated using a mouse transfer system. These studies indicated that macrophages which had taken up small amounts of antigen *in vitro* were able to induce both antibody production and delayed hypersensitivity upon transfer to a normal recipient. Furthermore, these studies indicated that macrophage-bound antigen was more immunogenic than free antigen (242-245).

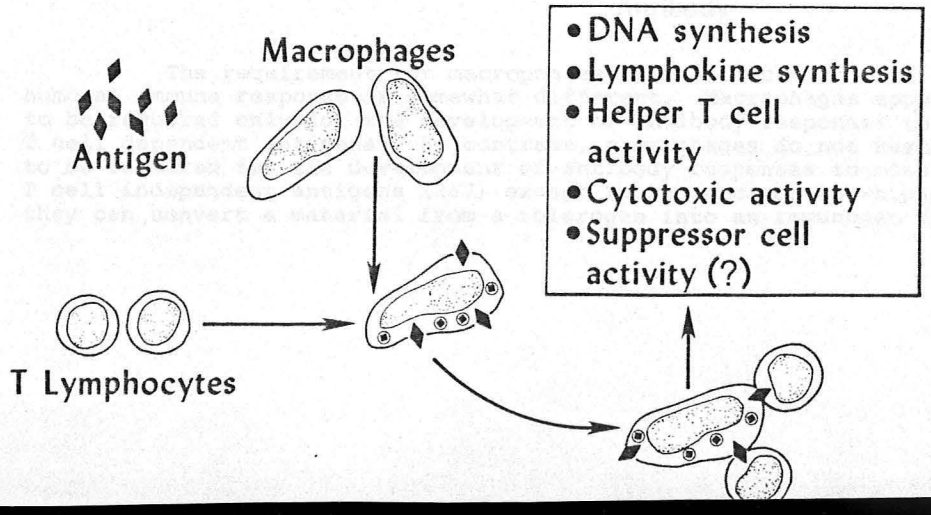
A number of *in vitro* experiments have established that antigen is taken up by macrophages by means of the endocytic processes described above (246). Autologous, homologous and heterologous proteins are taken up in a similar manner indicating that foreignness per se is not recognized by the macrophage (247). The physical properties of the antigen determine its rate of uptake and catabolism (248). Soluble materials are taken up by macrophages by pinocytosis (70). The rate of uptake varies with the degree of binding of the protein to the macrophage plasma membrane. In immune animals, the presence of antibody of the IgG class can augment the uptake of antigen by macrophages by means of Fc receptor binding (249).

The bulk of antigen taken up by macrophages is degraded by lysosomal hydrolases. However, a small fraction of antigen avoids degradation in, as yet, an unknown way and is maintained as the immunogenic moiety for prolonged periods of time (246).

Physical interactions between lymphocytes and macropages are common biological events necessary for the efficient expression of a wide variety of the physiologic functions of lymphoid cells, such as the maintenance of lymphocyte viability and functional integrity (175) and the promotion of the maturation of immature lymphocytes (250). These interactions are thought to be mediated by a macrophage receptor mechanism with the capacity to recognize and reversibly bind thymus-derived and bone marrow-derived lymphocytes (251, 252). The development of physical interactions between macrophages and lymphocytes is also critical for the development of a variety of immune responses (253).

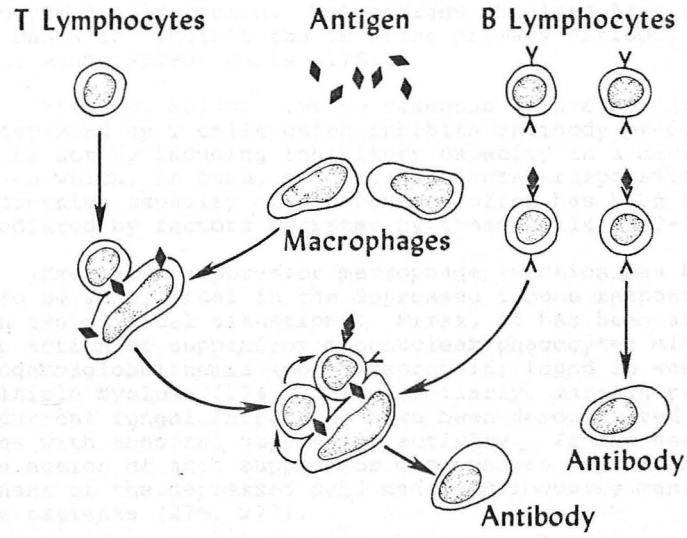
Clustering of lymphocytes about macrophages bearing an antigenic signal has been observed *in vivo* and during *in vitro* cultivation of lymphoid cells from a number of species (253-259). More detailed investigation has indicated the importance of physicial interaction between macrophages and lymphocytes for the *in vitro* induction of primary (258, 259) and secondary antibody responses (260), as well as the antigen mediated *in vitro* initiation of immune T lymphocyte proliferation (253-256). The clustering of lymphocytes about antigen bearing macrophages serves to provide a mechanism for the presentation of immunologically relevant antigen to lymphocytes (253) and for the functional interaction between helper T cells and B cells necessary to trigger an antibody response to many antigens (260-262).

MACROPHAGE-LYMPHOCYTE INTERACTION: INDUCTION OF T LYMPHOCYTE ACTIVATION



Macrophages are required for the activation of thymus-derived lymphocytes. While B lymphocytes bearing surface membrane immunoglobulin receptors can be activated directly by interaction with appropriate antigen (263), T lymphocytes require antigen to be presented by a macrophage in order to have effective triggering of either blastogenesis (233, 264, 265) lymphokine production (266) or helper cell induction (180). Effective T cell activation appears to be facilitated by the development of intimate physical interaction between macrophages and T lymphocytes (253).

MACROPHAGE-LYMPHOCYTE INTERACTION : INDUCTION OF ANTIBODY RESPONSES



The requirement for macrophages in the induction of a humoral immune response is somewhat different. Macrophages appear to be required only for the development of antibody responses to T cell dependent antigens. By contrast, macrophages do not seem to be required for the development of antibody responses to most T cell independent antigens (267) except in those cases in which they can convert a material from a tolerogen into an immunogen by

appropriate catabolism or concentration (268). Thus, macrophages appear to function in humoral immune responses by promoting the induction of antigen-specific helper T cells and providing a focus for collaboration between helper T cells and responding B cells.

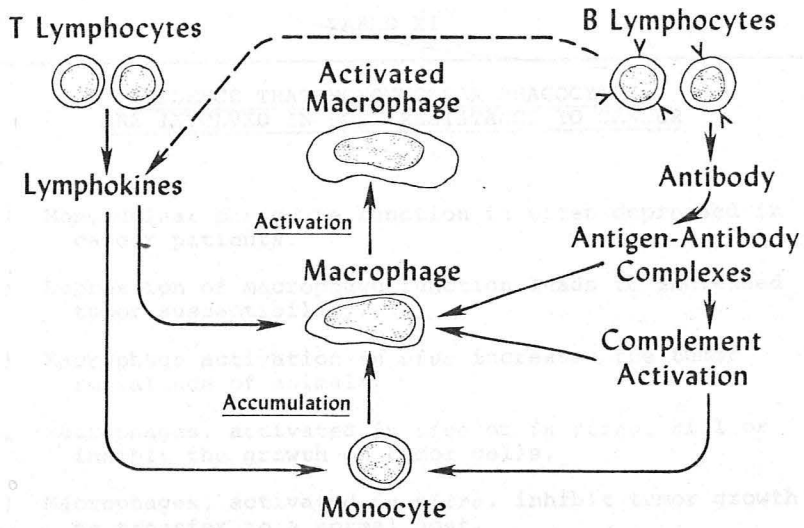
Macrophages have also been demonstrated to have the capability of modulating lymphocyte responsiveness in a number of *in vitro* situations. Thus, macrophages or their secreted products can inhibit lymphocyte responsiveness. For example, macrophages obtained from animals undergoing graft versus host reactions (269), or from animals injected with a number of adjuvants such as Corynebacterium parvum (270, 271), or from animals bearing certain tumors (272) have the capacity to suppress mitogen-induced T lymphocyte proliferation. Macrophages obtained from normal animals can also inhibit the *in vitro* primary antibody response of normal mouse spleen cells (175).

Finally, soluble immune response suppressor (SIRS), a factor secreted by T cells which inhibits antibody production, appears to act by inducing inhibitory capacity in a macrophage population which, in turn, suppresses humoral responsiveness (273). The suppressive capacity of macrophages often has been demonstrated to be mediated by factors secreted by these cells (182-185).

Excessive suppressor macrophage function has been suggested to be etiological in the depressed immune responsiveness found in two clinical situations. First, it has been suggested that the action of suppressor mononuclear phagocytes might explain the hypogammaglobulinemia (non-paraprotein) found in some patients with multiple myeloma (274, 275). Similarly, certain patients with recurrent fungal infections have been demonstrated to have monocytes with abnormal suppressor activity. It has been suggested that the action of such suppressor macrophages may be an important determinant of the depressed cell mediated immunity manifested by these patients (276, 277).

Macrophages, likewise, have been demonstrated to produce a number of factors which can augment or facilitate the responsiveness of various populations of lymphocytes in different experimental models (174). Thus, for example, macrophages or their secreted factors can maintain lymphocyte viability *in vitro* (175), augment the proliferative response of lymphocytes to various mitogens (177), facilitate the generation of appropriate helper T cells (180), or augment the differentiation of antigenically stimulated B cells into antibody secreting cells (178). In general, these factors have been characterized using *in vitro* correlates of immune responsiveness and their *in vivo* significance remains to be elucidated.

MACROPHAGE-LYMPHOCYTE INTERACTION:
MACROPHAGES AS EFFECTOR CELLS



Finally, mononuclear phagocytes play a critical role as effector cells in immune reactions. As indicated above, they can be directed to appropriate sites by the action of a number of chemotactic attractants, including products of activated T or B lymphocytes (59-65). Once concentrated at the inflammatory site, they can be localized to that site by the action of macrophage migration inhibitory factor and other lymphokines produced by activated T or B lymphocytes. Once localized, they have the potential to ingest and degrade foreign materials, to secrete a variety of enzymes capable of degrading cellular debris, and to kill microorganisms or tumor cells. Finally, they have the capacity to be "activated" by antigen-antibody complexes, complement factors, or products of stimulated lymphocytes resulting in a markedly enhanced capacity to act as nonspecific effector cells.

III. The Role of Mononuclear Phagocytes in Resistance to Tumors.

In recent years much interest has centered around the possibility that mononuclear phagocytes play an important role in host defense against the development and spread of neoplastic cells.

TABLE XI

EVIDENCE THAT MONONUCLEAR PHAGOCYTES ARE INVOLVED IN HOST RESISTANCE TO CANCER

1) Mononuclear phagocyte function is often depressed in cancer patients.

2) Depression of macrophage function leads to increased tumor susceptibility.

3) Macrophage activation *in vivo* increases the tumor resistance of animals.

4) Macrophages, activated *in vivo* or *in vitro*, kill or inhibit the growth of tumor cells.

5) Macrophages, activated *in vitro*, inhibit tumor growth on transfer to a normal host.

A relationship between tumor resistance and the function of the Mononuclear Phagocyte System was first noted by Stern in 1941 (278), who measured clearance of a vital dye in cancer patients. He was able to show that cancer patients were less able to clear the dye and also demonstrated that defects in clearance were correlated with widespread disease and poor response to therapy. Subsequently, a number of studies have indicated that other macrophage functions are deficient in some cancer patients, including chemotaxis (279), phagocytosis (280, 281), and bactericidal capacity (282). Moreover, it has recently been demonstrated that the presence of tumor cells in an animal causes the liberation of a small molecular weight factor which markedly inhibits the ability of the macrophages of that animal to resist infection with Listeria monocytogenes (283).

A number of studies have also suggested that animals with depressed macrophage function, induced for example by chemical agents, have a decreased ability to resist tumor cell growth (284).

A number of experimental models have been employed to explore the role of macrophages in tumor resistance. It was found that animals infected with microorganisms known to promote macrophage activation, such as Toxoplasma gondii, Besnoitia

jellisoni, BCG, or Listeria monocytogenes (285), either had a decreased incidence of spontenaous neoplasms or an enhanced ability to reject tumors compared to normal mice. It was subsequently shown that macrophages obtained from mice infected with Toxoplasma gondii inhibited the growth of syngeneic or allogeneic tumor cells *in vitro*, but had no effect on the growth of normal cells (286). Immunization of mice with complete Freund's adjuvant was also found to lead to the development of a population of activated macrophages which were nonspecifically tumoricidal *in vitro* (287). Normal macrophages can be induced to inhibit the growth of tumor cells by *in vitro* incubation with endotoxin, double stranded RNA (288), or secreted factors from sensitized thymus derived lymphocytes (289). Finally, macrophages activated *in vitro* have been shown to limit the growth of tumor cells *in vivo* when injected into a recipient animal, along with the tumor cells (290).

These experiments led to the idea that activated macrophages possessed a mechanism for distinguishing neoplastic from normal cells which was not immunologically specific and did not require pre-sensitization with tumor antigens. It was suggested that such a population of macrophages capable of selectively killing neoplastic cells might play an important role in tumor surveillance since they could eliminate neoplastic cells as they arose.

A number of studies have investigated the mechanism by which macrophages limit the growth of tumor cells. One mechanism of macrophage anti-tumor activity involves phagocytosis of tumor cells in the presence of specific opsonizing antibody (291). Another mechanism involves the induction of extracellular killing of tumor cells in the presence of specific antibody without phagocytosis (292, 293). In these two systems, killing of tumor cells is specific but the specificity is conferred by the antibody. In most models studied, inhibition of tumor cell growth is nonspecific in that a variety of tumor cell types are killed (188). The effector cell is an activated macrophage. Tumor cell growth inhibition occurs in the absence of antibody and is usually facilitated when direct contact between tumor cells and macrophages develops.

The mechanism by which activated macrophages kill or limit the growth of tumor cells is not clear. The macrophage may release cytotoxic factors (294, 295) or lysosomal enzymes.

One suggested mechanism by which tumor cells may be killed by activated macrophages involves the direct transfer of lysosomal enzymes from macrophages into the cytoplasm of the tumor cells during cell to cell contact (296).

Although there is considerable evidence to suggest that activated macrophages may play a role in host defense to neoplastic cell growth, there are a number of studies indicating the opposite. For example, several agents known to result in macrophage activation *in vivo* have been demonstrated to enhance rather than inhibit tumor cell growth (297).

IV. Role of Mononuclear Phagocytes in the Removal of Senescent Cells

Mononuclear phagocytes play a central role in the disposal of aging and dying cells, cell fragments and a variety of other soluble and particulate macromolecules. One example of this function is the removal of senescent erythrocytes from the circulation. A normal 70 Kg human possesses about 5×10^{13} erythrocytes, of which 3×10^{11} are removed from the circulation by mononuclear phagocytes each day. As a result, the Mononuclear Phagocyte System ingests and metabolizes about 2.7 Kg of hemoglobin per year (70).

Most of the removal of the senescent red cells is accomplished by the mononuclear phagocytes of the liver, bone marrow, and spleen (298,299). A number of possible mechanisms have been suggested to explain the ability of mononuclear phagocytes to distinguish normal from aged red cells. For example, some studies have suggested that the removal of aging red cells is related to the observed reduction in the net negative charge of their surface membranes (300), while others have suggested that the decreased deformability of aged red cells may increase their likelihood of being engulfed by mononuclear phagocytes in the sinusoids of the liver and spleen (301). This latter possibility may be important in explaining the decreased red cell survival in patients with diseases such as hereditary spherocytosis and sickle cell anemia.

The removal of aging red cells appears to involve an IgG-mediated mechanism. It has been shown that aging erythrocytes shed sialic acid from their surface membranes (302). Moreover, it has been demonstrated that removal of sialic acid from red blood cells results in the expression of antigens on their surface that react with naturally occurring antibodies present in normal human sera (303, 304). Finally, phagocytosis of aged human red blood cells by macrophages appears to require the participation of IgG molecules present in normal human serum (304). Therefore, the turnover of senescent red cells, like the clearance of infectious agents, may be regulated by Fc receptor-mediated phagocytosis.

After the red cell is internalized into the phagosomes of the macrophage, lysosomal fusion occurs and breakdown of the erythrocyte membrane and globin moeity of hemoglobulin is accomplished by the action of a number of lysosomal enzymes (123). The heme moiety is catabolized by macrophage heme oxygenase to biliverdin, which is promptly reduced to bilirubin by a separate enzyme, biliverdin reductase.

In macrophages heme oxygenase activity is normally minimal. However, a striking stimulation of enzyme activity is observed after macrophage exposure to erythrocytes, methemalbumin or hemoglobin (305, 306).

Mononuclear phagocytes are also involved in removing extravasated erythrocytes from other sites in the body, such as may occur locally in an ecchymosis.

Hemosiderosis refers to conditions characterized by the presence of increased quantities of iron hemosiderin within the macrophages of various organs. Hemosiderosis results from either increased breakdown of red cells in the body or excessive iron administration. Hemosiderin may accumulate in Kupffer cells, alveolar macrophages, or macrophages of spleen or other tissues depending on the site of breakdown of red cells. Hemosiderin accumulates in macrophages because these cells lack enzymes to digest this iron pigment (307). Hemosiderin-laden macrophages may be found in the liver, spleen and bone marrow as a result of prolonged parenteral iron administration or various chronic hemolytic anemias. Large numbers of hemosiderin-laden alveolar macrophages may be found in a variety of conditions associated with chronic extravasation of red cells into the alveoli, such as occurs with chronic congestive heart failure, pulmonary hemosiderosis, Goodpasture's syndrome and severe mitral stenosis.

In a number of pathologic conditions, removal of erythrocytes from the circulation is excessive. These situations may result either from increased macrophage activity in the presence of normal erythrocytes, or normal macrophage activity associated with abnormal erythrocytes, of either intra- or extracorpuscular origin.

A number of conditions characterized by splenomegaly, such as myeloproliferative diseases, lipid storage diseases, and tuberculosis may be associated with a shortened red cell survival. In a number of experimental models, induction of reactive hyperplasia of the mononuclear phagocytes of the liver and spleen leads to hemolytic anemia (308-311). In these situations, both healthy and senescent red cells are removed from the circulation in the liver and spleen as a result of the enhanced phagocytic activity associated with activation of the macrophages in these organs.

Hemolytic anemia is also associated with a number of hereditary intracorpuscular defects of erythrocytes, such as hereditary spherocytosis or sickle cell anemia in which red cells with abnormal deformability are removed with augmented efficiency (301).

Finally, hemolytic anemia may be associated with the abnormal production of anti-erythrocyte antibodies. The factors governing the removal of erythrocytes from the circulation in autoimmune hemolytic anemia has been investigated extensively in experimental animals and man by Frank, et al (312). They found that clearance of erythrocytes depended upon the nature of the immunoglobulin sensitizing the cells, the ability of the immunoglobulin to activate complement, and the state of activation of mononuclear phagocytes of the liver and spleen. Clearance was unlikely to result from nonspecific membrane damage owing to the activity of the antibody, but rather depended upon the opsonic properties of immunoglobulin and complement and the activity of the specific membrane receptors on the cells of the Mononuclear Phagocyte System.

MONONUCLEAR PHAGOCYTE DYSFUNCTION

Because mononuclear phagocytes play such an important role in host defense, factors which impair the function of these cells may well predispose the host to an increased risk of infection. A number of factors have been demonstrated to impair mononuclear phagocyte function, some of which have been listed in Table XII.

The alveolar macrophage is the cell primarily responsible for defending the lung against the effects of inhaled microorganism and other environmental pollutants (332). Impaired alveolar macrophage function may, therefore, render the host more susceptible to bacterial infection and possibly other pathological processes of the lung. For example, the finding that Sendai virus pneumonia of mice causes an impairment of the bactericidal capacity of the alveolar macrophages of these animals may explain the clinical observation that viral infections of the lung in humans often predispose to the development of bacterial pneumonias (315).

A number of common environmental and industrial pollutants have been demonstrated to impair the function of alveolar macrophages. An interesting example is the effect of silica on macrophage function. Silica is extremely toxic to macrophages causing their rapid death (322). The material is phagocytized by

TABLE XII

FACTORS WHICH MAY INTERFERE WITH THE FUNCTION OF CELLS OF THE MONONUCLEAR PHAGOCYTE SYSTEM

Factor	Species	Mononuclear Phagocyte Tested	Function Inhibited	Reference
I. Infectious Agents:				
a) Ectromelia, LCM hepatitis virus	mice	Kupffer cells	carbon clearance	313
b) LCM virus	mice	Splenic	lymphocyte accessory cell function	314
c) Sendai virus	mice	Alveolar	bactericidal capacity	315
II. Environmental Factors:				
a) Marijuana smoke	rats	Alveolar	bactericidal capacity	316
b) Tobacco smoke	rabbits, mice	Alveolar	bactericidal capacity	317, 318
	humans	Alveolar	response to MIF	319
c) Ozone	rats	Alveolar	bactericidal capacity	320
d) Nickel	rabbits	Alveolar	phagocytosis	321
e) Silica	mice	Peritoneal	all (kills cells)	322
III. Host Factors:				
a) Immune complexes	mice	Peritoneal	ingestion of IgG opsonized particles	323
b) Erythrophagocytosis	mice	Peritoneal	phagocytosis, bactericidal capacity	324
IV. Drugs:				
a) Corticosteroids	humans	blood monocytes	chemotaxis, bactericidal capacity	325, 326
b) Gold compounds	humans	blood monocytes	lymphocyte accessory cell function	327
V. Miscellaneous				
a) Uremia	humans	blood monocytes	phagocytosis	328, 329
b) Systemic lupus erythematosus	humans	blood monocytes	phagocytosis, glass adherence	330
c) Chronic granulomatous disease	humans	blood monocytes	bactericidal capacity	331
d) Various cancers, Mucocutaneous moniliasis, Wiskott-Aldrich Syndrome, Chediak-Higashi Syndrome	humans	blood monocytes	chemotaxis	279

macrophages and incorporated within phagolysosomes. The ingested silica lyses the lysosomal membrane causing the release of lysosomal enzymes into the cytoplasm of the cell which leads to its rapid death.

The dying macrophages have been demonstrated to release a material which has the capacity to stimulate fibroblasts to synthesize collagen (333). This may explain the pulmonary fibrosis characteristic of chronic silicosis.

The presence of silicosis has also been correlated with an increased incidence of tuberculosis (334). This may be related to the observation that exposure of cultured macrophages to sublethal concentrations of silica potentiates the growth of M. tuberculosis (335, 336). The effects of silica-induced macrophage dysfunction on overall host defense is also indicated by the observations that exposure to silica increases susceptibility to infection by a number of pathogenic viruses (337) and that silicosis is associated with an increased incidence of neoplastic disease (338).

While tobacco smoke directly depresses the bactericidal capacity of alveolar macrophages *in vitro* (317, 318), a number of studies have indicated that alveolar macrophages obtained from smokers manifest normal phagocytosis and bacterial killing (339, 340). Alveolar macrophages from smokers differ from normals, however, in that they do not respond appropriately to lymphokines (341). A recent report has also suggested that alveolar macrophages from cigarette smokers spontaneously secrete elastase, while alveolar macrophages from nonsmokers do not (341). This may be important in the pathogenesis of emphysema.

A number of observations have indicated that animals with hemolytic anemia are more susceptible to infection with Salmonella (342). This susceptibility may be explained by the observation that simultaneous exposure of mouse macrophages to opsonized erythrocytes and S. typhimurium led to an inhibition in the capacity of the macrophages to phagocytize and kill the microorganisms (324). This may be a factor in the increased rate of Salmonella infections in patients with sickle cell anemia.

Patients with systemic lupus erythematosus have an increased rate of infectious complications (343). Mononuclear phagocytes from patients with SLE may be deficient in their capacity to kill microorganisms (330). This depressed bactericidal capacity appears to be mediated by a serum factor found in these patients.

Patients with uremia likewise have an increased rate of infections. One explanation may involve a depression of mononuclear phagocyte function (328, 329), which is reversed with hemodialysis (329).

THE INVOLVEMENT OF MONONUCLEAR PHAGOCYTES IN OTHER DISEASES

The cells of the Mononuclear Phagocyte System are also subject to malignant transformation as indicated in Table XIII.

TABLE XIII

MACROPHAGE INVOLVEMENT IN OTHER DISEASES (69)

I. Neoplastic Processes:

a) Monocytic leukemia.
b) Malignant histiocytosis
   Hand-Schüller-Christian Disease,
   Letterer-Siwe Disease,
   Histiocytic Medullary Reticulosis
c) Hodgkin's Disease

II. Storage Diseases:

a) Non-digestible substances.
b) Enzyme deficiencies.

Of interest is the recent observation that the Reed-Sternberg cell, most characteristically found in Hodgkin's disease, is likely to be a malignant mononuclear phagocyte (344).

Mononuclear phagocytes also play a role in a number of storage disease or conditions in which materials accumulate within cells. Accumulation within these cells occurs when macrophages ingest materials which they cannot degrade. Two forms of storage diseases affect mononuclear phagocytes. The first is caused by ingestion of nondigestible substances, and includes conditions such as anthracosis or asbestosis (345). These diseases can also be characterized as chronic granulomatous inflammatory conditions since the presence of the nondigestible foreign material leads to the accumulation of mononuclear phagocytes, formation of giant cells and epithelioid cells and the development of granulomata. The location of the lesion is determined by the route of entry of the foreign material.

Thus, the development of pneumoconiosis results from the inhalation of nondigestible materials and their subsequent ingestion by alveolar macrophages, while post-operative talc or starch granulomata result from the exposure of peritoneal macrophages to these materials during surgery (346).

Storage disorders may result from increased cell turnover of various types. For example, hemosiderosis results from an excess of hemosiderin in the macrophages as indicated above.

Other storage disorders result from the accumulation of potentially degradable materials in the cells of individuals with an absence of specific digestive enzymes. Gaucher's disease, Niemann-Pick's disease, and Tay Sach's disease are lipidoses which result from genetic defects in digestive enzymes. Because of the deficiency of a lysosomal enzyme, undigested material accumulates in the lysosomes of cells. The location of lipid-laden macrophages in the lipidos s is dependent on the normal phagocytic function of tissue macrophages in different organs. For example, typical Gaucher cells resulting from the accumulation of incompletely digested membranes of ingested senescent erythrocytes and leukocytes are usually found in liver, spleen and bone marrow; whereas macrophages in other organs which do not usually ingest these cells are less effected (347).

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