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Answered and unanswered questions about macrophages

R VAN FURH

University Hospital, Department of Infectious Disease, Leiden, The Netherlands

Eli Metchnikoff, who was born in 1845 and died in 1916, was one of the first to write about phagocytes. He described the uptake of spores of a primitive fungus *(Monosporu bicuspidata)* by cells of the fresh-water flea *(Daphnia magna)* and later the uptake of a needle of a fern by a larva of a starfish *(Bipinnaria)* [1]. But Metchnikoff was not the first to observe phagocytosis. Ernst Haeckel had already described the uptake of Indigo dye by cells of *Thetis fimbria*, a small marine organism [2]. In the same period in which phagocytosis was described, Julius Cohnheim was the first to describe the migration of leucocytes from the circulation into tissues after injury [3]. His study done in the frog was the first to yield results comparable to those of modern research on inflammation, since Cohnheim described the role of chemotaxis in the supply of cells to a site of inflammation or infection. Another historical event concerns the description of opsonins, which are serum factors that promote the ingestion of bacteria by professional phagocytes, i.e., granulocytes, monocytes, and macrophages, by Almroth Wright and Stewart Douglas [4]. Today it is known that these opsonins are immunoglobulins (antibodies) and complement and in some respect fibronectin, that cover the bacteria and interact with specific binding sites on the surface of phagocytes. More recently, the mechanism by which opsonized bacteria are taken up by phagocytes, which is called the zipper mechanism, was described by Sam Silverstein [5].

In the present contribution I shall briefly review our studies on the origin and kinetics of macrophages as well as the functions of these cells and point out questions that remain to be answered.

Origin and Kinetics of mononuclear Phagocytes

Mononuclear phagocytes form a cell line that originates from the pluripotent stem cell in the bone marrow. The most immature cell of this line is the monoblast (Fig. 1). In the mouse, the cell cycle of the monoblast is about 12 hr, and after division one cell gives rise to two promonocytes. The promonocyte too divides only once (cell-cycle time

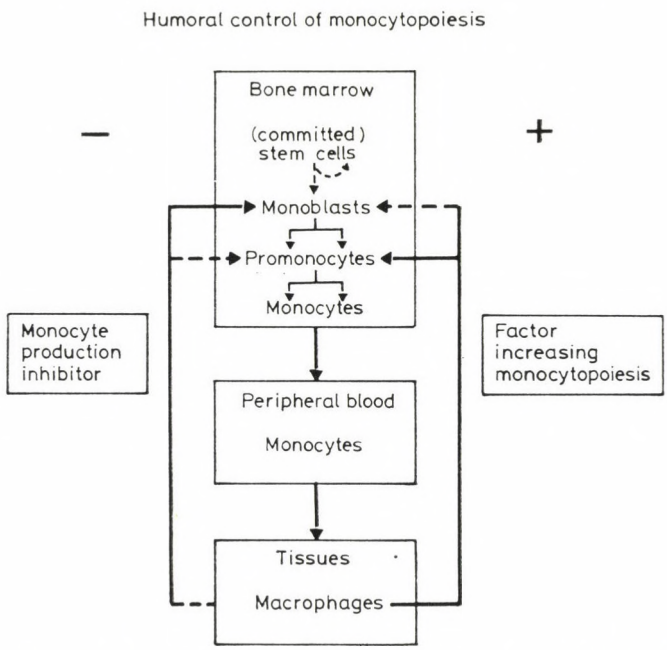

FIG. 1. Schematic representation of the origin and kinetics of mononuclear phagocytes and the humoral control (positive and negative feedback mechanisms) of the production of monocytes

about 16 hr) and gives rise to two monocytes (Fig. 1). Monocytes do not divide further, and leave the bone marrow randomly within 24 hr after they are formed. These cells remain relatively long in the circulation (mouse: half-time about 17 hr; man: half-time about 71 hr) compared with granulocytes (half-time about 7 hr), and leave this compartment randomly.

During the last twenty years, considerable attention has been given to the origin of the macrophages. The bone marrow origin of macrophages in the peritoneal cavity, liver, spleen, and lung, as well as of synovial type-A cells and osteoclasts, has been proven by a large number of studies [6]. Kinetic studies with in vivo-labeled monocytes done in normal, in monocytopenic and in irradiated mice have provided proof that the monocytes migrate from the blood into the tissues, where they become macrophages (Fig. 1) [7–10]. In vitro labeling studies with the DNA precursor ^{3}H-thymidine have shown that 5% or less of macrophages at various sites synthesize DNA. Not much is known about the characteristics of the mononuclear phagocytes that divide in the tissues and body cavities. These cells are not resident macrophages but have very recently, less than 24 to 48 hr before harvesting, arrived in the tissues from the bone marrow. The current view is that in the normal steady state the maintenance of the population of macrophages in a tissue compartment de-

pends on the influx of monocytes from the circulation and on local division of mononuclear phagocytes that also derive from the bone marrow and divide once in the tissues. Calculations have shown that on average 75% of the macrophage population is supplied by the influx of monocytes and 25% by local division of (immature) mononuclear phagocytes. Based on these data, in the mouse the calculated mean turnover time of macrophages in the tissues lie between 7 and 14 days, which is much shorter than the turnover time previously reported.

In the normal steady state the combination of a constant influx of monocytes into tissues, where they become macrophages, and a constant local production, implies a constant cell death in the tissues and/or a constant efflux of cells from the tissue compartments. Almost nothing is known about this point. It is known that lung macrophages leave the body via air spaces, and there is evidence that macrophages migrate to the local lymph nodes and perhaps die at that site.

Another important point which is often neglected in the study of experimental or pathological lesions in which macrophages are involved is whether these cells accumulate or proliferate in the tissues. These terms are often used erroneously: proliferation should be reserved for cases in which the increase in the number of cells is known to be due to the division of cells already present at, or recruited to, a site and the term accumulation for increases due to the migration of (non-dividing) cells from other sites (e.g., the circulation).

Regulation of monocyte production

As soon as tissue injury occurs, the mechanisms by which injurious agents are eliminated start to operate. In general, granulocytes appear first at the site of the lesion, and after a short delay the number of exudate macrophages in the inflammatory exudate increases. During various kinds of inflammation the number of circulating monocytes increases as well. When a rather large number of macrophages is required at the site of inflammation, a regulatory mechanism is needed to augment the rate of division of the monocyte precursors in the bone marrow.

Investigations have shown that plasma and sera collected during the onset of an inflammatory reaction contain a factor, called factor increasing monocytopoiesis (FIM), that stimulates monocytopoiesis [11–14]. This factor is synthesized and secreted by macrophages at the site of inflammation and then transported via the circulation to the bone marrow where it exerts its stimulatory action (Fig. 1). FIM is a small protein with a molecular mass of about 20 K dalton, is cell line specific, is not species specific, is no complement or clotting factor, has no chemotactic activity, and is not CSF-M or IL-1. The mole-

cular structure of FIM is unknown as yet.

During the second phase of an inflammatory response the circulation contains a serum factor that inhibits monocytopoiesis (Fig. 1). This factor, monocyte production inhibitor (MPI) [15], has a molecular mass of approximately 250 K dalton; the site of its production has not yet been established. Although FIM and MPI are not detectable under steady-state conditions with the available assay methods, it is conceivable that they regulate monocytopoiesis under steady-state conditions as well.

Mononuclear phagocyte system

On the basis of the data then available, the concept of the Mononuclear Phagocyte System (MPS) [16] was put forward in 1969. Later research confirmed the assumptions underlying this concept, and supplementary evidence was also obtained. The cells assigned to the mononuclear phagocyte system at present are shown in Table I.

Several types of cell (i.e., dendritic cells, interdigitating cells in lymphoid tissues, follicular and germinal-centre dendritic cells, epidermal Langerhans cells, and veiled cells in lymph) all differ from monocytes and macrophages in a number of morphologic and functional characteristics and, furthermore, their origin has not yet been definitely established [17, 18]. Consequently, these cells have not yet been assigned definitely to the MPS, even though some of them (e.g., the interdigiting cell, the Langerhans cell, and the veiled cells) are good candidates.

Table I

Cells belonging to the mononuclear phagocyte system (MPS)

Bone marrow	*Tissues*	*Body acvities*
Monoblasts	Macrophages occurring in:	Pleural macrophages
Promonocytes	connective tissue (histiocytes)	Peritoneal macrophages
Monocytes	skin (histiocytes; Langerhans cells?)	
	liver (Kupffer cells)	
		Inflammation
Blood	spleen (red pulp macrophages)	Exudate macrophages
Monocytes	lymph nodes (free and fixed macrophages; interdigitating cells?)	Epithelioid cells
		Multinucleated giant cells
	thymus	
	bone marrow (resident macrophages)	
	bone (osteoclasts)	
	synovia (type A cell)	
	lung (alveolar and tissue macrophages)	
	mucosa-associated lymphoid tissues	
	gastro-intestinal tract	
	genito-urinary tract	
	endocrine organs	
	central nervous system (macrophages, (reactive) microglia, CSF macrophages)	

Opsonization of particles

Research done in the last few decades has revealed that opsonization is achieved by the coating of particles (e.g., micro-organisms) with specific antibodies of the IgG class, in particular the subclasses IgG1 and IgG3, with or without complement, or by IgM antibodies together with complement. The $(Fab)_2$ sites of the IgG molecules bind to the particle, thus exposing their Fc parts to the surface to the phagocytes. Since phagocytes have membrane receptors for the Fc part of IgG, they can recognize and bind IgG-coated particles. Among the other immunoglobulins, IgA has been reported to have some opsonic activity too [19–21], albeit much less than IgG. IgD and IgE, as far as is known at present, are not involved in the opsonic process despite the presence of IgE receptors on mononuclear phagocytes [22]. IgM itself has no opsonic capacity, but the binding of IgM antibodies to particles promotes activation of the complement systems, which leads to the deposition of C3b on the particles and thus yields micro-organisms coated with IgM + C3b. IgG-coated bacteria too can activate complement, which leads to IgG + C3b--coated bacteria. Deposition of C3b on micro-organisms can also occur in the absence of immunoglobulins. This occurs via the alternative pathway of complement activation, which is initiated by structures on the cell walls of micro-organisms [23, 24]. Particles like zymosan can also be coated by complement synthesized and secreted by macrophages [25]. Since phagocytes have C3b receptors, C3b-opsonized particles can attach to the membrane of these cells and be ingested [26, 27].

Although the recognition of particles coated with IgG and/or C3b via their Fcγ and C3b receptors is the main mechanism underlying the ingestion of foreign material (e.g. micro--organisms), which is called immune phagocytosis [28–31], attachment of particles can also be promoted by other serum factors, such as fibronectin [32–35]. Fibronectin enhances the ingestion mediated via C3b and/or Fcγ receptors [36]. Non-immune phagocytosis is less efficient than immune phagocytosis and refers to the ingestion of non-opsonized particles [37], particles opsonized by nonspecific proteins [32–34], particles with a lectin-like structure [38], and particles with a modified surface membrane [39]. This type of ingestion occurs via nonspecific binding sites on the phagocyte membrane.

Mechanisms of phagocytosis

When a micro-organism has been recognized and has become attached to the phagocytes, ingestion can occur. Recently, the mechanism underlying the ingestion of particles by macrophages has been studied in detail (29, 30, 40) (Fig. 2). These authors showed that one singlepoint receptor--ligand interaction between particle and phagocyte is not enough to trig-

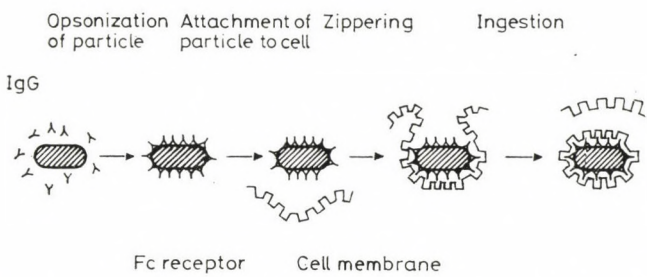

FIG. 2. Postulated mechanism for the ingestion of IgG-coated micro-organisms (////////) by a zipper phenomenon. Adapted from Griffin et al (29)

ger complete ingestion of the particle; for that, the phagocyte membrane must surround the particle in a zipper-like way by continous receptor-opsonin interactions. When the tips of the pseudopodia of a phagocyte surrounding a particle make contact with each other, the micro-organism is lodged in a phagosome. The membrane of this vacuole then fuses with lysosomes, which gives rise to a phagolysosome.

INTRACELLULAR KILLING OF BACTERIA

Serendipity led to the observation that when monocytes with ingested bacteria were incubated in the presence of medium without serum, no intracellular killing occurred. This indicated that serum proteins stimulate intracellular killing [41]. The assay used to measure the intracellular killing of micro-organisms in vitro includes the removal of non-ingested bacteria by repeated washes followed by incubation of cells containing phagocytosed bacteria for 60 or 120 min at 37 °C before determination of the number of viable intracellular micro-organisms with a microbiological method [42].

Analysis of the observation concerning serum in relation to intracellular killing showed that in the presence of IgG monocytes kill about 40–50% of the ingested bacteria and in the presence of complement components C3, C3b, and B/Bb about 30–40%, but when both IgG and complement are present 85% of the ingested bacteria are killed within 120 min. Blocking and other experiments proved that the Fcγ part of IgG interacts with its specific receptor in the cell membrane, and that C3/C3b interacts with its membrane receptor as well [41, 43]. It is of interest that for monocytes the membrane stimulation necessary for optimal intracellular killing can also be activated by a non-physiological stimulus, e.g., concanavalin A, for which binding sites, i.e., the mannose residues, are present in the cell membrane [44].

Catalase-positive and catalase-negative bacteria differ as to the degree of intracellular killing in the absence of extracellular IgG [45]. Ingested catalase-positive bacteria are not or virtually not killed in the absence of

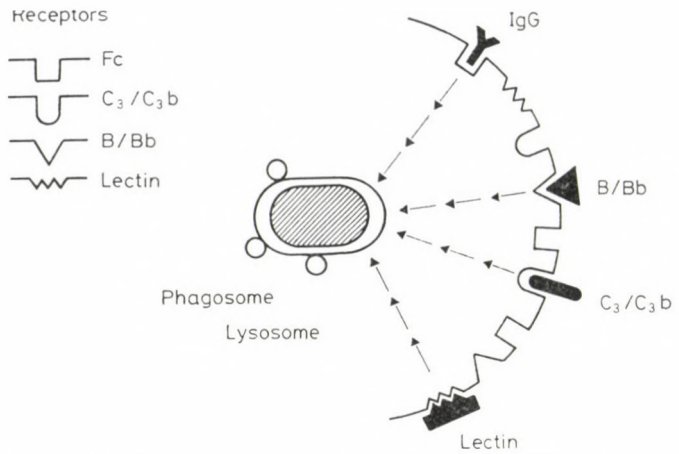

FIG. 3. Schematic representation of the stimulation of the intracellular killing by extracellular stimuli via an interaction with their respective membrane receptors. For granulocytes this stimulation was found in the presence of IgG and C3b; for monocytes in the presence of IgG, C3/C3b, B/Bb and lectins

serum whereas catalase-negative bacteria are killed rather effectively under these conditions.

In sum, these studies have shown that optimal intracellular killing of bacteria ingested by granulocytes and monocytes/macrophages requires continous stimulation of these cells by extracellular serum factors (IgG, C3/C3b, and B/Bb) interacting with their specific membrane receptors [41, 43, 45, 46] (Fig. 3).

Macrophage Activation

The phenomenon of cellular immunity has been known for more than eighty years, and is best illustrated by the experiments of Robert Koch in 1882 [47]. When normal guinea pigs are inoculated subcutaneously with a large number of live tubercle bacilli, the wound first closes and seemingly heals, but after about 10 to 14 days extensive ulceration develops and persists until the animal dies. When a small number of tubercle bacilli are injected intracutaneously the animal's defences can cope with these micro-organisms and it will survive because a state of immunity has developed. When such an animal has recovered and is re-infected with a large number of tubercle bacilli, it will not die. This increased resistance to infection with tubercle bacilli, based on an altered immunity, can be demonstrated with the tuberculin test. When a small amount of an extract of tubercle bacilli (called tuberculin) is applied to the skin, a local inflammatory reaction becomes manifest in two to three days. Histologically, the reaction is characterized by an infiltrate containing lymphocytes and macrophages. This type of response is called a delayed hypersensitivity reaction, because two to three

days elapse before the reaction reaches a maximum.

In 1945, Merril Chase [48] was the first to demonstrate that the cellular immunity to tubercle bacilli and the delayed hypersensitivity reaction expressing the altered state of cellular immunity can be transferred from immune guinea pigs to normal animals by lymphocytes but not by serum. Studies by Mackeness [49, 50] and Blanden et al. [51] showed that live L. monocytogenes and BCG activate macrophages that express enhanced microbicidal activity against various micro-organisms. This state of acquired immunity is called cellular or cell-mediated immunity. Recent research has shown that lymphokines produced and secreted by T4 (helper) lymphocytes are responsible for the activation of macrophages [52 – 55].

In general, it has been assumed that activated macrophages express enhanced microbicidal activity against all types of micro-organism irrespective of the kind of stimulus. Recent research has shown that activated macrophages exert their increased microbicidal activity only toward certain types of micro-organism depending on the kind of stimulus.

Activation of macrophages occurs during an infection with BCG or L. monocytogenes. These activated macrophages fulfill two generally accepted criteria for macrophage activation [56], i.e., inhibition of the intracellular replication of Toxoplasma gondii and enhanced oxidative metabolism as reflected by the increased oxygen consumption and H_2O release after stimulation of the cells with PMA [57]. The rate of intracellular killing of L. monocytogenes by murine macrophages activated by BCG and PPD was greater by a factor of about 1.7 – 2.0 than that of the resident macrophages [57]. The results of in vivo studies, i.e., a decrease in the number of intravenously-injected L. monocytogenes in the liver and spleen of BCG-infected mice are consistent with the in vitro results [57]. BCG-PPD-activated macrophages did not kill S. typhimurium more rapidly than normal resident macrophages [57] and the intracellular killing of S. aureus and E. coli by activated macrophages was also not more efficient than that by resident macrophages [57]. Thus, activation of macrophages by live bacteria is a selective process with regard to their bactericidal effector functions.

Interferon-γ is assumed to be the most important component of the T lymphocyte-derived lymphokines required for the induction of enhanced oxidative and toxoplasmastatic activity in macrophages [58 – 60]. However, resident macrophages activated with interferon-γ either in vivo or in vitro did not kill ingested Listeria monocytogenes [61] and S. typhimurium (to be published) at a higher rate than normal resident macrophages. A finding which was recently confirmed [62]. Also the growth of L. monocytogenes and S. typhimurium in the spleen and liver of mice treated with interferon-γ was

not retarded [61]. From these results it became evident that interferon-γ, although adequate for the induction of some manifestations of macrophage activation, did not enable activated macrophages to kill bacteria more rapidly.

In sum, recent studies have shown that the interactions between activated macrophages and micro-organisms are dependent on the kind of activation and the type of micro-organism.

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