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MECHANISMS *in* RESPIRATORY TOXICOLOGY

Volume II

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Mechanisms in Respiratory Toxicology

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PREFACE

This second volume of *Mechanisms in Respiratory Toxicology* is not meant to stand by itself. Rather much of the material discussed can only be fully appreciated in the context of the subjects treated in Volume I. Volume I deals with the structural elements of the lung, the ways toxic agents gain access to the lung, and the way the lung reacts to chemical injury.

This volume discusses endogenous modulating factors, pulmonary defense mechanisms, bioactivation of toxic agents as well as the many long-term consequences of pulmonary injury, namely emphysema and fibrosis. Again emphasis is placed on the general principles underlying injury and disease development rather than on discussion of individual toxic chemicals.

Hopefully, the two volumes will provide useful information as well as an incentive to the experimental toxicologist to study further the many fascinating aspects of lung damage produced by chemicals.

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Modifying Factors and Events

Chapter 1

ALVEOLAR MACROPHAGE TOXICOLOGY

Arnold R. Brody and Gerald S. Davis

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I. INTRODUCTION

The pulmonary alveolar macrophage (AM) utilizes its phagocytic and lytic properties to provide effective defense of the respiratory membrane against inhaled particles and microorganisms.[1] The AM participates in lung defense, inflammation, and immune responses by

1. Isolating ingested particles by means of phagocytosis
2. Acting as a vehicle for physical transport of substances out of the lung
3. Detoxifying inhaled and phagocytized material
4. Presenting antigens to lymphocytes
5. Releasing factors which attract other inflammatory cells
6. Responding to immunologic signals from sensitized lymphocytes

There is a growing body of evidence suggesting that abnormal macrophage function or macrophage death can mediate certain pulmonary diseases.[1-3] In this chapter, we will consider the toxic responses of macrophages to a variety of inhaled particles and gases and discuss the known mechanisms of macrophage toxicology as they relate to pulmonary disease.

II. ORIGIN, FATE, AND VARIABLE MORPHOLOGY OF THE PULMONARY MACROPHAGE

It is now well understood that pulmonary macrophages originate from bone marrow-derived, blood-borne monocytes which differentiate into large aerobic cells upon arrival in the lung. Recent reviews by Brain et al.,[3] Sorokin,[4] and Green et al.[1] describe in detail the origin and fate of lung macrophages and their role in pulmonary host defenses. Briefly, it has been shown that dividing promonocytes of the bone marrow give rise to circulating monocytes which leave the bloodstream at a rapid rate (8.4 hr half-time in man[5]) and enter perivascular connective tissues. The macrophages of the alveolar airspace may originate directly from blood monocytes[3,6-8] or from a local proliferating cell population within the lung.[9] It appears that these interstitial macrophages may or may not divide, depending on a variety of stimuli such as increased load of phagocytized particles, with some of the progeny migrating to the air spaces and others remaining in the interstitium.[3,10] This hypothesis requires further confirmation.

Morphologic studies of normal and pathologic tissues have shown that interstitial macrophages and monocytes move through alveolar basement membranes and between alveolar lining cells during migration to the air spaces,[11,12] as shown in Figure 1. It is not clear whether the different morphologic and functional types of AMs which comprise this heterogenous population all arise from a single type of monocyte precursor and whether the different cell types represent intermediate sequences or final stages of differentiation. The factors governing macrophage proliferation and differentiation also are unknown.

Once a macrophage has entered the alveolar space, the choices for its pathway out of the lung are limited. The particles, gases, and other substances which the AM confronts in the alveolus may, in part, determine its lifespan and fate. Most AMs exit from the lung by way of the mucociliary escalator to be ultimately swallowed or expectorated. It remains unknown whether particle-laden macrophages can reenter the interstitial space from the alveolus in reverse of the interepithelial migration route. The question then arises as to how particle-laden macrophages collect in the pulmonary interstitium and lymph nodes if they did not carry the dust there. Animal and in vitro

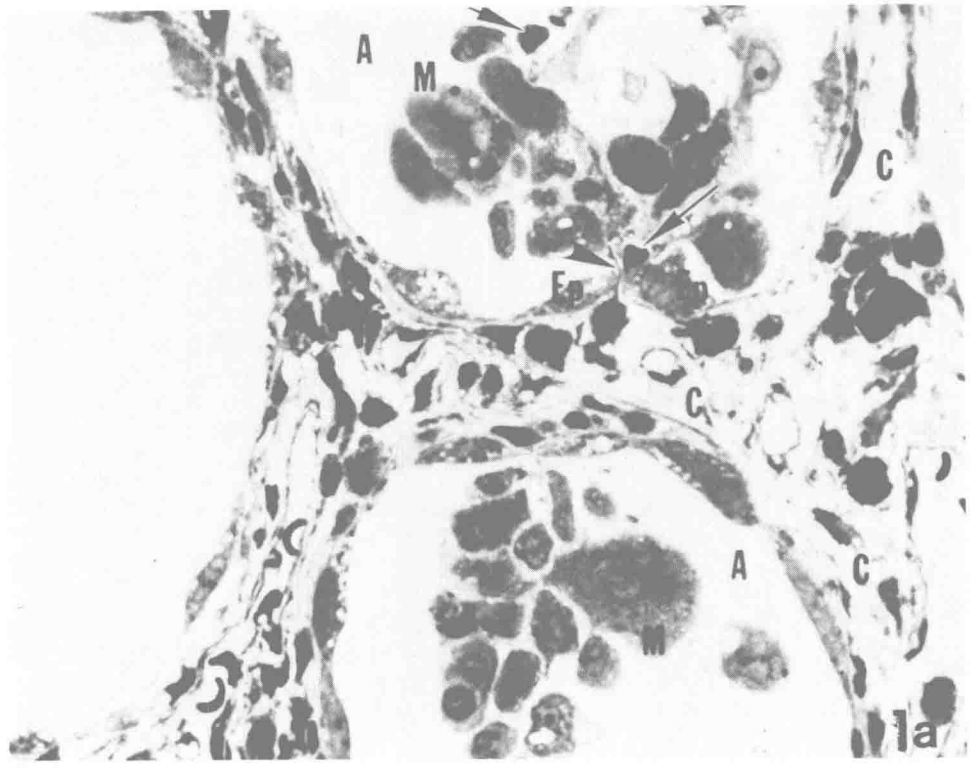

FIGURE 1A. Light micrograph of plastic-embedded lung tissue from an individual with interstitial fibrotic lung disease. Macrophages (M) and lymphocytes (arrows) are in the small airspaces (A) lined by epithelial cells (Ep). Increased connective tissue (C) is seen in the interstitium. An interstitial macrophage has a cytoplasmic process (arrowhead) extending between two epithelial cells and into the airspace. Perhaps this cell is migrating into the alveolar space. (Original magnification × 320.)

models of particle exposure have shown that particulates can cross the alveolar and bronchial epithelium directly, thus entering the interstitium without transport by macrophages.[13,14] Thus, particle-laden interstitial macrophages which accumulate following exposure could have phagocytized the dust in the interstitium. They may have carried the dust with them from the air spaces, although morphologic evidence has not yet been presented to confirm this hypothesis. Furthermore, Lauweryns and Baert[15] demonstrated that particles may move from the pulmonary interstitium to small lymphatics without the aid of macrophages. Adamson and Bowden[16] found no evidence of carbon-laden macrophages moving from the air spaces to interstitium. Our work has shown consistently that migrating macrophages have a configuration suggestive of movement toward the air spaces (see Figure 1). Thin pseudopods appear to lead the larger mass of the cell which contains the nucleus and most of the organelles.

There seems to be little doubt that the majority of AMs are cleared by means of the mucociliary escalator. The mechanism by which the macrophages move onto the escalator is poorly understood, and it is not known if different subpopulations of cells (e.g., necrotic or particle-laden) are cleared at different rates.

There is no evidence that pulmonary macrophages migrate back into the vasculature. Again, the presence of particle-laden macrophages in the blood and lymphatic vessels as well as in lymph nodes and subpleural connective tissue could reflect the phagocytosis of extracellular particles by resident or circulating phagocytes. It is likely that many such particles were ingested by macrophages which subsequently died, thus re-

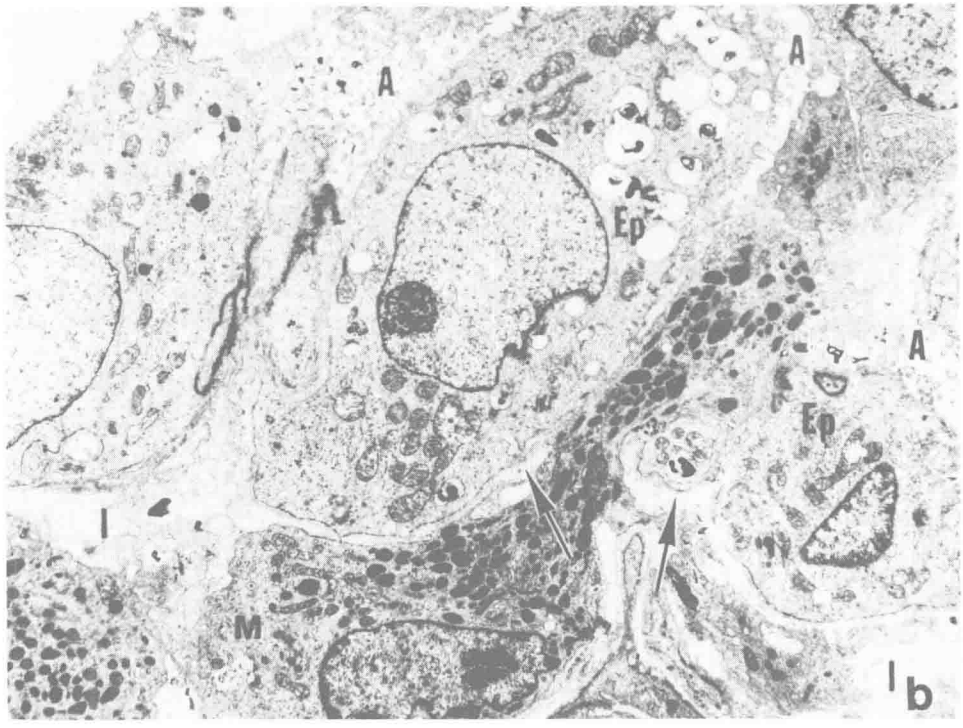

FIGURE 1B. Transmission electron micrograph from lung tissue similar to that described above. A macrophage (M) with numerous electron-dense lysosomes is in the process of migrating from the interstitium (I). It has passed through the basement membrane (arrows) and between cuboidal epithelial lining cells (Ep) on the way to a small airspace (A). (Original magnification × 7000.)

leasing the particles for phagocytosis by new cells at different sites. This is a common event caused by toxic particulates and will be discussed in more detail below.

Some populations of macrophages may remain in the lung for long periods of time. It is known that exposure to a diffuse-dust aerosol results in focal accumulations of the particulates,[17] and certain tissue macrophages appear to sequester particulates in various anatomic compartments in the lung (see Figure 2). The ultimate fate of these cells and intracellular particles and how long they remain in the lung are not known. It is likely that the pathogenic nature of some particles is altered by increased residence time in the lung. Some particles could be rendered inert, while others might exhibit enhanced cytoxic properties.

AMs are cells of varying morphology and states of activation. Light and electron microscopic techniques have shown that they generally are large cells (15 to 30 μm) with a single kidney-shaped nucleus, numerous lysosomes, and a well-developed rough endoplasmic reticulum (see Figure 1). These internal features are quite variable, depending upon the phagocytic activity and environment of the cell. There seem to be several subpopulations of macrophages, although we know very little about this phenomenon.[3] It is difficult to study AMs *in situ*, as most fixation techniques displace the cells from their normal position on the alveolar walls. Within these limitations, it is possible to recognize macrophages *in situ* as being flat or round, ruffled or smooth, and in varying combinations of these conditions (see Figure 3). Interestingly enough, AMs collected from human and animal lungs by bronchoalveolar lavage show similar surface variability in vitro (see Figure 4). We do not know the significance of this morphologic variance, but studies are in progress to address the basic question of how

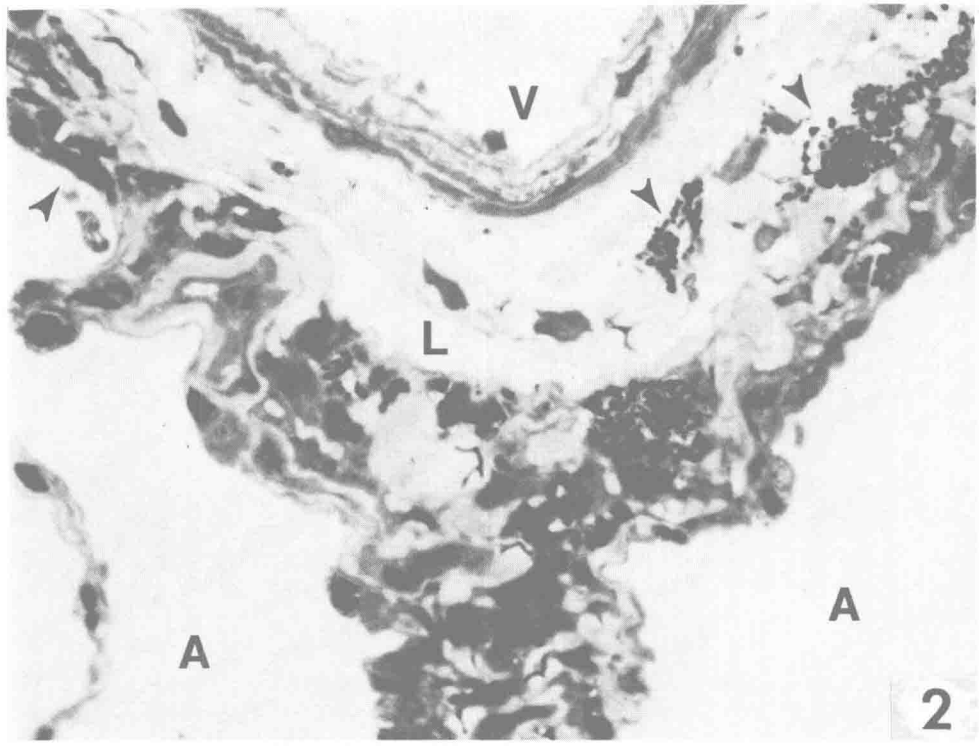

FIGURE 2. Light micrograph of plastic-embedded lung tissue from a cigarette smoker. It is not unusual to find accumulations of anthracotic pigment (arrowheads) in macrophages and connective tissue of perivascular and perilymphatic regions. The lumens of a small arterial vessel (V), a lymphatic channel (L), and air spaces (A) are seen. (Original magnification × 320.)

macrophage form relates to function. For example, human AMs which are flat in vitro phagocytize more iron particles than do round cells[18] (see Figure 4).

The AM resides in a unique microenvironment. It functions in the humidified gas phase of the alveolar space, adherent to the surface and bathed in the lipoproteins and serum constituents of the alveolar lining material. It is a highly aerobic cell, dependent on oxidative phosphorylation and relatively high oxygen tensions.[19] It cannot metabolize or phagocytize effectively in an hypoxic liquid environment[20] in which polymorphonuclear (PMN) leukocytes perform normally. It is distinctly different from the PMN or the peritoneal macrophage, and studies using these latter cell types may not accurately predict AM behavior.

In summary, available data indicate that most AMs originate from the bone marrow and are borne by the blood to the lung where they migrate to the interstitium. Here the cells may or may not divide, depending upon various stimuli, and those phagocytes destined to be AMs migrate through basement membranes and between epithelial cells to the small airspaces. A variety of morphologic and functional types of AMs may be recovered from the airspaces. An apparently distinct population of phagocytes remains in the pulmonary interstitium where they undergo mitotic division. Macrophages generally are cleared from the lung by the mucociliary escalator of the airways. Other clearance pathways may be operative, but are poorly understood.

III. ALVEOLAR MACROPHAGES AND PARTICLES

The AM confronts and ingests a variety of particles in its role as the primary phagocyte of the lung (see Figure 5). The events which accompany phagocytosis and the

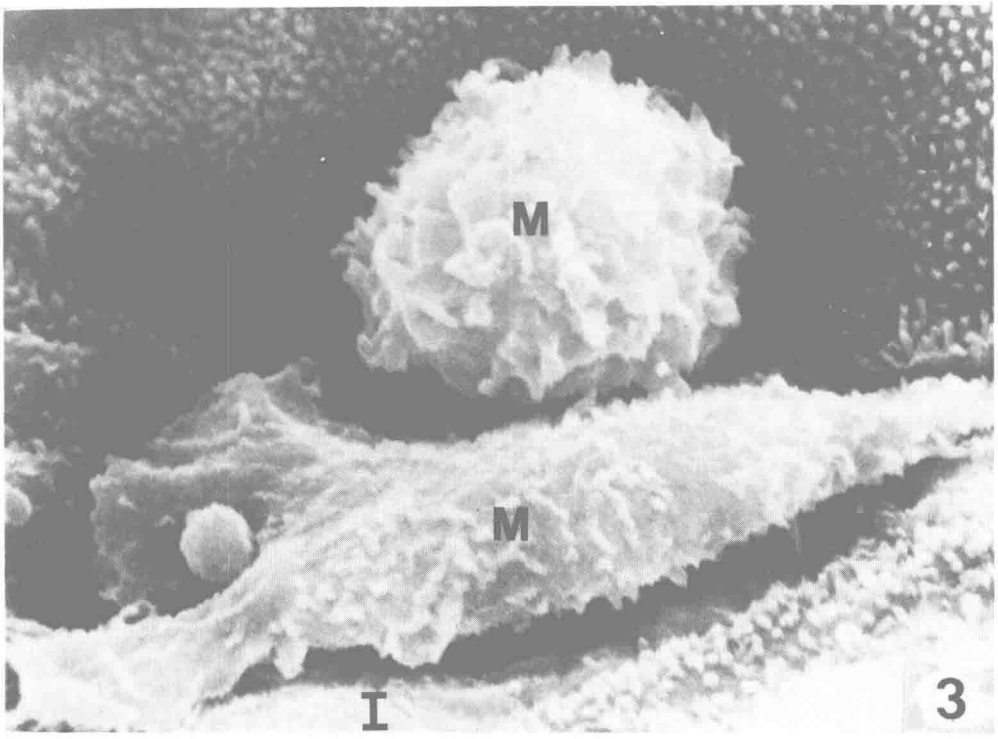

FIGURE 3. Scanning electron micrograph of two AMs (M) in an air space from normal human lung. A cuboidal Type II epithelial cell (II) with numerous microvilli is seen in the background. The migrating macrophage in the foreground is on an attenuated Type I (I) cell. These macrophages appear healthy with numerous surface ruffles and intact membranes. (Original magnification × 8600.)

effects of ingested particles on the macrophages may be indirect determinants of respiratory disease. The AM contains a rich store of lysosomal enzymes with proteolytic, elastolytic, and inflammatory properties.[21,22] Digestive enzymes and other factors derived from macrophages produce emphysema,[23,24] pulmonary fibrosis,[25-27] and inflammation.[25,28] It is well known that the AM is responsible for initial defense of the lung against inhaled microorganisms.[29]

Both organic and inorganic particles which reach the respiratory surface are ingested rapidly by AMs. Many toxic particles can express their effects through macrophages inasmuch as phagocytosis results in the release of lysosomal enzymes and other substances. Such active substances may be released from macrophages consequent to cell disintegration, as is seen following silica ingestion (see discussion below), or may be selectively released without cell injury, as occurs during phagocytosis of starch granules.[30] We have studied the release of cell contents from AMs during phagocytosis of a variety of particles. The appearance of certain marker enzymes was measured in the medium surrounding cells in monolayer tissue culture. Beta-glucuronidase (β-G) and other enzymes were used as markers of lysosomal enzyme release, while lactic dehydrogenase (LDH) activity was used as an index of leakage of cytoplasmic contents, resulting from loss of cell membrane integrity. Appearance of β-G in the supernatant medium without increase LDH activity was interpreted as selective lysosomal enzyme release without cell injury. Parallel increases of both these enzymes in the medium was interpreted as indicating cytotoxicity. Table 1 summarizes the effects of phagocytosis of several crystalline, organic, and mineral particles on guinea pig AMs.