Macrophage Damage in Relation to the Pathogenesis of Lung Diseases

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Pulmonary macrophages are important since their migratory patterns and behavior are often pivotal events in the pathogenesis of pulmonary disease. Alveolar macrophages act to decrease the probability of particle penetration through epithelial barriers, and their phagocytic and lytic potentials provide most of the known bactericidal properties of the lungs. Macrophages are also involved in immune responses and in defense against neoplasms. Increased inert or infectious particles stimulate the recruitment of additional macrophages. Most free cells containing particles eventually reach the airways and are quickly carried to the pharynx and swallowed.

In addition, evidence has now accumulated that macrophages play a part in the pathogenesis of pulmonary diseases. For example, the ingestion of some particles by macrophages causes a release of lysosomal enzymes into the macrophage cytoplasm. These enzymes may kill the macrophage, and dead or dying macrophages release a substance which attracts fibroblasts that elicit fibrogenic responses. Other toxic particles, such as cigarette smoke, may lead to a release of proteases and other toxic enzymes. All particles are capable of competitive inhibition of phagocytosis in macrophages and many may be cytotoxic and further depress phagocytosis. In addition, connective tissue macrophages may contribute to lung disease by concentrating and storing potent carcinogens or other toxic particles close to a reactive bronchial epithelium for long periods. Thus, even though macrophages serve as a first line of defense for the alveolar surface, they may also be capable of injuring the host while exercising their defensive role.

Introduction

Pulmonary macrophages are important since their migratory patterns, phagocytic behavior, and secretory potential are often pivotal events in the pathogenesis of pulmonary disease. Their protective role is widely appreciated and our understanding of it growing. Even though macrophages are the first line of defense for the airway and alveolar surfaces, we believe that they can also injure the host while exercising their defensive roles. This review will briefly summarize both classical and more recent aspects of the protective posture of pulmonary macrophages and then will describe accumulating evidence that macrophages also play a part in the pathogenesis of pulmonary diseases.

The respiratory tract is the most common route for entry of toxic particles from modern urban and occupational environments. The same thinness and delicacy of the air-blood barrier which allows rapid

exchange of oxygen and carbon dioxide also reduces its effectiveness as a barrier to inhaled carcinogens, toxic particles, allergens, noxious gases, and microorganisms. Growing evidence suggests that most pulmonary diseases are either initiated by, or at least aggravated by, the inhalation of particles and gases and that the role of environmental factors in the development of respiratory disease is important. First, we will describe briefly the positive contributions of pulmonary macrophages.

Helpful Effects of Macrophage Function

Macrophages are usually credited with keeping the alveolar surfaces clean and sterile. They ingest inhaled particles and pathogens as well as endogenous effete cells and even "worn-out" surfactant. Alveolar macrophages are large, mononuclear, phagocytic cells found on the alveolar surface. They do not form part of the continuous epithelial layer, which is made up of pulmonary surface epithelial cells (type I pneumonocytes) and great alveolar cells

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(type II pneumonocytes). Rather the alveolar macrophages rest on this lining. Considerable interest still exists in the origin and kinetics of pulmonary macrophages. A primary question is whether they are normally derived directly from bone marrow via the blood monocytes or from an intrapulmonary population of macrophages and precursors capable of self-replication. Several investigators suggest that both pathways can occur, depending on the conditions. Alveolar macrophages are ultimately derived from cells in the bone marrow; however, a resident population in the lungs can be maintained by secondary cell renewal systems within the lung.

Thus, there are multiple overlapping strategies for maintaining the macrophage population. In normal unchallenged animals, the immediate precursors are supplied by a cell renewal system in the pulmonary interstitium. Division of existing alveolar macrophages may also contribute to the maintenance of the pool of free cells. Demand for more macrophages, as a result of infection or large numbers of inhaled particles, may be met by increased multiplication of free macrophages, release of pre-existing cells from reservoirs within the lungs, increased production from macrophage precursors in the lung interstitium, and increased flux of monocytes from the blood to the lung.

Pulmonary macrophages are largely responsible for the normal sterility of the lung (1) and for protecting the respiratory tract against a wide variety of foreign materials (2-4). Their phagocytic and lytic potentials provide most of the known bactericidal properties of the lungs. During acute infection or injury they are also supplemented by other leukocytes. Increased inert or infectious particles stimulate the recruitment of additional macrophages. Like other phagocytes, alveolar macrophages are rich in lysosomes, subcellular organelles 0.5 μm or less in diameter (5). The lysosomes attach themselves to the phagosomal membrane surrounding the ingested pathogen. Then the lysosomal and phagosomal membranes become continuous and the lytic enzymes kill and digest the bacteria. Among the enzymes known to be present in lysosomes are proteases (5), acid ribonuclease (6), β-glucuronidase (7), acid phosphatase (6, 7), lysozyme (8), β-galactosidase (9), and phospholipases (10).

Not only do pulmonary macrophages ingest and kill pathogens but they also deal decisively with nonliving, insoluble dust and debris. This function is also essential, since rapid endocytosis of insoluble particles prevents particle penetration through the alveolar epithelia and facilitates alveolar-bronchiolar transport. Schiller (11) and, later, Sorokin and Brain (12) found little evidence that macrophages laden with dust can re-enter the alveolar wall; only free particles appear to penetrate. Thus phagocytosis plays an important role in the prevention of particle entry into the fixed tissues of the lung. If particles leave the alveolar surface and penetrate the tissues subjacent to the air-liquid interface (type I and II cells, interstitial and lymphatic tissues), their removal is slowed. Those remaining on the surface are cleared with a biological half-time estimated to be 24 hr in humans, while particles that have penetrated into "fixed" tissues are cleared with half-times ranging from a few days to thousands of days. Therefore, the extent to which macrophages influence the probability of particle penetration into fixed tissues is critical in determining the clearance times of particles from the nonciliated regions of the lungs.

The re-entry of alveolar macrophages into lymphatic pathways and connective tissue has often been suggested but rarely observed. Some investigators consider the presence of particle-containing macrophages in these compartments to be compelling evidence. However, distinguishing between the entry of alveolar macrophages and the entry of bare particles which are subsequently ingested by connective tissue macrophages already present is difficult. During alveolar clearance, some noningested particles may follow lymphatic or vascular channels from alveoli into the peribronchial, perivascular, or subpleural adventitiae and thus penetrate the connective tissue of the lung. They are then stored by resident macrophages. This pathway may be more common when conditions favor increased lymphatic permeability (e.g., pulmonary edema).

Evidence is rapidly accumulating to indicate that macrophages have roles besides phagocytosis. The diverse activities of pulmonary macrophages which relate them to other cells and processes are being increasingly recognized (13). They are involved in both host defense and host-damaging reactions. The available data show that both outcomes are possible, but predicting and controlling these reactions remain difficult. Beyond that, they secrete a variety of substances which interact with multi-enzyme cascades and other cells such as lymphocytes, fibroblasts and other macrophages. As will be discussed later, some of these secretions are involved in connective tissue turnover, e.g., collagenase, elastase, and lysosomal enzymes; others affect lymphoid cells by helping to regulate mitogenesis and differentiation. Macrophages release additional products such as interferon, lysozyme, and certain components of complement that are involved in defense processes.

Other biologically active materials secreted by macrophages include an angiogenesis factor, plasminogen activator, prostaglandins, nucleosides, cyclic nucleotides, pyrogens, granulopoietins, and factors influencing fibroblast proliferation and tumor

growth. Still other agents may interact with humoral enzyme systems such as the clotting, complement, fibrinolytic, and kinin-generating system.

Pulmonary macrophages are also involved in the induction and expression of several forms of cell-mediated and humoral immunity; they are essential for delayed hypersensitivity reactions, and may be involved in allograft rejection and the pathogenesis of autoimmune diseases. The response of macrophages in the lung to antigens in the respiratory tract may vary. They may prevent excessive antigen stimulation by ingesting and catabolizing inhaled foreign proteins and antigens derived from bacteria, viruses, fungi, and other microbes. Alternatively, they may preserve and present antigens to lymphocytes and act cooperatively with components of the immune system.

Their observed potential for recognizing and destroying neoplastic cells, thus preventing the development of cancer, is another remarkable property that merits further study (14). More than two decades ago, Gorer (15) noted that macrophages were prominent during the rejection of tumor cells. Since then, other investigators (16, 17) have shown that macrophages can selectively damage tumor cells. The stimulation of macrophages by infection can further enhance the ability of macrophages to prevent tumor growth (18, 19).

This growing list of macrophage properties and functions suggests that macrophages are much more than resident garbage men. Continued exploration of these functions is required to appreciate better the biologic potential of pulmonary macrophages and to gain insight into when they help or hurt us.

Harmful Effects of Macrophage Function

In addition to a variety of protective postures which help the host, macrophages may also be participants in the pathogenesis of lung disease. We will begin by considering how their normal behavior may occasionally have untoward effects. We will then consider other types of responses that may involve damage or unusual events. The normal activity and movement of pulmonary macrophages may cause harm. Because the macrophages are actively phagocytic, inhaled toxic, radioactive, or carcinogeneic particles become concentrated within pulmonary macrophages. What begins as a diffuse and relatively even exposure becomes highly localized and nonuniform. "Hot spots" of high dosage are formed which may exceed the thresholds for certain effects and cause damage.

Similarly, in the airways adherence of macrophages to the epithelium may increase epithelial exposure to inhaled toxic materials. More importantly, perhaps, this close association with the bronchial epithelium can lead to transbronchial transport of inhaled particles and subsequent reingestion by subepithelial connective tissue macrophages (20). These cells, like their relatives in the alveolar and airway compartments, also segregate, retain, and perhaps metabolize carcinogenic and other toxic particles.

"Hot spots" may also be associated with damage and perhaps with enhanced epithelial transport. These, in turn, lead to increased access of toxic particles to the connective tissue compartment. Alveolar epithelial defenses may also be breached at the alveolar level, but because of lymphatic pathways, particles may end up in similar sites. Particles gaining access to the lymphatics are probably cleared slowly and thus they may attain great significance in the pathogenesis of many lung diseases. Months and years after exposure to particles, these connective tissue burdens may constitute the major reservoir of retained particles. In addition, connective tissue macrophages may contribute to progressive damage by concentrating and storing potent toxic particles for long periods.

Another way in which macrophages may be involved is through diminution or failure of their defensive role. A number of investigators, using both *in vivo* or *in vitro* bactericidal or phagocytic assays, have shown that macrophage function can be compromised by environmental insults and pathological changes. Such diverse agents as silica, immunosuppressives, ethanol intoxication, cigarette smoke, air pollution, and oxygen toxicity, can dramatically depress the ability of pulmonary macrophages to protect their host. Sometimes the agent or factor acts directly on the macrophage producing a damaged or even a dead cell. In other cases (e.g., high concentrations of inhaled particles) the mechanism can be competitive inhibition in which the phagocytic machinery becomes saturated even in the absence of cytotoxicity. In other instances, particularly those situations involving pulmonary edema or altered acid-base balance, the macrophages may be undamaged, but their activity may be depressed because of an indirect effect on their milieu, the airway, or alveolar microenvironment. Nonetheless, it is important to realize that macrophage failure or damage is not always a cause of the disease in question; in many instances, alterations in macrophage function may simply reflect the onset and progression of the disease. For example, changes in macrophage activity during pulmonary edema associated with oxygen toxicity fall in this class. It is not the macrophage's failure to ingest particles or bacteria which causes

the edema; rather the reverse. Many investigators have similarly misinterpreted associations between other altered respiratory defense mechanisms and lung disease. For example, the presence of altered mucociliary transport in lung disease does not necessarily imply that depressed mucociliary transport has caused the lung disease or even contributed to its pathogenesis. Defective mucus movement may be a result of the disease rather than a cause. Carefully designed experiments are needed to make these distinctions.

Macrophage Damage and Pulmonary Connective Tissue: Proteolysis

There are situations in which pulmonary macrophages not only fail but are directly implicated in the pathogenesis of pulmonary diseases. Two important examples both involve pulmonary connective tissue (21, 22). Connective tissue proteins have a fundamental role in lung structure and function. Collagen and elastin help maintain alveolar, airway and vascular stability, limit lung expansion and contribute to lung recoil at all lung volumes. Elastin makes the major contribution to the lung's mechanical properties at low and middle lung volumes and also helps to tether and orient collagen fibers. Changes in elastin and collagen architecture result ultimately in small airway obstruction by decreasing the elasticity of structures supporting the conducting airways. Other associated inflammatory changes result in plugging, narrowing, and obliteration of small airways. Changes can be observed in the lumen as well as in the epithelial walls. Two groups of lung disease are associated with aberrations of normal collagen and elastin balance: emphysematous and fibrotic disorders. To what extent these associations involve changes in the rate and nature of synthesis, or of degradation of these proteins remains speculative. Let us first review the relationship of macrophages to proteolysis.

Growing understanding of the pathogenesis of emphysema has focused attention on the natural balance between elastase and antielastase which exists in the respiratory tract. Elastase is one of many lysosomal and cytoplasmic enzymes involved in the intracellular killing and digestion of pathogens. Although these enzymes constitute an important aspect of the lung's defensive posture, when kept in a chronically activated state, their digestive capacity may damage pulmonary tissues. Release of lysosomal enzymes, particularly proteases, from activated macrophages and leukocytes promote the development of emphysema. Release occurs as a consequence of cell death, cell injury, exocytosis, or

regurgitation while feeding. Increased deposition of inert or infectious particles acts to recruit additional macrophages and thus the effect may be reinforced.

Interest in proteolytic injury was stimulated because of knowledge about pulmonary emphysema associated with inborn α_1-antitrypsin-inhibitor deficiency in humans (23). Imbalances between proteolytic activity and its control or inhibition have important implications as a generalized mechanism of lung injury in other pulmonary pathological states caused by air pollution, or the inhalation of occupational dusts.

Macrophages secrete enzymes capable of connective tissue degradation. Both collagenase and elastase activity can be detected in fluids from macrophage cultures (24-26). The release of both enzymes is influenced by activation and is greater from exudative peritoneal macrophages than from resident peritoneal macrophages; it is also stimulated by phagocytosis. Enzyme secretion is stimulated by cytochalasin B, colchicine, and vinblastine. The epidemiological and clinical relationships between smoking and chronic obstructive pulmonary disease are probably mediated by release of elastase from macrophages and neutrophils. There is also evidence that exposure to smoke causes increased synthesis and release of elastolytic enzymes from these cells (27, 28). Importantly, culture media from alveolar macrophages of smokers demonstrated greater elastase activity than those obtained from nonsmokers. These findings tend to confirm the suspected role of PM in the pathogenesis of pulmonary emphysema in smokers.

Smokes and other pollutant particles characteristic of work and urban environments also act to recruit more cells, to activate them, and to release proteolytic enzymes. Macrophages and polymorphonuclear leukocytes may then be centrally involved in many stages of the development of pulmonary disease. Oxidant radicals produced by macrophages and neutrophils may also be involved. These highly reactive oxygen byproducts may damage lung tissue directly; they may also have an indirect effect by damaging macrophages which subsequently release toxic or proteolytic enzymes. The extent of damage in the latter case depends on the number of additional macrophages recruited, on the extent of their activation, and on the degree to which elastase, oxidants, and other materials are secreted or released from macrophages. Pathogenesis of emphysema may also involve damage to the epithelium which provides greater access of elastase to the elastin.

A number of animal models have been produced which support the proteolytic theory of emphysema. A lesion very similar to emphysema can be produced

by intratracheal instillation of aerosolization of non-specific proteolytic enzymes such as papain or by elastase (29-30). Homogenates of neutrophils or pulmonary macrophages have a similar effect (32, 33) as does elastase present in purulent sputum (34, 35). A number of investigators have described the anatomical and physiological development of emphysematouslike lesions following intratracheal instillation of elastase. Generally, the lesion is characterized by an initial phase of enzymatic degradation of elastin in the lung followed by a more gradual architectural derangement characterized by expanded airspaces and/or destruction of some alveolar units. The relationship between relatively short-term animal models where the lesion develops in a matter of weeks and the human disease which requires 10 to 40 years is still a mystery.

The role of α-1-antitrypsin in man has emphasized the importance of not only proteases but also antiproteases. When porcine pancreatic elastase is instilled into hamsters with no serum or with serum from α-1-antitrypsin-deficient people, the result is a lesion resembling emphysema. However, when identical amounts of pancreatic elastase are added with serum from normal individuals, no change occurs. Thus the maintenance of a normal balance between elastase and elastase inhibitors is critical. The body's defenses against excessive elastase levels include inhibitors present in alveolar and airway lining fluid and the ingestion and degradation of elastase by macrophages. It is not known to what extent these antiprotease inhibitors are derived locally and systemically.

There may be changes in other inhibitors. Changes in the composition of the lung fluid in the interstitium may be important. The concentration of antiproteases at the site of injury may be changed and may be unrelated to those measured in the serum or even the lavage fluid. In any event, a key event appears to be disruption of the natural elastase-antielastase homeostasis may reflect increased protease and/or decreased inhibitor activity, resulting in a sustained burden of unopposed proteases and destruction of alveolar structures.

Therapies should focus on the reduction of unopposed proteases. They could attempt either to regulate the production and release of neutrophil and macrophage proteases or to augment inhibitor activity by stimulating endogenous production through the use of synthetic antielastase agents, or through replacement therapy utilizing elastase recovered from blood or other biological fluids. Further studies are needed to examine mechanisms of release of proteolytic enzymes, their effects on lung cells and tissues, and the protection afforded by serum and lung antiproteases.

Macrophage Damage and Pulmonary Connective Tissue: Fibrogenesis

Dead or dying macrophages may release substance(s) which can attract fibroblasts and elicit fibrogenic responses. Dust particles of appropriate size, shape, chemical composition, and durability, may deposit on alveolar surfaces and stimulate production of excess collagen in the alveolar membrane. In such fibrotic diseases as asbestosis and silicosis, progressive fibrogenesis may continue long after the exposure to dust particles has stopped. Excessive collagen may make the lungs stiffer than normal, severely decreasing the vital capacity and increasing the muscular forces required for breathing.

Asbestos, glass, and other fibrous dusts all have been shown to stimulate collagen synthesis (36, 37). Fibers over 5 μm in length are sometimes incompletely ingested by macrophages (38) and may lead to macrophage death or release of mediators. Growth of fibroblasts *in vitro* has been shown to require a solid supporting particle of critical minimum dimensions (39).

There is some evidence that fibrogenesis may involve macrophages and occur as a two-step process (40, 41). This especially applies to highly fibrogenic particles such as silica which have very symmetrical shapes. Silica has not been shown to exert a direct stimulatory effect on fibroblasts. Rather, the interaction of a particle with a macrophage is thought to release factors which stimulate local production of collagen by fibroblasts. It is unlikely that macrophages differentiate into collagen-synthesizing fibroblasts (42, 43) and the addition of silica to cultured fibroblasts does not stimulate collagen biosynthesis or release (37).

A number of investigators have produced evidence showing that macrophages can produce a factor or factors which, in turn, influence the proliferation (44) and biosynthetic activity (45) of fibroblasts. After adding silica particles to mouse macrophages, Heppleston and Styles (40) reported the presence of a factor which stimulated chick fibroblasts to produce collagen. Using rabbit pulmonary macrophages and WI-38 fibroblasts, Burrel and Anderson (46) showed the same response. More recently, Allison (47) summarized other experiments carried out in his laboratory supporting the two-stage theory of fibrogenesis utilizing an *in vivo* preparation.

Diffusion chambers bounded by Millipore filters were placed in the peritoneal cavities of mice for a month or more. If the chambers contained only peritoneal macrophages or silica particles, no fibrogenesis occurred. But if both silica and peritoneal macrophages were placed in the chamber, the result

was marked thickening of the parietal and visceral pleura with significant collagen deposition beneath the mesothelial cells. Allison (47) concluded that "a factor released from surviving macrophages stimulated by silica passes out of the diffusion chamber and stimulates collagen synthesis by fibroblasts."

Silica and asbestos present an added hazard of being cytotoxic to alveolar macrophages. Within a few minutes they can lyse cells by direct interaction with the plasma membrane, or if successfully ingested, in several hours cause rupture of secondary lysosomes, releasing lysosomal hydrolases into the cytoplasm (41). The resulting dead macrophages can become focal points for further fibrogenesis. In addition, the particles are released anew on the alveolar surface to cause more irritation. Not all published experiments, however, support the two-stage theory. Harrington et al. (48) added culture medium from hamster macrophages incubated with silica and observed decreased collagen biosynthesis. Additional research is needed to explore these possible species differences. To understand fibrosis, the role of lymphocytes, macrophages, fibroblasts, and the effect of fibrogenic agents on collagen balance and on the replication and differentiation of each parenchymal cell type need to be studied (49).

Other Effects

The responses of the lung to inhaled antigens and allergens and the area of environmental allergic respiratory disease is also emerging as an important area which may involve macrophages. Considerable evidence is available which shows that inhaled organic chemicals, dusts, molds, and animal proteins can cause a variety of lung responses such as allergic asthma, extrinsic allergic alveolitis, immune complex disease and other phenomena. Since excellent discussions of these diseases are available (50), we will not review them here except to point out that macrophages may sometimes be involved. Little is known about the degradation of proteins deposited on the respiratory tract surfaces. No doubt, in many instances macrophages and pulmonary clearance defend the body against excessive antigenic stimulation. However, there may also be circumstances when clearance pathways cooperate with the immune system and preserve and present immunogenic molecules to the immune system. Thus the issue of how and when pulmonary macrophages suppress or enhance the immunogenicity of antigens must be confronted.

Other pulmonary diseases may also involve macrophages. Pulmonary-alveolar proteinosis, a disease characterized by the presence of lipoproteinaceous material in the alveoli, may alter macrophage function (3, 4). Golde et al. (51) have suggested that phagocytosis of excessive quantities of the characteristic lipoproteinaceous material may damage macrophages and contribute to the frequent infectious complications of this disease. Gee et al. (52) have speculated on possible relationships between sarcoidosis and macrophages. They noted that the disease is often associated with elevations of serum levels of lysozyme or angiotensin-converting enzyme or both. They believe that the elevated lysozyme levels are caused by increased secretion of the enzyme by monocytes and lung macrophages. In addition, human pulmonary macrophages recovered from patients with sarcoid have more angiotensin-converted enzyme than in those recovered from normals. They believe there is evidence for macrophage activation in sarcoidosis.

Conclusion

Thus, even though macrophages serve as a first line of defense for the alveolar surface, they may also be capable of injuring the host while exercising their defensive role. Support for studying the effects of air pollution on macrophages should be continued. Such studies are essential in determining mechanisms of biological response and as part of systematic studies of dose-response relationships. Systematic exposure of animals to materials produced by technological advance followed by evaluation of macrophage function or damage will permit assessment of some of the effects of these materials. Additional knowledge is needed about the ultrastructural and biochemical alterations in alveolar macrophages following exposure to physical, chemical, and infectious agents. The effects of parameters which may modulate the pulmonary response to atmospheric and industrial contaminants should be measured. The effects of altered physiological states and concurrent illness should be systematically explored. Frequently, important contributions to our understanding of environmental disease have depended upon the existence of appropriate animal models.

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