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TOXICOLOGICAL ASPECTS OF ALTERATIONS OF PULMONARY MACROPHAGE FUNCTION

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INTRODUCTION

Pulmonary macrophages are similar to mononuclear phagocytes throughout the body, such as those found in the peritoneal cavity or liver. Yet, pulmonary macrophages face unique challenges. Found on the inner surfaces of the respiratory tract, they come in direct contact with toxic particles and gases as well as pathogens contained in the inspired air. Their mobility, phagocytic capacity, and bactericidal properties are essential to the maintenance of clean and sterile alveoli.

Since these cells are accessible by bronchopulmonary lavage, they have been studied extensively in vitro. Our knowledge of the cell biology and toxicology of phagocytic cells has been extended by experiments with isolated pulmonary macrophages. These cells are of interest since their migratory patterns, phagocytic behavior, and secretory potential are pivotal events in the pathogenesis of pulmonary disease. Even though macrophages are an essential line of defense for airway and alveolar surfaces, they can also injure the host. For these reasons, the toxicology of pulmonary macrophages is an essential aspect of how toxic agents injure the lung (1).

Under certain conditions, macrophages are damaged as a consequence of particle ingestion, and the nature of that damage is relevant to pulmonary disease. In response to phagocytic stimuli, macrophages secrete lysosomal hydrolases, proteases, and antiproteases, chemotactic factors, interferon, and other mediators (2). Excessive or prolonged secretion, or secretion in an inappropriate site, can cause tissue damage. An imbalance between protease and antiprotease levels in the lungs can lead to emphysema [reviewed by P. J.

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Stone (3)]. Macrophages may be involved in this process either by secretion of the neutral protease, elastase (4), or by binding and releasing the polymorphonuclear neutrophil elastase (5). In vitro studies show that macrophages, after stimulation with a variety of agents such as crystalline silica (6) or cold-insoluble globulin (7), produce factors that stimulate fibroblast proliferation and/or collagen production. The production of such factors in vivo are relevant to wound healing (8) and are important in fibrogenesis. Macrophages appear to be involved in the pathogenesis of atherosclerosis (9); they may also have a role in identifying and destroying neoplastic cells (10). Finally, pulmonary macrophages appear to present antigen in vivo to T cells and thereby trigger T cell–dependent immune responses (11). In turn, the phagocytic activity of macrophages may be modified by local antibody production (12).

In this review we describe the major classes of pulmonary macrophages and discuss their origin, function, and quantitation. After discussing how toxic particles and gases affect pulmonary macrophages, we summarize evidence that macrophages may contribute to the pathogenesis of pulmonary diseases.

CLASSES OF PULMONARY MACROPHAGES

Pulmonary macrophages encompass at least three types: the best known is the alveolar macrophage. Alveolar macrophages are large, mononuclear, phagocytic cells found on alveolar surfaces. They are not part of the continuous epithelial layer of Type 1 and Type 2 cells, rather, alveolar macrophages rest on this lining covered by surfactant. Figure 1 shows a hamster alveolar macrophage containing ingested iron oxide particles. Another kind of pulmonary macrophage is the airway macrophage (13). Airway macrophages can be found in the conducting airways, both large and small. They may be present as passengers on the mucus escalator, or they may be found beneath the mucus lining, adhering to the bronchial epithelium (14).

Interstitial macrophages comprise a third subdivision of pulmonary macrophages. They are found in the various connective tissue compartments of the lung, including alveolar walls, lymph nodes, and peribronchial and perivascular spaces. In addition to these three primary types, other minor macrophage compartments exist. For example, macrophages are found in the pleural space. In some species, there may also be intravascular macrophages similar to Kupffer cells in the hepatic sinusoids. In ruminants, these macrophages are very prominent (15).

Even macrophages from a single compartment need not be homogeneous. Both in structure and in function, heterogeneity is common. Many investigators have described considerable variability in macrophages recovered by bronchoalveolar lavage. For example, Godleski et al (16) have shown that hamster lung macrophages vary considerably in regard to the amount of antigen present

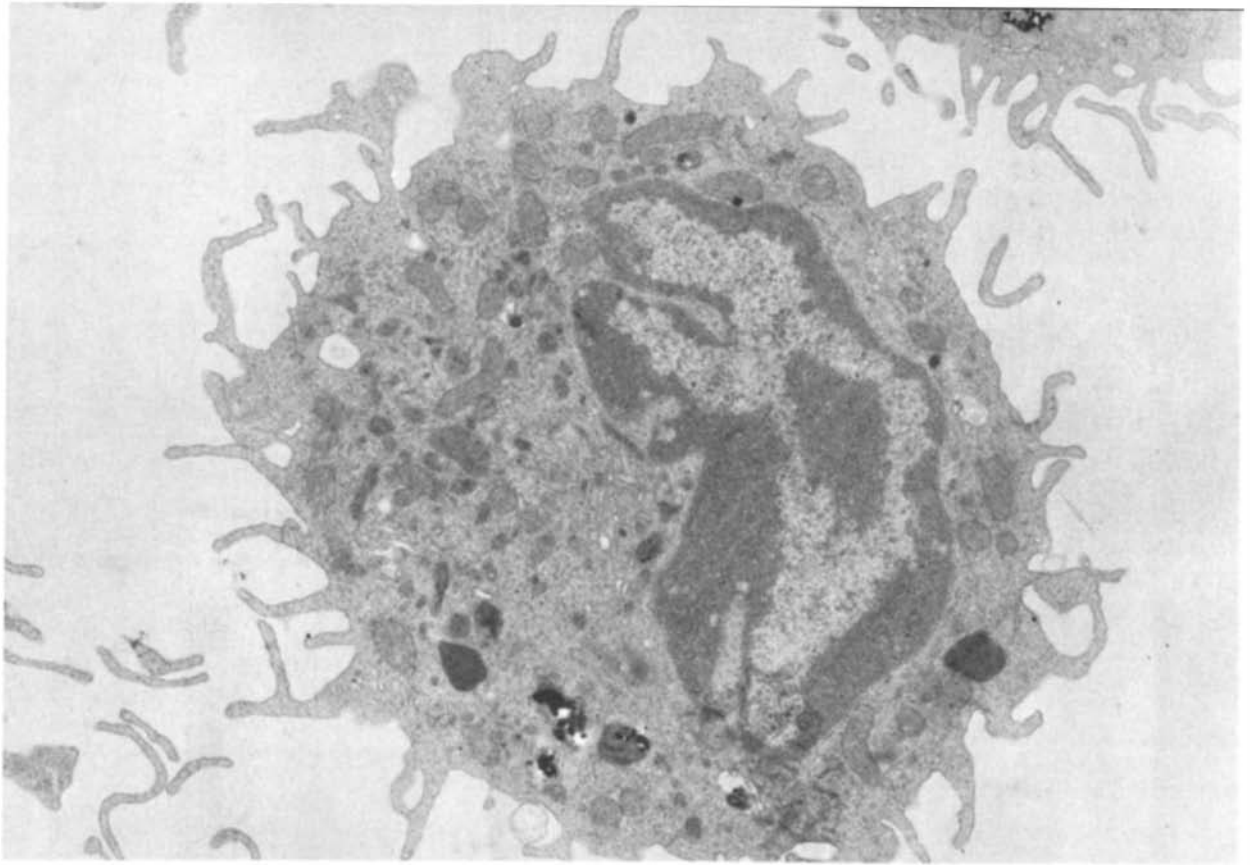

Figure 1 Hamster alveolar macrophages recovered by bronchoalveolar lavage after incubation in vitro with submicrometric particles of magnetite (Fe_3O_4). These electron-dense particles can be seen in the phagolysosomes on the lower left side of the nucleus and in the phagolysosome in part of another alveolar macrophage visible in the upper right corner. Many small pseudopods extend from the plasma membrane. 10,800 X. (Courtesy of Dr. Peter Valberg and Ms. Rebecca Stearns)

on their plasma membranes. This antigen is not present on macrophages in other organs, and increasing amounts of antigen on alveolar macrophages appear to correlate with the length of time that they have been resident in the alveolar space (17).

ORIGIN OF PULMONARY MACROPHAGES

The origin and kinetics of pulmonary macrophages have received extensive study (18, 19). The data demonstrate that multiple strategies for maintaining the macrophage population in the lungs exist. 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 a pool of free cells. Demand for more macrophages, as a result of infection or deposition of large numbers of inhaled particles, may be met by (*a*) increased multiplication of free macrophages, (*b*) release of preexisting cells from reservoirs within the lungs, (*c*) increased production from macrophage precursors in the lung interstitium, and (*d*) an increased flux of monocytes from the blood to the lung. Newborn animals have relatively few pulmonary macrophages; those present are immature. However, within a week or so normal numbers of fully activated macrophages develop in the lungs (20).

FUNCTION OF PULMONARY MACROPHAGES

Particle Clearance

Macrophages keep the surfaces of the lungs clean and sterile. They ingest inhaled pathogens and particles as well as endogenous effete cells and even "worn-out" surfactant (21). Several reviews of these functions are available (18, 19, 22–25).

Although large numbers of infectious particles are continuously deposited in the lungs, the alveolar surface is usually sterile. During acute infection or injury they are also supplemented by other leukocytes. Increased 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 (see Figure 1). Among the enzymes known to be present in lysosomes are proteases (26), acid ribonuclease (26), β-glucuronidase (27), acid phosphatase (26, 27), lysozyme (28), β-galactosidase (29), and phospholipases (30). 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. Macrophages also are responsible for the intracellular killing of parasites such as African trypanosomes and malarial parasites (31). Lung macrophages are also involved in the response to viral infections (32–34).

More important to the microbicidal activity of macrophages than lytic enzymes are the oxygen-dependent cytotoxic systems. Although best described in neutrophils (35, 36), these mechanisms are also present in pulmonary macrophages. Phagocytosis triggers increased oxygen consumption and the generation of oxygen radicals such as superoxide (O_2^-), hydroxyl radicals, singlet oxygen, and H_2O_2. These highly reactive oxygen derivatives modify macromolecules of pathogens. Lipids, proteins, and nucleic acids may be modified by (myelo)peroxidase and halide-mediated oxygen-dependent reactions (36). Damaging oxidizing agents can also include several halogen derivatives such as chlorine, chloridium ions, and hypochlorous acid. Macrophages also contain ingredients that can protect them against the damaging effects of these oxidizing agents. Protective substances include vitamin E, ascorbic acid, and the glutathione redox system as well as catalase and superoxide dismutase.

Not only do pulmonary macrophages ingest and kill pathogens but they also deal with nonliving, insoluble dust and debris. Figure 1 shows how iron oxide is taken up into membrane-bound vesicles in the macrophage cytoplasm. This function is essential, since rapid endocytosis of insoluble particles prevents particle penetration through alveolar epithelia and facilitates alveolar-bronchiolar transport (14).

Secretion and Regulation

Macrophages have many functions besides phagocytosis of particles and microbes. They secrete a variety of substances that interact with multi-enzyme cascades and with other cells such as lymphocytes, fibroblasts, neutrophils, and other macrophages. Thus, the macrophage both responds to and regulates its external environment. Werb (37) has recently reviewed materials secreted by macrophages. Interactions between alveolar macrophages and lymphocytes may be involved in the suppression or induction of immunologic pulmonary disease. Unanue (38) has reviewed other aspects of the immunoregulatory function of the macrophage and has described factors that regulate the expression of I-region-associated antigens (Ia) by macrophages. Macrophages are involved in the presentation of antigens and interact with the helper-inducer set of T lymphocytes. Finally, interactions between macrophages and lymphocytes are modified by exposure to inhaled particles such as tobacco smoke (39).

As we discuss below, 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, fibronectin, lysozyme, certain components of complement, and antiproteases. 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. Macrophages are known to secrete plasminogen activator and a variety of arachidonic acid oxygenation products such as prostaglandins F_2 and E_2 and thromboxane B_2 (37). Finally, macrophages secrete interleukin-1 and a variety of mitogens for T and B cells.

MEASURING THE PHAGOCYTIC PROPERTIES OF PULMONARY MACROPHAGES IN SITU

The capacity of the lung to ingest and kill pathogens via its macrophages has been widely studied. After a bacterial aerosol exposure, the progress of intrapulmonary bacterial killing can be assayed by counting bacterial colony forming units (CFU) in pour plate cultures containing samples of lung homogenates from animals sacrificed at various times. The results are then compared to the number of CFU seen immediately after the bacterial challenge. This technique was first developed by Laurenzi, Berman, First & Kass (40) and has been used extensively to study the effects of toxic agents, clinical syndromes, and environmental factors on intrapulmonary killing. For example, Green & Kass (41) demonstrated the depressant effects of ethanol and hypoxia. Other depressants that have been studied include NO_2 (42) and cigarette smoke (43, 44). Goldstein et al (45) showed that the toxic effects of ozone on killing were more a consequence of defects in intracellular bactericidal activity than of impaired bacterial ingestion.

Brain & Corkery (46) have devised a technique for estimating the extent of in situ phagocytosis of radioactive particles administered to rodents by intratracheal (i.t.) instillation or by inhalation. Their approach is based on analysis of how particles and macrophages wash out of the lung during repeated lung lavage. They established that pulmonary lavage removes macrophages in a pattern distinctly different from that of free particles (those not associated with macrophages or fixed tissues).

Within hours after the i.t. instillation of an ^{198}Au colloid, Brain & Corkery (46) observed that the curve describing the washout of radioactivity began to lose the shape characteristic of free particles and started to mimic the washout of the macrophages; this suggests a transfer of particles from the free state to a cell-associated state. A mathematical curve fitting gave rise to an index, λ: the fraction of particles phagocytosed. In hamsters, λ is usually around 50–75% by 2 hr, more than 95% by 10 hr, and nearly 100% at 24 hr.

This assay has proven to be a useful way to examine the impact of particles on the function of pulmonary macrophages in vivo. Brain & Corkery (46) examined the effects of preexposure to iron oxide, colloidal carbon, and coal dust on the endocytosis of colloidal gold. For all three materials, endocytosis measured 2 hr postexposure was significantly depressed. However, when the

hamsters were given the test gold particles 24 hr postexposure, only the coal dust group exhibited depressed endocytosis. Brain & Corkery concluded that all dusts can competitively inhibit endocytosis, but only some exhibit a sustained toxic effect on macrophage function. Beck et al (47) have incorporated the λ assay into a comprehensive in vivo hamster bioassay designed to assess the toxicity of particulates for the lungs. The λ assay discriminates between relatively nontoxic dusts like Fe_2O_3 and Al_2O_3 and the highly fibrogenic dust alpha quartz (silica). Its cytotoxicity for macrophages is reflected by the depression in λ. At the highest dose of alpha quartz studied, less than 30% of the gold was ingested in 90 min compared to more than 60% in the controls.

A novel approach to describing macrophage function in situ has recently been described. Brain et al (48) and Gehr et al (49) have described how both phagocytosis of iron oxide particles and macrophage motility can be monitored noninvasively with magnetometric methods. When magnetic forms of iron oxide are instilled or inhaled into the lungs, the retained particles can be used as an in vivo tracer for phagocytosis, cytoplasmic motility, and particle clearance [reviewed by Brain et al (50)]. These particles can be magnetized and aligned by an external magnetic field; the remanent magnetism coming from them can then be measured at the surface of a human's or animal's chest. Immediately following magnetization, the field from the lungs begins to decay (relaxation). Interestingly, the characteristics of this decay change with time after aerosol exposure. These time-dependent changes in relaxation are related to the progression of particle ingestion by macrophages in situ (48, 49).

Relaxation is caused by the random reorientation of particles away from their initially aligned state. Our in vivo experiments suggest that the rotational forces applied to the particles come largely from intracellular movements of phagosomes and lysosomes that contain the particles (48, 49). We believe that these movements arise from contractions of the cytoskeleton, which orchestrate cell motion required for functions such as phagocytosis, secretion, or ameboid movement. The hypothesis that organelle motion is a dominant mechanism for relaxation has now been confirmed in studies of hamster pulmonary macrophages observed in vitro (51, 52). Cultured macrophages that had previously ingested magnetic particles exhibited relaxation that was quantitatively similar to that seen in vivo, demonstrating that cardiac and respiratory movements are not essential for particle misalignment. Cytochalasin B (51) or D (52) slowed relaxation as did cold, formalin fixation, or nocodazole (52). Each of these interventions compromises the contractile capabilities of the cytoskeleton.

HARVESTING PULMONARY MACROPHAGES

Recovering macrophages from the lungs is important for two reasons: first, a change in macrophage number is one important index of response to inhaled particles and gases. Second, in vitro studies of pulmonary macrophages ex-

posed to toxic agents require the isolation of pure populations of macrophages from the lungs of animals and humans, but success in isolating pulmonary macrophages depends on the particular subclass involved. Alveolar macrophages, for example, represent a relatively accessible cell population. Unfortunately, airway and connective tissue macrophages in the lungs cannot be recovered with the same ease and purity.

Alveolar macrophages are usually recovered by bronchoalveolar lavage. After filling all or part of the lungs with saline via the airways, one can withdraw the fluid. The recovered saline brings with it both cells and molecules that are contained in airway and alveolar lining fluid. Not all free cells recovered by lung lavage meet the definition of alveolar macrophages discussed earlier. Some Type 1 and Type 2 pneumonocytes, airway epithelial cells, and contaminating red and white blood cells may also be harvested. Airway macrophages are always present, being more prevalent in the initial washes, less so in the later washes.

Lung lavage to recover macrophages was first used by Gersing & Schumacher (53) and has been used extensively since (54–56). Brain & Frank (57) attempted to make the technique more sensitive and reproducible by utilizing multiple lung washings and by identifying and controlling the factors influencing macrophage yields.

Excised lungs, whole lungs in situ, or parts of lungs in situ can be lavaged. Usually, lungs of small animals are washed in situ since the possibility of causing leaks in the lungs is reduced. Following exsanguination, the neck is opened and the trachea cannulated. The chest wall or diaphragm should be opened to allow the lungs to empty themselves of as much air as possible. Washes are then carried out as just described. In most mammalian species, yields of 3–15 million cells/g lung are obtained when the lungs are washed 12 times (19).

Lungs of living animals, especially large ones such as calves and dogs, may also be lavaged. Instilled saline not recovered will be absorbed into the capillaries. Following topical anesthesia of the upper airways, a cuffed endotracheal tube is introduced through the larynx and placed in the left or right bronchus or even in smaller airways. The cuff is then inflated to create a tight seal. The intubated lung or lobe may then be lavaged while the remaining lung meets the ventilatory demands of the animal. Smaller subdivisions of the lung may be lavaged by using smaller-caliber endotracheal tubes; tubes without inflatable cuffs may be simply wedged in an appropriately sized airway. Since only a small percentage of the lungs is washed, the lavage of different lung segments can yield different results. This is more likely when injury or disease is nonuniformly distributed in the lungs. Recoveries of injected saline may be less in animals possessing considerable collateral ventilation (e.g. dog).

Similar procedures have been used to recover macrophages from human

subjects. With the advent of flexible fiberoptic bronchoscopy, access to the lower respiratory tract has become relatively easy and nontraumatic. Segmental lobes can be lavaged to obtain evidence of particle exposure or to obtain human macrophages for in vitro study. Lung lavage in humans generally uses volumes ranging from 100 to 1000 ml (58, 59).

To obtain quantitatively consistent recoveries of macrophages it is necessary to control all aspects of the harvesting procedure. Gas-freeing the lungs, the length of the postmortem delay time, wash volume, leakage, pathological changes, and the number of washes all influence the results (19). So does age, sex, lung weight, and body weight (19). Additional observations (57) dealt with the effects of wash osmolarity and temperature, and duration of the washing cycle. Mechanical factors are also involved in the recovery of free cells from the alveolar surface and airways. Massage of the excised lungs or of the chest wall when the lungs are washed in situ increases macrophage recovery.

Lung lavage is often used to quantify changes in macrophage pool size following challenges with toxic materials, lung infection, or lung injury. Lung lavage is more reliable and sensitive with multiple washings. The mean cumulative yield increases at a faster rate than does the standard error (60). Even though individual washes vary, differences tend to cancel each other so that the cumulative total yield becomes less variable. Lungs should be washed at least six times to achieve maximum sensitivity.

It is not surprising that a cell system so intimately involved with inhaled materials responds to the quantity of particles presented to it. Brain exposed hamsters and rats (23, 60) to a wide variety of particulates, including carbon, coal dust, barium sulfate, triphenyl phosphate, chrysotile, iron oxide, and cigarette smoke, and observed increased macrophage numbers. Beck et al (47) have described changes in macrophage populations after exposing hamsters to iron oxide, aluminum oxide, volcanic ash, and silica. Macrophages in man also respond to chronic exposure to inhaled tobacco smoke (61). Brain (60) observed that smaller particles tend to be more effective stimuli than larger particles. The numbers of macrophages released may be related more to particle number or to particle surface area than to particle mass.

When interpreting changes in the numbers of macrophages present in the lungs, one must be aware of two assumptions. The ratio of the cells harvested to those actually present in situ is usually assumed to be the same in controls and in exposed groups, but the possibility exists that the treatment has influenced the efficiency of recovery. If toxic particles provoke an inflammatory response, bronchoconstriction, or atelectasis, altered cell harvest efficiency may occur.

One should also remember that the pool size of macrophages is dynamically determined. The equilibrium number of cells present at a point in time is a function of the input and output history of the pool. For example, if the pool size decreases, it may be due to decreased production or recruitment of free cells, or

to accelerated clearance of macrophages in the lungs. If input and output of free cells both increase, the pool size can remain constant in spite of increased release of alveolar macrophages onto the lung surface. Accelerated or depressed lysis of macrophages also influences the equilibrium number of free cells.

MEASURING THE PHAGOCYTIC PROPERTIES OF PULMONARY MACROPHAGES IN VITRO

Many methods are available to assess in vitro endocytosis by macrophages. A review describing the merits and limitations of different approaches has been published by Kavet & Brain (62). Once harvested, phagocytic cells are usually studied as adherent monolayers or as cells in suspension. Certain principles apply to endocytic assays regardless of which type of culture system is employed. First, one should characterize the cell population introduced into the incubation medium. It is important to quantify (*a*) the number (or concentration) of cells present, (*b*) the percentage of each cell type present, and (*c*) the fraction of phagocytes that are viable. Particle uptake per cell (or per cell mass) will be improperly estimated if nonmacrophages or nonviable macrophages are included. The recovered cells should be purified or appropriate corrections should be made when the endocytic rates are calculated.

Second, in both suspension and monolayers, it is necessary to be able to (*a*) arrest phagocytosis and (*b*) separate the cells from unphagocytosed particles. With monolayers, these two aims are simultaneously achieved with a thorough rinse of the coverslip or culture dish. In a suspension system, arrest and separation usually require a two-step process. Phagocytic arrest can be accomplished by rapidly chilling the culture, by adding inhibitors such as iodoacetate, sodium fluoride, or N-ethylmaleimide, or by diluting suspensions to the point at which the probability of cell-particle contact approaches zero. When the cells and particles in suspension assays have different densities and/or sedimentation rates, separation of arrested cells from remaining free particles can be accomplished by centrifugation. Filtration can also be used to separate cells and free particles, provided that all cells are trapped by the filter and all free particles pass through.

A new assay for pulmonary macrophage endocytosis that uses flow cytometry was recently developed (63). Macrophages recovered by lung lavage are incubated with fluorescent latex particles. Then a cytofluorograph is used to characterize the uptake of particles by small samples of pulmonary macrophages. A linear relationship exists between the number of fluorescent particles associated with each cell and the intensity of fluorescent light emitted by each cell. Particle uptake is significantly inhibited by removing divalent

cations with ethylenediaminetetraacetic acid and lowering the incubation temperature (64).

PATHOPHYSIOLOGY OF PULMONARY MACROPHAGES

In addition to protecting the host, macrophages also participate in the pathogenesis of lung disease. Because macrophages are actively phagocytic, inhaled toxic, radioactive, or carcinogenic particles become concentrated within pulmonary macrophages. What begins as a diffuse exposure becomes highly localized and nonuniform. "Hot spots" of high dosage are formed that may exceed the thresholds for certain effects and cause damage. Macrophages may also metabolize chemicals and change them to a more toxic form.

When macrophages adhere to the airway epithelium they may increase epithelial exposure to inhaled toxic materials. More importantly, this close association with the bronchial epithelium can lead to transbronchial transport of inhaled particles and subsequent reingestion by connective tissue macrophages (65). 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 be associated with damage to the epithelial barriers and thus with enhanced epithelial transport. These, in turn, could lead to increased access of toxic particles to the connective tissue compartment. Epithelial defenses may also be breached at the alveolar level; because of lymphatic pathways, particles may arrive in similar sites. Particles gaining access to the lymphatics are cleared slowly, thus increasing their contribution to the pathogenesis of many lung diseases. Years after exposure to particles, these connective tissue burdens may constitute the major reservoir of retained particles. 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 and 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 depress the ability of pulmonary macrophages to protect their host. For example, diesel exhaust may depress the phagocytic activity of these cells (66). Sometimes the agent or factor acts directly on the macrophage, producing a damaged or even a dead cell. For example, the ingestion of lead oxide particles is followed by swelling of the mitochondria, nuclear membrane, and endoplasmic reticulum as well as the appearance of precipitation complexes within the nuclear chromatin and cytoplasm (67).

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. Then again, particularly in 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. However, macrophage failure or damage is not always a cause of the disease in question; sometimes changes in macrophages 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 that causes the edema; rather, the presence of edema alters macrophage function.

There are situations in which pulmonary macrophages not only fail but contribute directly to the pathogenesis of pulmonary diseases. Two important examples involve pulmonary connective tissue (68, 69). Connective tissue proteins have an essential 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. Two groups of lung disease are associated with aberrations of normal collagen and elastin balance: emphysematous and fibrotic disorders.

Emphysema

Studies of the pathogenesis of emphysema have focused attention on the balance between elastase and anti-elastase in the respiratory tract (70). Elastase is involved in wound healing and in the disposal of damaged cells and debris. Although these enzymes are useful, when chronically present their digestive capacity may damage pulmonary tissues. Release of lysosomal enzymes, particularly proteases, from activated macrophages and other leukocytes promotes the development of emphysema. Release occurs as a consequence of cell death, cell injury, exocytosis, or regurgitation while feeding. Increased deposition of particles acts to recruit additional macrophages; thus the effect may be reinforced. For example, Warheit et al (71) have recently demonstrated that inhaled chrysotile asbestos fibers that deposit at alveolar duct bifurcations activate complement. In turn, complement activation enhances the inflammatory response by attracting more macrophages into the region. Macrophages also secrete substances that are chemotaxic for neutrophils (72).

Interest in proteolytic injury was stimulated because of knowledge about pulmonary emphysema associated with inborn α_1-antitrypsin-inhibitor deficiency in humans (73). Imbalances between proteolytic activity and its inhibition have important implications as a general mechanism of lung injury. Macrophages secrete enzymes capable of connective tissue degradation. Both collagenase and elastase activity can be detected in fluids from macrophage

cultures (74–77). The release of both enzymes is stimulated by cell activation and by phagocytosis. There is evidence that exposure to smoke causes increased synthesis and release of elastolytic enzymes from these cells (78–80). Importantly, culture media from alveolar macrophages of smokers contained greater elastase activity than media obtained from macrophages of nonsmokers. Smokers have higher levels of elastase activity in bronchoalveolar lavage fluid than do nonsmokers (81). These findings help confirm the suspected role of macrophages in the pathogenesis of pulmonary emphysema in smokers. Generally macrophages contribute less elastase to lavage fluid than do neutrophils. However, connective tissue macrophages that release elastase in direct contact with elastin may be very important.

Other pollutant particles characteristic of work and urban environments also act to recruit more cells, to activate them, and to release proteolytic enzymes. For all these situations, the extent of damage depends on the number of additional macrophages recruited, on the extent of their activation, and on the degree to which elastase and other toxic materials are secreted or released from macrophages. Pathogenesis of emphysema may also be facilitated by damage to epithelial barriers that provides greater access of alveolar elastase to elastin in the interstitium.

A number of animal models have been produced that support the proteolytic theory of emphysema. A lesion very similar to emphysema can be produced by intratracheal instillation or aerosolization of nonspecific proteolytic enzymes such as papain or by elastase (82–84). Homogenates of neutrophils or pulmonary macrophages have a similar effect (4, 85), as does elastase present in purulent sputum (86, 87). In all these models, the lesion is usually characterized by an initial phase of enzymatic degradation of elastin in the lungs, a subsequent resynthesis to control levels, and then a more gradual architectural derangement leading to expanded airspaces and/or destruction of alveoli.

The role of antiproteases should be noted. 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. Macrophages bind PMN elastase, although some of the enzyme may remain active.

We have previously described how reactive metabolites of oxygen such as superoxide anions, hydroxyl radicals, and hydrogen peroxide are used by macrophages and neutrophils to kill microorganisms. However, these agents may also cause damage. For example, they may damage cell membranes or essential metabolic enzymes. They may reduce the activity of endogenous

protease inhibitors and thus allow the activity of extracellular proteases to go unchecked. Oxygen radicals may also have an indirect effect by damaging other phagocytic cells and causing additional release of toxic and proteolytic enzymes (88). Recently, Weitberg et al (89) have demonstrated that human phagocytes that produce oxygen radicals may also produce cytogenic damage in cultured mammalian cells. It is conceivable that chronic inflammatory states characterized by persistent increases in macrophages may contribute to cancer by this mechanism.

Fibrosis

Fibrogenesis also involves macrophage damage. Dead or dying macrophages may release substance(s) that 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 wall. In such fibrotic diseases as asbestosis and silicosis, progressive fibrogenesis may continue long after inhalation of dust particles has stopped. A continued influx of new macrophages is frequently a prominent feature of the fibrogenic process (90). Excessive collagen or alterations in types of 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 have been shown to stimulate collagen synthesis (91, 92). Fibers over 5 μm in length are sometimes incompletely ingested by macrophages (93) 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 (94).

There is evidence that fibrogenesis involves macrophages and occurs as a two-step process (95, 96). Silica does not exert a direct stimulatory effect on fibroblasts (92). Rather, the interaction of a particle with a macrophage is thought to release factors that then stimulate local production of collagen by fibroblasts.

A number of investigators have produced evidence showing that macrophages can produce a factor or factors that, in turn, influence the proliferation (97) and biosynthetic activity (98) of fibroblasts. After adding silica particles to mouse macrophages, Heppleston & Styles (96) reported the presence of a factor that stimulated chick fibroblasts to produce collagen. Using rabbit pulmonary macrophages and WI-38 fibroblasts, Burrell & Anderson (99) showed the same response. Aalto & Kulonen (6) reported that macrophages damaged by quartz in vitro release factors that stimulate collagen synthesis by fibroblasts in culture. Using a diffusion chamber implanted into mouse peritoneal cavities, Bateman et al (100) observed that when mouse peritoneal macrophages were incubated with either chrysotile asbestos or silica, factors that diffused through

a Nucleopore membrane produced fibrosis. It is possible that generation of similar fibrogenesis stimulating factors by quartz-damaged pulmonary macrophages in vivo may play a role in the development of fibrosis in the lungs, but such factors have not yet been isolated from the developing silicotic lung (101).

Emerging areas of research interest that may involve macrophages include the responses of the lung to inhaled antigens and allergens, and environmental allergic respiratory disease. Current evidence 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. Excellent discussions of these diseases are available (102); they suggest that macrophages may be involved.

CONCLUSION

Research in inhalation toxicology must not only delineate dose-response relationships, it must also elucidate mechanisms of lung injury. Pulmonary macrophages defend alveolar and airway surfaces, but they are also capable of injuring the host while exercising their defensive role. Further studies are needed of the ultrastructural and biochemical features of normal pulmonary macrophages as well as their alterations following exposure to physical, chemical, and infectious agents.

Literature Cited

1. Witschi H., Nettesheim, P., eds. 1982. *Mechanisms in Respiratory Toxicology*, Vol. II. Boca Raton, Fla: CRC
2. Hunninghake, G. W., Gadek, J. E., Szapiel, S. V., Strumpf, I. J., Kawanami, O., et al. 1980. The human alveolar macrophage. In *Methods in Cell Biology*, Vol. 21A, ed. C. C. Harris, B. F. Trump, G. D. Stoner, pp. 95–112. New York: Academic
3. Stone, P. J. 1983. The elastase-antielastase hypothesis of the pathogenesis of emphysema. See Ref. 70, pp. 405–12
4. Mass, B., Ikeda, I., Meranze, D. R., Weinbaum, G., Kimbel, P. 1972. Induction of experimental emphysema: cellular and species specificity. *Am. Rev. Respir. Dis.* 106:384–91
5. McGowan, S. E., Stone, P. J., Calore, J. D., Snider, G. L., Franzblau, C. 1983. The fate of neutrophil elastase incorporated by human alveolar macrophages. *Am. Rev. Respir. Dis.* 127:449–55
6. Aalto, M., Kulonen, E. 1979. Fractionation of connective-tissue-activating factors from the culture medium of silica-treated macrophages. *Acta Pathol. Microbiol. Scand. Sect. C.* 87:241–50
7. Martin, B. M., Gimbrone, M. A., Majeau, G. R., Unanue, E. R., Cotran, R. S. 1981. Monocyte/macrophage-derived growth factor production: modulation by cold insoluble globulin and extracellular matrix. *Arteriosclerosis* 1:361a
8. Diegelman, R. F., Cohen, I. K., Kaplan, A. M. 1981. The role of macrophages in wound repair: a review. *Plast. Reconstr. Surg.* 68:107–13
9. Watanabe, T., Hirata, M., Yoshikawa, Y., Nagafuchi, Y., Toyoshima, H., Watanabe, T. 1980. Role of macrophages in atherosclerosis: sequential observations of cholesterol-induced rabbit aortic lesion by the immunoperoxidase technique using monoclonal antimacrophage antibody. *Lab. Invest.* 53:80–90
10. Kan-Mitchell, J., Hengst, J. C. D., Kempf, R. A., Rothbart, R. K., Simons, S. M., et al. 1985. Cytotoxic activity of human pulmonary alveolar macrophages. *Cancer Res.* 45:453–58

11. Holt, P. G., Leivers, S. 1985. Alveolar macrophages: antigen presentation activity in vivo. *Aust. J. Exp. Biol. Med. Sci.* 63:33–39
12. Harmsen, A. G., Bice, D. E., Muggenburg, B. A. 1985. The effect of local antibody responses on in vivo and in vitro phagocytosis by pulmonary alveolar macrophages. *J. Leuk. Biol.* 37:483–92
13. Brain, J. D., Gehr, P., Kavet, R. I. 1984. Airway macrophages: The importance of the fixation method. *Am. Rev. Respir. Dis.* 129:823–26
14. Sorokin, S. P., Brain, J. D. 1975. Pathways of clearance in mouse lungs exposed to iron oxide aerosols. *Anat. Rec.* 181:581–626
15. Warner, A. P., Brain, J. D. 1984. Intravascular pulmonary macrophages in ruminants actively participate in reticuloendothelial clearance of particles. *Fed. Proc.* 43:1001 (Abstr.)
16. Godleski, J. J., Mortara, M., Joher, M. A., Kobzik, L., Brain, J. D. 1984. Monoclonal antibody to an alveolar macrophage surface antigen in hamsters. *Am. Rev. Respir. Dis.* 130:249–55
17. Harbison, M. L., Godleski, J. J., Mortara, M., Brain, J. D. 1984. Correlation of lung macrophage age and surface antigen in the hamster. *Lab. Invest.* 50:653–58
18. Brain, J. D. 1985. Macrophages in the respiratory tract. In *Handbook of Physiology—The Respiratory System I. Circulation and Nonrespiratory Functions*, ed. A. P. Fishman, A. B. Fisher, pp. 447–71. Bethesda, Md: American Physiological Society
19. Brain, J. D., Godleski, J. J., Sorokin, S. P. 1977. Structure, origin and fate of the macrophage, In *Respiratory Defense Mechanisms* (Lung Biology in Health and Disease, Monograph 5), ed. J. D. Brain, D. F. Proctor, L. Reid, pp. 849–52. New York: Marcel Dekker
20. Zeidler, R. B., Kim, H. D. 1985. Phagocytosis, chemiluminescence, and cell volume of alveolar macrophages from neonatal and adult pigs. *J. Leuk. Biol.* 37:29–43
21. Eckert, H., Lux, M., Lachmann, B. 1983. The role of alveolar macrophages in surfactant turnover. *Lung* 161:213–18
22. Bowden, D. H. 1973. The alveolar macrophage. *Curr. Top. Pathol.* 55:1–36
23. Brain, J. D. 1970. Free cells in the lungs: Some aspects of their role, quantitation, and regulation. *Arch. Intern. Med.* 126: 447–87
24. Hocking, W. G., Golde, D. W. 1979. The pulmonary-alveolar macrophage. *N. Eng. J. Med.* 301:580–87
25. Hocking, W. G., Golde, D. W. 1979. The pulmonary-alveolar macrophage. *N. Eng. J. Med.* 301:639–45
26. Cohn, Z. A., Wiener, E. 1963. The particulate hydrolases of macrophages. I. Comparative enzymology, isolation, and properties. *J. Exp. Med.* 18:991–1008
27. Leake, E. S., Gonzalves-Ojeda, D., Myrvik, Q. N. 1964. Enzymatic difference between normal alveolar macrophages and oil-induced peritoneal macrophages obtained from rabbits. *Exp. Cell Res.* 33:553–61
28. Sorber, W. A., Leake, E. S., Myrvik, Q. N. 1974. Isolation and characterization of hydrolase-containing granules from rabbit lung macrophages. *J. Reticuloendothel. Soc.* 16:184–92
29. Yarborough, D. J., Meyer, O. T., Dannenberg, A. M. Jr., Pearson, B. 1967. Histochemistry of macrophage hydrolases. III. Studies of β-galactosidase, β-glucuronidase and aminopeptidase with inodolyl and naphthyl substrates. *J. Reticuloendothel. Soc.* 4:390–408
30. Franson, R. C., Waite, M. 1973. Lysosomal phospholipases A_1 and A_2 of normal and bacillus Calmette Guerin-induced alveolar macrophages. *J. Cell Biol.* 56:621–27
31. Sethi, K. K. 1982. Intracellular killing of parasites by macrophages. *Clin. Immunol. Allergy* 2:541–65
32. Rodgers, B. C., Mims, C. A. 1982. Role of macrophage activation and interferon in the resistance of alveolar macrophages from infected mice to influenza virus. *Infect. Immun.* 36:1154–59
33. Rose, R. M., Crumpacker, C., Waner, J. L., Brain, J. D. 1982. Murine cytomegalovirus pneumonia: Description of a model and investigation of pathogenesis. *Am. Rev. Respir. Dis.* 125:568–73
34. Rose, R. M., Crumpacker, C., Waner, J. L., Brain, J. D. 1983. Treatment of murine cytomegalovirus pneumonia with acyclovir and interferon. *Am. Rev. Respir. Dis.* 127:198–203
35. Babior, B. M. 1980. The role of oxygen radicals in microbial killing by phagocytes. In *The Reticuloendothelial System. A Comprehensive Treatise*, Vol. II, *Biochemistry and Metabolism*, ed. A. J. Sbarra, R. R. Strauss, pp. 339–54. New York: Plenum
36. Klebanoff, S. J. 1980. Myeloperoxidase-mediated cytotoxic systems. See Ref. 35, pp. 279–308
37. Werb, Z. 1983. How the macrophage

regulates its extracellular environment. *Am. J. Anat.* 166:237–56
38. Unanue, E. R. 1982. Symbiotic relationships between macrophages and lymphocytes. In *Macrophages and Natural Killer Cells,* ed. S. J. Normann, E. Sorkin, pp. 49–63. New York: Plenum
39. DeShazo, R. D., Banks, D. E., Diem, J. E., Nordberg, J. A., Baser, Y., et al. 1983. Bronchoalveolar lavage cell–lymphocyte interactions in normal nonsmokers and smokers. *Am. Rev. Respir. Dis.* 127:545–48
40. Laurenzi, G. A., Berman, L., First, M., Kass, E. H. 1964. A quantitative study of the deposition and clearance of bacteria in the murine lung. *J. Clin. Invest.* 43:759–68
41. Green, G. M., Kass, E. H. 1964. Factors influencing the clearance of bacteria by the lung. *J. Clin. Invest.* 43:769–76
42. Goldstein, E., Eagle, M. C., Hoeprich, P. D. 1973. Effect of nitrogen dioxide on pulmonary bacterial defense mechanisms. *Arch. Environ. Health.* 26:202–4
43. Laurenzi, G. A., Guarneri, J. J., Endriga, R. B., Carey, J. P. 1963. Clearance of bacteria by the lower respiratory tract. *Science* 142:1572–73
44. Spurgash, A., Ehrlich, R., Petzold, R. 1968. Effect of cigarette smoke on resistance to respiratory infection. *Arch. Environ. Health* 16:385–90
45. Goldstein, E., Lippert, W., Warshauer, D. 1974. Pulmonary alveolar macrophage. Defender against bacterial infection of the lung. *J. Clin. Invest.* 54:519–28
46. Brain, J. D., Corkery, G. C. 1977. The effect of increased particles on the endocytosis of radiocolloids by pulmonary macrophages *in vivo:* competitive and toxic effects. In *Inhaled Particles and Vapours,* IV, ed. W. H. Walton, pp. 551–64. London: Pergamon
47. Beck, B. D., Brain, J. D., Bohannon, D. 1982. An *in vivo* hamster bioassay to assess the toxicity of particulates for the lungs. *Toxicol. Appl. Pharmacol.* 66:9–29
48. Brain, J. D., Bloom, S. B., Valberg, P. A., Gehr, P. 1984. Behavior of magnetic dusts in the lungs of rabbits correlates with phagocytosis. *Exp. Lung Res.* 6: 115–31
49. Gehr, P., Brain, J. D., Nemoto, I., Bloom, S. B. 1983. Behavior of magnetic particles in hamster lungs: estimates of clearance and cytoplasmic motility. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* 55:1196–202
50. Brain, J. D., Gehr, P., Valberg, P. A., Bloom, S. B., Nemoto, I. 1985. Biomagnetism in the study of lung function. In *Biomagnetism: Applications and Theory,* ed. H. Weinberg, G. Stroink, T. Kattila, pp. 378–87. New York: Pergamon
51. Gehr, P., Brain, J. D., Bloom, S. B. 1983. Magnetometry: a tool to study intracellular movement. *J. Cell Biol.* 97: 194a
52. Valberg, P. A. 1984. Magnetometry of ingested particles in pulmonary macrophages. *Science* 224:513–16
53. Gersing, R., Schumacher, H. 1955. Experimentelle Untersuchungen über die Staubphagozytose. *Beitr. Silikose-Forsch.* 25:31–34
54. LaBelle, C. W., Brieger, H. 1959. Synergistic effects on aerosols. II. Effects on rate of clearance from the lung. *Arch. Indust. Health* 20:100–5
55. LaBelle, C. W., Brieger, H. 1960. The fate of inhaled particles in the early post-exposure period. 1:432–37
56. Myrvik, Q. N., Leake, E. S., Fariss, B. 1961. Studies on pulmonary alveolar macrophages from the normal rabbit: A technique to procure them in a high state of purity. *J. Immunol.* 86:128–32
57. Brain, J. D., Frank, R. 1973. Alveolar macrophage adhesion: wash electrolyte composition and free cell yield. *J. Appl. Physiol.* 34:75–80
58. Burns, D. M., Shure, D., Francoz, R., Kalafer, M., Harrel, J., et al. 1983. The physiologic consequences of saline lobar lavage in healthy human adults. *Am. Rev. Respir. Dis.* 127:695–701
59. Low, R. B., Davis, G. S., Giancola, M. S. 1978. Biochemical analyses of bronchoalveolar lavage fluids of normal healthy volunteers. *Am. Rev. Respir. Dis.* 118:863–76
60. Brain, J. D. 1971. The effects of increased particles on the number of alveolar macrophages. In *Inhaled Particles* III, ed. W. H. Walton, pp. 209–25. London: Unwin
61. Plowman, P. N. 1982. The pulmonary macrophage population of human smokers. *Ann. Occup. Hyg.* 25:393–405
62. Kavet, R. I., Brain, J. D. 1980. Methods to quantify endocytosis: a review. *J. Reticuloendothel. Soc.* 27:201–21
63. Parod, R. J., Brain, J. D. 1982. Uptake of latex particles by macrophages: characterization using flow cytometry. *Am. J. Physiol. 245 (Cell Physiol. 14):* C220–26
64. Parod, R. J., Brain, J. D. 1983. Uptake of latex particles by pulmonary mac-

rophages: role of calcium. *Am. J. Physiol. 245 (Cell Physiol. 14):* C227–34
65. Watson, A. Y., Brain, J. D. 1979. Uptake of iron oxide aerosols by mouse airway epithelium. *Lab. Invest.* 40:450–59
66. Castranova, V., Bowman, L., Reasor, M. J., Lewis, T., Tucker, J., Miles, P. R. 1985. The response of rat alveolar macrophages to chronic inhalation of coal dust and/or diesel exhaust. *Environ. Res.* 36:405–19
67. DeVries, C. R., Ingram, P., Walker, S. R., Linton, R. W., Gutknecht, W. F., Shelburne, J. D. 1983. Acute toxicity of lead particulates on pulmonary alveolar macrophages. Ultrastructural and microanalytical studies. *Lab. Invest.* 48:35–44
68. Harington, J. S., Allison, A. C. 1977. Tissue and cellular reactions to particles, fibers, and aerosols retained after inhalation. In *Handbook of Physiology, Section 9, Reactions to Environmental Agents,* ed. H. L. Falk, S. D. Murphy, pp. 263–83. Bethesda, Md: Am. Physiol. Soc.
69. Turino, G. M., Rodriquez, J. R., Greenbaum, L. M., Mandl, I. 1974. Mechanisms of pulmonary injury. *Am. J. Med.* 57:493–505
70. Snider, G. L. 1983. *Emphysema.* In *Clinics in Chest Medicine,* Vol. 4. No. 3. Philadelphia: Saunders
71. Warheit, D. B., George, G., Hill, L. H., Snyderman, R., Brody, A. R. 1985. Inhaled asbestos activates a complement-dependent chemoattractant for macrophages. *Lab. Invest.* 52:505–14
72. Snella, M-C. 1985. Manganese dioxide induces alveolar macrophage chemotaxis for neutrophils in vitro. *Toxicology* 34:153–59
73. Laurell, C. B., Erikson, S. 1963. The electrophoretic α_1-globulin pattern of serum in α_1-antitrypsin deficiency. *Scand. J. Clin. Lab Invest.* 15:132–40
74. Wahl, L. M., Wahl, S. M., Mergenhagen, S. E., Martin, G. R. 1975. Collagenase production by lymphokine-activated macrophages. *Science* 187:261–63
75. Werb, Z., Gordon, S. 1975. Elastase secretion by stimulated macrophages. Characterization and regulation. *J. Exp. Med.* 142:361–77
76. Wharton, W. 1983. Human macrophage-like cell line U937-1 elaborates mitogenic activity for fibroblasts. *J. Reticuloendothel. Soc.* 33:151–56
77. White, R., Lin, H. S., Kuhn, C., III. 1977. Elastase secretion by peritoneal exudative and alveolar macrophages. *J. Exp. Med.* 146:802–8
78. Kuhn, C. III, Senior, R. M. 1978. The role of elastase in the development of emphysema. *Lung* 155:188–97
79. Kuhn, C. III, Senior, R. M., Pierce, J. A. 1982. The pathogenesis of emphysema. In *Mechanisms in Respiratory Toxicology,* Vol. 2, ed. H. Witschi, P. Nettesheim, pp. 155–211. Boca Raton, Fla: CRC
80. Rodriguez, F. J., White, R. R., Senior, R. M., Levine, E. A. 1977. Elastase release from human alveolar macrophages: comparison between smokers and nonsmokers. *Science* 198:313–14
81. Janoff, A., Raju, L., Dearing, R. 1983. Levels of elastase activity in bronchoalveolar lavage fluids of healthy smokers and nonsmokers. *Am. Rev. Respir. Dis.* 127:540–44
82. Johanson, W. G. Jr., Pierce, A. K. 1972. Effects of elastase, collagenase, and papain on structure and function of rat lungs *in vitro. J. Clin. Invest.* 51:288–93
83. Kaplan, P. D., Kuhn, C., Pierce, J. A. 1973. The induction of emphysema with elastase. I. The evolution of the lesion and the influence of serum. *J. Lab. Clin. Med.* 82:349–56
84. Snider, G. L., Hayes, J. A., Franzblau, C., Kagan, H. M., Stone, P. J., Korthy, A. K. 1974. Relationship between elastolytic activity and experimental emphysema-inducing properties of papain preparations. *Am. Rev. Respir. Dis.* 110:254–62
85. Weinbaum, G., Marco, V., Ikeda, T., Mass, B., Meranze, D. R., Kimbel, P. 1974. Enzymatic production of experimental emphysema in the dog: route of exposure. *Am. Rev. Respir. Dis.* 109: 351–57
86. Lieberman, J. 1973. Involvement of leukocytic proteases in emphysema and antitrypsin deficiency. *Arch. Environ. Health* 27:196–200
87. Lieberman, J., Gawad, M. A. 1971. Inhibitors and activators of leukocytic proteases in purulent sputum: digestion of human lung and inhibition by alpha$_1$-antitrypsin. *J. Lab. Clin. Med.* 77:713–27
88. Riley, D. J., Kerr, J. S. 1985. Oxidant injury of the extracellular matrix: potential role in the pathogenesis of pulmonary emphysema. *Lung* 163:1–13
89. Weitberg, A. B., Weitzman, S. A., Destrempes, M., Latt, S. A., Stossel, T. P. 1983. Stimulated human phagocytes produce cytogenetic changes in cultured mammalian cells. *N. Engl. J. Med.* 308:25–29
90. Tryka, A. F., Godleski, J. J., Brain, J.

D. 1984. Alterations in alveolar macrophages in hamsters developing pulmonary fibrosis. *Exp. Lung Res.* 7:41–52
91. Davis, J. K. G. 1973. Are ferruginous bodies an indication of atmospheric pollution by asbestos? In *Biological Effects of Asbestos*. ed. P. Bogovski, J. C. Gilson, V. Timbrell, J. C. Wagner, pp. 238–42. Lyons, France: IARC
92. Richards, R. J., Morris, T. G. 1973. Collagen and mucopolysaccharide production in growing lung fibroblasts exposed to chrysotile asbestos. *Life Sci.* 12:441–51
93. Allison, A. C. 1973. Effects of asbestos particles on macrophages, mesothelial cells and fibroblasts. See Ref. 91, pp. 89–93
94. Maroudas, N. G. 1973. Chemical and mechanical requirements for fibroblast adhesion. *Nature* 244:353–54
95. Allison, A. C., Harington, J. S., Birbeck, M. 1966. An examination of the cytotoxic effects of silica on macrophages. *J. Exp. Med.* 124:141–54
96. Heppleston, A. G., Styles, J. A. 1967. Activity of a macrophage factor in collagen formation by silica. *Nature* 214: 521–24
97. Leibovich, S. J., Ross, R. 1976. A macrophage-dependent factor that stimulates the proliferation of fibroblasts *in vitro*. *Am. J. Pathol.* 84:501–4
98. Aho, S., Kulonen, E. 1977. Effect of silica-liberated macrophage factors on protein synthesis in cell-free systems. *Exp. Cell Res.* 104:31–38
99. Burrell, R., Anderson, M. 1973. The induction of fibrosis by silica-treated alveolar macrophages. *Environ. Res.* 6:389–94
100. Bateman, E. D., Emerson, R. J., Cole, P. J. 1982. A study of macrophage mediated initiation of fibrosis by asbestos and silica using a diffusion chamber technique. *Br. J. Exp. Pathol.* 63:414–25
101. Reiser, K. M., Last, J. A. 1979. Silicosis and fibrogenesis: Fact and artifact. *Toxicology* 13:51–72
102. Kirkpatrick, C. H., Reynolds, H. Y., eds. 1976. *Immunologic and Infectious Reactions in the Lung* (Lung Biology in Health and Disease, Monograph 1). New York: Dekker