Mechanisms, Measurement, and Significance of Lung Macrophage Function.

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Macrophages exist throughout the body. They have critical roles in the peritoneal cavity, bone marrow, skin, spleen, liver, and elsewhere. Their migratory patterns, phagocytic behavior, immunologic roles, and secretory potential are pivotal to both defense mechanisms and to the pathogenesis of disease. Macrophages have been implicated recently in such diverse disease processes as arthritis, AIDS, and juvenile onset diabetes. It is important to recognize the existence of other lung macrophages besides alveolar macrophages. Macrophages exist in small and large airways above and below the mucus. They may release chemotactic factors and a variety of mediators. They ingest and degrade antigens and are microbicidal. Interstitial macrophages are in direct contact with the extracellular matrix as well as other cells in pulmonary connective tissue such as fibroblasts. Thus, release of mediators or enzymes by interstitial macrophages can have a profound effect. Pulmonary intravascular macrophages are resident cells within the pulmonary capillaries of some species. They avidly remove particles and pathogens from circulating blood and secrete inflammatory mediators. Finally, pleural macrophages are involved in the fate and consequences of inhaled particles, especially fibers. A key attribute of macrophages is motility. Movement is an essential step in phagocytosis. There can be no particle binding or ingestion unless macrophage-particle contact occurs. To what extent and by what mechanisms do alveolar macrophages move on the alveolar epithelium? We have used optical methods as well as magnetometry to describe macrophage motility. Lung macrophages express an array of contractile proteins that are responsible for spreading, migration, phagocytosis, and the controlled intracellular motions of phagosomes and lysosomes. We have used magnetometric probing of cytoplasmic motion and rheology for both *in vivo* studies of macrophage function in animals and humans as well as in *in vitro* studies of macrophages. The cytoskeleton of macrophages and resulting motile events are key in understanding the role of macrophages in relation to particle phagocytosis, translocation, and solubilization.

Introduction

Resident macrophages are central in defending the lungs against the assaults of particles and pathogens in inspired air. Particles are not only ingested but undergo gradual dissolution within the phagolysosomes of macrophages (*1*). The phagocytic and microbicidal potential of macrophages is one of the major reasons why the lungs remain clean and sterile. Macrophages may also prevent allergy by ingesting and catabolizing inhaled foreign proteins. Alternatively, during some lung infections macrophages may preserve and present antigens to lymphocytes and act cooperatively with other components of the immune system to enhance the immune response. At other times lung macrophages recognize and destroy neoplastic cells, thus preventing the development of cancer. Alveolar macrophages may also ingest effete type 1 and type 2 epithelial cells, red blood cells, and perhaps even some of the "worn out" surfactant (*2*).

Role of Macrophages

Regulatory Role

During the last several decades, a mountain of evidence indicates that macrophages have roles that extend far beyond phagocytosis. They are secretory and regulatory cells. They can initiate and prolong inflammatory responses; they can stimulate the synthesis of extracellular matrix proteins. Thus, macrophages both respond to their microenvironment and control the activities of other cells such as neutrophils, lymphocytes, and fibroblasts. Macrophages can secrete such diverse substances as lysosomal enzymes, interferon, components of complement, angiogenesis factor, plasminogen activator, cyclic nucleotides, leukotrienes, prostaglandins, inflammatory cytokines, and granulopoietins. Still other macrophage products may interact with complex systems such as those controlling clotting, fibrogenesis, fibrinolysis, as well as those regulating kinin and complement fragment generation.

Failure of Macrophage Function

Some of the activities of macrophages reflect protective postures that help prevent lung disease, but at other times macrophages may be involved in processes leading to lung damage (*3*). In many instances, their defensive role can be compromised. Many investigators have shown that such diverse agents as viruses, silica, immunosuppressives, ethanol intoxication, cigarette smoke, air pollution, hypoxia, and hyperoxia can depress the ability of pulmonary macrophages to protect their host. Sometimes the agent or factor acts directly to kill or damage

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the macrophage. In other instances, particularly those situations involving pulmonary edema or altered acid–base balance, the macrophages themselves may be undamaged, but their activity may be indirectly depressed because of changes in their milieu, the pulmonary microenvironment. Recently, macrophages have been implicated in the transmission and pathology of the human immunodeficiency virus (HIV-1). Monocytes and macrophages can be persistently infected with HIV over long periods of time despite host-cell immune responses (*4–5*). Such infected cells can produce virus and also exhibit reduced function, thus leading to some of the pathology characteristic of AIDS (*6*).

Pathogenic Role

There are also situations in which pulmonary macrophages not only fail but are themselves implicated in the pathogenesis of pulmonary diseases. For example, the ingestion of particles (e.g., cigarette smoke), microbes, or endotoxin causes the release of lysosomal enzymes and oxygen radicals into the macrophage cytoplasm or the external environment. These substances may damage surrounding cells or other macrophages; then dead or dying macrophages release substances that can attract fibroblasts and elicit fibrogenic responses. This extracellular release of proteases and oxygen radicals can also alter the extracellular matrix or the activity of a variety of enzymes. When smokes or other particles act to recruit more cells, to activate them, and to release proteolytic enzymes and oxygen radicals, then macrophages may be centrally involved in the development of lung disease. The same inhaled toxins may also elicit similar responses from other white blood cells such as polymorphonuclear leukocytes. Thus, even though macrophages defend the lungs, they can also injure the host while exercising their defensive role.

Types of Lung Macrophages

In the past, lung macrophages were usually exclusively equated with alveolar macrophages. The terms should not be used interchangeably because macrophages exist not only in alveolar ducts and spaces, but also in other anatomic locations in the lungs (*7*). They are present in airways (*8,9*), connective tissue (*10,11*), the pleural space (*12,13*), and even in pulmonary capillaries. I now briefly review these different types of lung macrophages.

Airway Macrophages

Alveolar macrophages are frequently reviewed (*2*) and well studied, in part because they are readily accessible by bronchoalveolar lavage (BAL). Some workers assume that all macrophages recovered by BAL are alveolar macrophages. Nevertheless, airway macrophages are present in both large and small conducting airways, and many are recovered during routine lavage. Similarly, when lungs are fixed via the airways, most airway macrophages are displaced (*8*). If, however, lungs are more carefully fixed by submersion, intravascular fixation, or by vapor inhalation, many macrophages can be seen in the bronchial tree. Some are visible as cells suspended in the mucous blanket; they are presumably being transported to the pharynx where they will be swallowed. However, macrophages can also be seen beneath the mucous and serous layers where they are adherent to the bronchial epithelium and may reside for longer periods of time (*9*).

If macrophages exist in airways, what might they be doing there? Macrophages in large and small airways may release mediators that attract lymphocytes, neutrophils, or mast cells into the airways and regulate their activity there. By release of proteolytic enzymes and oxygen radicals, macrophages and other leukocytes may modify the barrier properties of the airway epithelium. Airway macrophages may ingest and degrade antigens deposited in airways, thereby suppressing the antigenicity of foreign proteins, or they may retain selected antigens for presentation to other parts of the immune system.

Macrophages may have an important role in regard to killing pathogens deposited in airways. A number of investigators, beginning with Laurenzi and colleagues (*14*), demonstrated experimentally that inhaled bacteria that deposit in the lungs quickly lose their ability to form colonies. Although this disappearance of colony-forming units has been attributed to alveolar macrophages, little is known about the precise deposition patterns of inhaled bacteria. It is likely that airway macrophages may be involved in killing bacteria deposited in small and large airways. Too frequently, we have arbitrarily described mucociliary transport and macrophages as two distinct systems. We have assumed that mucociliary transport operates only in airways and macrophages operate only in alveoli. It seems likely that the two systems overlap and work cooperatively. Alveolar–bronchiolar transport needs to be explored, and the ways in which macrophages and mucociliary transport mechanisms work together in the airways, particularly peripheral airways, should be better defined.

Pleural Macrophages

One of the macrophages least studied in the lungs is the pleural macrophage. Zlotnik et al. (*12*) compared pleural macrophages in mice to macrophages recovered by BAL and from the peritoneum. They concluded that pleural macrophages were more similar to peritoneal macrophages than to alveolar macrophages. In part, this may reflect the lower Po_2 values in the peritoneal and pleural cavities compared to the alveolar microenvironment. Ackerman et al. (*13*) showed that carrageenan can cause dramatic increases in the numbers of pleural macrophages. The role of pleural macrophages in health and disease is largely unknown. Agostoni (*15*) even suggested that these cells serve as tiny "roller bearings" that facilitate movements of the parietal and visceral pleura. The paucity of studies on pleural macrophages is documented by the absence of the word "macrophage" in the index of a book entitled *The Pleura in Health and Disease* (*16*).

Connective Tissue Macrophages

Substantial numbers of macrophages also exist in the connective tissue of the lungs, and several investigators have begun to isolate and characterize these cells (*10,17*). Morphometric studies show that the number of macrophages within the interstitium of normal and injured lungs approximates or exceeds the number of alveolar macrophages (*18–20*). It is noteworthy that increased numbers of lung interstitial macrophages appear within the active lesions of injured lungs. Antigenic differences

between interstitial and alveolar macrophage populations have been observed in hamsters (*21*), rats (*22*), and humans (*23*).

Importantly, it is interstitial macrophages, not alveolar macrophages, that are in direct contact with matrix and other cells in pulmonary connective tissue. Release of mediators or enzymes by these macrophages may have a greater effect than those released by their sister cells in the alveolar space. For example, elastase secreted by an interstitial macrophage directly onto an elastin fiber may be much more damaging than elastase released into the alveolar space. Investigators have begun to characterize the functional capacities of these cells [e.g., superoxide anion production (*24*)], but more information about the ability of interstitial macrophages to secrete proteolytic enzymes or inflammatory mediators is needed. We also need to characterize their proliferation and differentiation. *In vitro* analyses of interstitial macrophages must involve enzymatic or mechanical disruption of lung tissue followed by purification steps. It is important to consider the likely presence of residual alveolar macrophages in such preparations. One approach is to use antigenic distinctions between alveolar and interstitial macrophages and flow cytometry to identify and isolate purified interstitial macrophages (*17*). Another type of connective tissue macrophage is found in lymph nodes where macrophages are frequently in close proximity to mast cells and lymphocytes.

Pulmonary Intravascular Macrophages

Since 1984, our laboratory has published a series of papers demonstrating that abundant resident macrophages within pulmonary capillaries of sheep, calves, goats, and cats avidly remove particles and pathogens from circulating blood (*25,26*). A recent review (*27*) summarizes the cell biology and pathogenic role of pulmonary intravascular macrophages (PIMs). Morphometry demonstrated that normal sheep had more macrophages in pulmonary blood vessels than in their alveolar spaces (*28*). Pulmonary intravascular macrophages are large (20–80 μm diameter), mature macrophages that are bound to the endothelium of pulmonary capillaries. PIMs have morphologic features characteristic of differentiated macrophages including an indented nucleus, lysosomal granules, pseudopods, phagosomes and phagolysosomes, tubular micropinocytosis vermiformis structures, and a fuzzy glycocalyx (Fig. 1*A*). These ultrastructural features, especially the phagocytic vacuoles and micropinocytosis vermiformis, indicate the well- differentiated state of PIMs. They are not simply adherent monocytes. Phagosomes are a prominent feature in PIM cytoplasm, suggesting an active role for these cells in surveillance of the circulation. Thus, PIMs are a member of that portion of the mononuclear phagocyte system (MPS) with access to the circulating blood. In a number of species, we have seen erythrophagocytosis by PIMs.

PIMs form membrane-adhesive complexes with underlying endothelial cells (Fig. 1*A*). These adhesions have an intercellular separation of 12–15 nm, and electron-dense material is present both in the intercellular space and subjacent to the plasma membrane of both cells. A significant number of PIMs with easily demonstrated phagocytic function have been found in a number of species, including calves, sheep, pigs, goats, and cats (*25,28,29*). Figure 1*B* shows a macrophage in an alveolus of a sheep so that pulmonary intravascular and alveolar macrophages can be compared.

Physiologic and Pathophysiologic Role of PIMs

PIMs actively ingest particles such as iron oxide and gold colloid from the circulating blood (*26,28*). Importantly, when such pathogenic agents as gram-negative bacteria and endotoxin are sequestered in the lungs, the subsequent inflammatory response is also localized there. We have found pulmonary inflammatory changes, including neutrophil recruitment, intravascular fibrin deposition and endothelial cell injury, as early as 1 hr following localization of bacteria or endotoxin in the lungs (*30,31*). Figure 2*A* shows the appearance of sheep capillaries after intravenous injection of *Pseudomonas aeruginosa*. Figure 2*B* demonstrates that the cell responsible for pulmonary uptake of the circulating bacteria is the PIM.

We believe that rapid ingestion of pathogenic materials leads to secretion of inflammatory mediators from pulmonary intravascular macrophages. These mediators may include such cytokines as tumor necrosis factor, interleukin-1, platelet-activating factor, and a range of substances that may recruit and activate neutrophils. Oxygen radicals and proteolytic enzymes from activated macrophages and from neutrophils and platelets recruited there by PIMs may then cause local tissue injury. Thus, these macrophages may be central to the chain of events leading to altered ventilation and perfusion, and finally to respiratory distress.

New Methods for Studying Lung Macrophages

This is an exciting time to be studying macrophages. New tools are becoming available to supplement classic approaches such as ultrastructure, biochemistry, and *in vitro* cell culture. Not only do we have an extensive repertoire of bioassays and immunologic assays for studying macrophage mediators, but also the tools of molecular biology such as the polymerase chain reaction now allow us to measure very small quantities of the RNA message responsible for the synthesis of these mediators. Moreover, *in situ* hybridization can be used to identify the cellular anatomic sites of mediator synthesis and to compare them to the distribution of disease within the lung. Flow cytometry is emerging as a valuable tool to study phagocytosis by macrophages and its associated oxidative burst (*32*). Interestingly, Kobzik et al. (*33*) have shown that the extent of the oxidative burst elicited by particle ingestion depends on whether opsonins are present. When opsonins are present, the generation of potentially toxic oxygen metabolites increases with increasing particle ingestion. However, during opsonin-independent phagocytosis (perhaps characteristic of the fate of some dusts in the lungs) there is a downregulation of the alveolar macrophage oxidative response.

Magnetometric Methods for Measuring Macrophage Motility

Magnetic particles and sensitive magnetometers also serve as a new tool in cell biology (*34,35*). Iron oxide particles can be in-

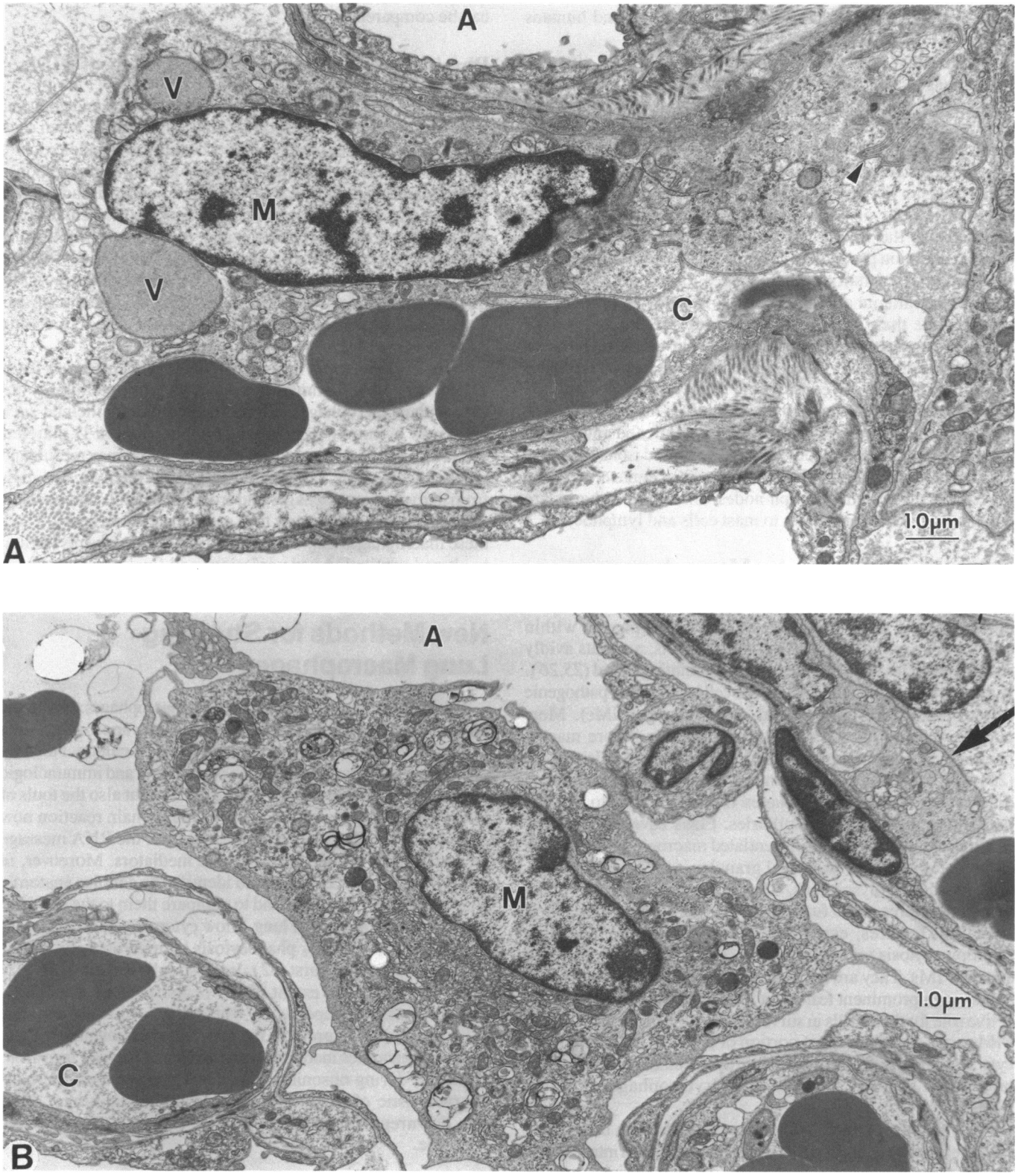

FIGURE 1. Comparison of sheep pulmonary intravascular and alveolar macrophages. (*A*) Pulmonary intravascular macrophage (M) within the capillary lumen (C) in sheep lung. The macrophage is closely apposed to the underlying capillary endothelium and has two prominent phagocytic vacuoles (V) and tubular membrane invaginations of characteristic *Micropinocytosis vermiformis* (arrowhead); alveolar space (A). (*B*) Sheep alveolar macrophage (M) within the alveolar space (A); capillary lumen (C). A portion of an intravascular macrophage (arrow) is seen within an adjacent capillary lumen in the upper right. Reprinted by permission from *Laboratory Investigation* (*28*).

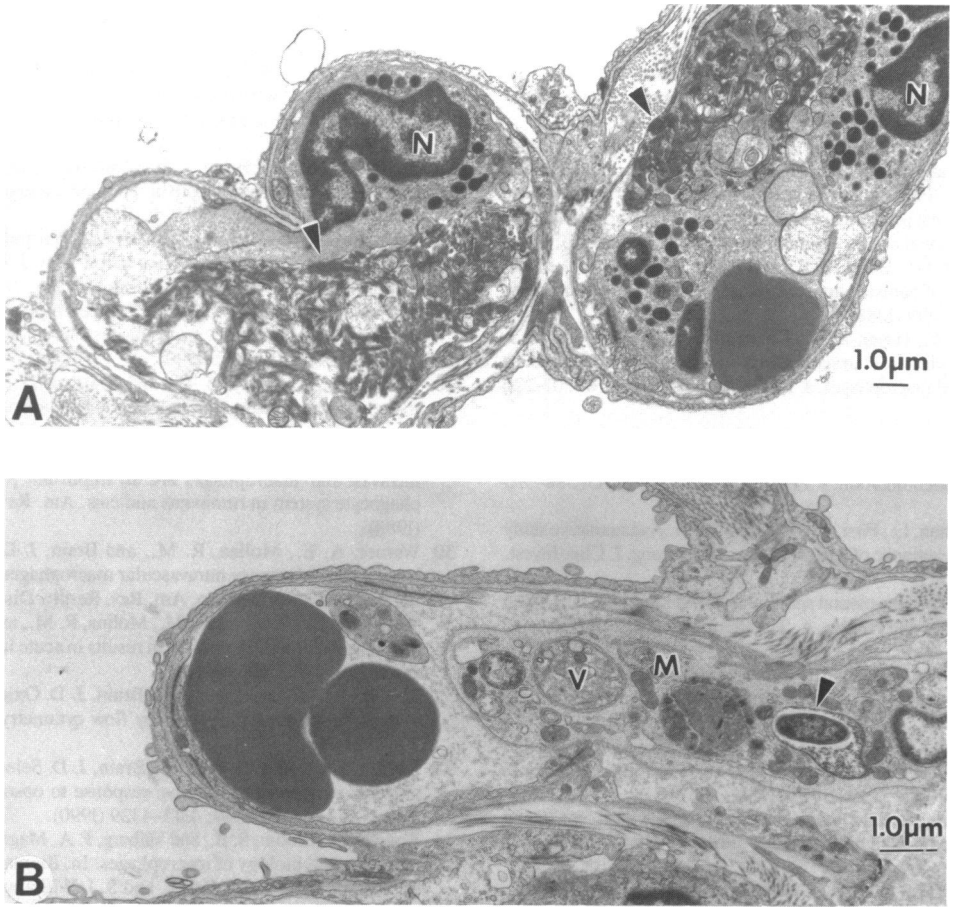

FIGURE 2. Pulmonary capillaries from sheep lungs 1 hr after intravascular injection of *Pseudomonas aeruginosa*; epon-embedded, uranyl acetate and lead citrate stains. (*A*) Ultrastructure of congested capillaries showing neutrophil (N) and fibrin (arrowhead) accumulation. (*B*) Pulmonary intravascular macrophage (M) showing phagocytic vacuole (V) and as ingested bacterial cell (arrowhead). Reprinted by permission from *American Review of Respiratory Disease* (*30*).

troduced into the lungs by inhalation or intratracheal instillation of magnetite or gamma hematite. They are then ingested by lung macrophages (*36*). In species lacking PIMs, such as the rat, these particles are ingested by hepatic and splenic macrophages after intravenous injection (*37*). However, when species with abundant PIMs are studied, these lung cells can be readily labeled by intravenous injection of magnetic dust.

Magnetic particles are easily recognized in living or fixed cells studied by light microscopy. They are also easily visualized by electron microscopy because of their electron density. Because the particles are magnetic, the motion of particle-containing organelles (primarily phagosomes and phagolysosomes) can be either measured or manipulated externally (*34,35*). Magnetic fields from particles ingested by mononuclear phagocytes in the lungs can be measured with fluxgate or with SQUID (superconducting quantum interference device) magnetometers. We have developed methods for using these magnetic particles to monitor the progression of phagocytosis, to characterize organelle motion, and measure cytoplasmic viscosity in normal and compromised cells.

Conclusion

Macrophages and other phagocytic cells occupy a central role in the pathogenesis of lung injury. These cells prevent infection and are involved in wound healing, but they also contribute to lung disease when activated and/or damaged. Evidence suggests that phagocytic cells in lung capillaries have a key role in the response to bacteremia or septicemia. Continuing development of new methods will inevitably lead to additional insights about how macrophages are involved in lung injury and respiratory failure.

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