# Inflammatory and Immune Processes in the Human Lung in Health and Disease: Evaluation by Bronchoalveolar Lavage

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**Bronchoalveolar lavage is an invaluable means of accurately evaluating the inflammatory and immune processes of the human lung. Although lavage recovers only those cells and proteins present on the epithelial surface of the lower respiratory tract, comparison with open lung biopsies shows that these constituents are representative of the inflammatory and immune systems of the alveolar structures. With the use of these techniques, sufficient materials are obtained from normal individuals to allow characterization of not only the types of cells and proteins present but their functions as well. Such observations have been useful in defining the inflammatory and immune capabilities of the normal lung and provide a basis for the study of lung disease. Lavage methods have been used to characterize inflammatory and immune processes of the lower respiratory tract in destructive, infectious, neoplastic, and interstitial disorders. From the data already acquired, it is apparent that bronchoalveolar lavage will yield major insights into the pathogenesis, staging, and therapy decisions involved in these disorders. (Am J Pathol 97:149–206, 1979)**

THE PRESENCE of inflammatory and immune effector cells within the alveolar structures of the human lung in health and disease has been appreciated for some time. However, the full extent of the inflammatory and immune armamentarium within the human lung only became apparent with the use of bronchoalveolar lavage to recover the cellular and protein constituents of the lower respiratory tract. The concept behind the use of bronchoalveolar lavage to study inflammatory and immune mechanisms in the alveolar structures can be summarized as follows: although inflammatory and immune effector cells and related proteins are present throughout the alveolar structures (Text-figure 1; Figures 1–3), the cells and noncellular components present on the epithelial surface of the alveoli are representative of the inflammatory and immune system of the entire lower respiratory tract. As we will see later in this review, this is now known to be a valid concept, at least in normal individuals and in those with a variety of chronic lung diseases. The tracheobronchial tree, therefore, is a "window" to the inflammatory and immune

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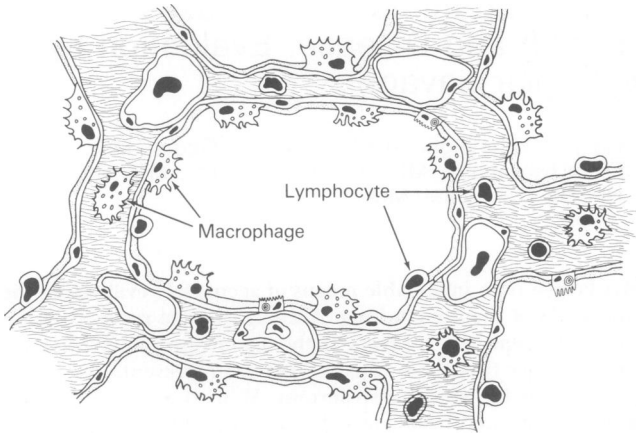


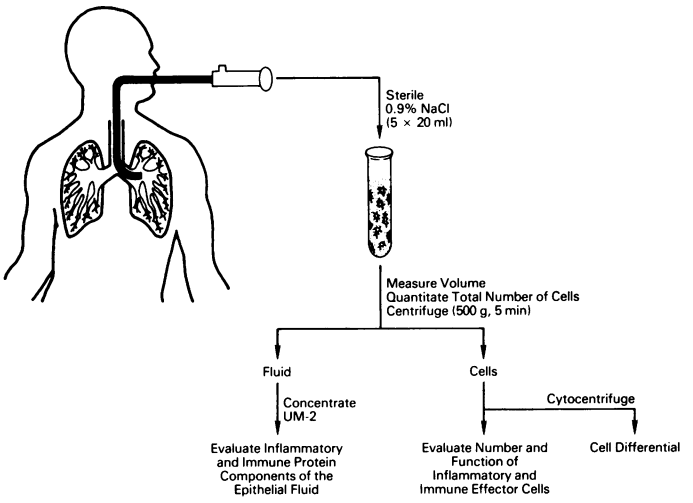

TEXT-FIGURE 1—Inflammatory and immune effector cells and related proteins of the alveolar structures of the normal lung. Most of the inflammatory and immune effector cells of the normal lung are alveolar macrophages; a smaller proportion of these cells are lymphocytes. Polymorphonuclear leukocytes (neutrophils, eosinophils, and basophils) are rarely found. Within the lung, the inflammatory and immune effector cells are present both on the epithelial surface of the alveoli and within the interstitium. Various proteins that are capable of participating in inflammatory and immune reactions are also found on the epithelial surface of the alveoli and within the interstitium of the lung.

systems of the alveolar structures, and bronchoalveolar lavage allows safe, repetitive sampling of various components of the inflammatory and immune system at the site of their action. Over the past 10 years, these techniques have allowed enormous insight into how the human pulmonary inflammatory and immune systems function both in health and disease.

#### Techniques of Bronchoalveolar Lavage

In early studies, sampling of the inflammatory and immune system of the lower respiratory tract was done through a rigid bronchoscope [1-4] or by a large balloon-tipped catheter wedged into a primary or secondary branch of the bronchial tree.[5-9] With the development of the fiberoptic bronchoscope, however, such evaluation can now be carried out with no risk and little discomfort to the subject. In the past 5 years, the Pulmonary Branch of the National Heart, Lung, and Blood Institute has performed more than 400 lavages using the following technique [10-16] (Text-figure 2).

After the upper airways and trachea are anesthetized with a xylocaine spray, an 8-mm fiberoptic bronchoscope (Olympus Model BF-B2, Olympus Corporation of America, New Hyde Park, NY) is inserted through the nose into the tracheobronchial tree. Following routine evaluation of the respiratory tract, the tip of the bronchoscope is wedged into a subsegmental bronchus of the lingula or right middle lobe. Other lobes can be used, but lavage of the upper lobe is more difficult because of the tight




TEXT-FIGURE 2—Methods of obtaining inflammatory and immune effector cells and related proteins from the alveolar structures by bronchoalveolar lavage. Following local anesthesia of the airways with 2% lidocaine spray, a fiberoptic bronchoscope is inserted into the lung and wedged into a peripheral airway of the right middle lobe or lingula. Sterile saline (5 × 20 ml aliquots) is then instilled through the bronchoscope and recovered by suction. The volume of fluid and the total number of cells recovered are quantitated, and the cells are separated from the fluid by centrifugation (at 500g for 4 minutes). Prior to evaluation of the fluid for the presence of various proteins, the fluid is concentrated with the use of a UM-2 Amicon ultrafiltration membrane. The cells obtained from bronchoalveolar lavage are utilized to quantitate the number and function of various inflammatory and immune effector cells. The proportion of the brochoalveolar cells that are alveolar macrophages, lymphocytes, or polymorphonuclear leukocytes are enumerated on a Wright–Giemsa–stained cytocentrifuge preparation of the cells.

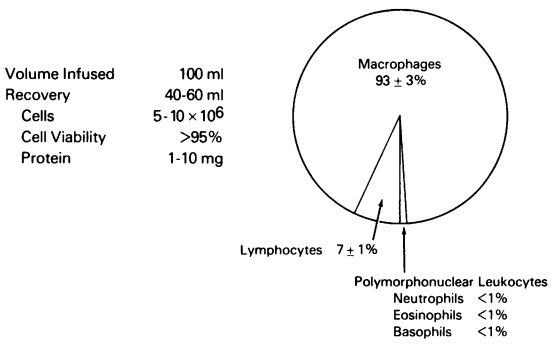
bend in the bronchoscope imposed by the anatomy of the upper lobe bronchi, and the amount of fluid recovered from the lower lobes is slightly less than that from the middle lobes. Once the bronchoscope is wedged, 20 ml of 0.9% sterile saline is inserted in the suction port by the use of a syringe on a three-way stopcock. The fluid is immediately pulled back with suction with the use of 50–100 mm Hg of negative pressure from a usual clinical suction apparatus (Ohio Intermittent Suction Unit, Ohio Medical Products, Madison, Wis), and the fluid is collected in a 50-ml specimen trap (Cheesebrough-Ponds Inc., Greenwich, Conn). This process of lavage and suction is then repeated five times (total fluid, 100 ml). In general, 40–60% of the infused volume is recovered.[10–16] In patients with destructive lung disease, particularly panacinar emphysema, the recovery is less, mostly because the bronchial walls collapse when the suction is applied. Although our routine is to use a total of 100 ml of lavage, we occasionally use up to 300 ml fluid if it is necessary to obtain larger numbers of inflammatory and immune cells in a particular subject. Although some in-

vestigators use larger amounts, in our experience increased patient morbidity may result, particularly local atelectasis and, occasionally, transient fevers.

Following lavage, the fluid is immediately placed at 4 C and the volume measured. If mucus is present (a rare occurrence in individuals without inflammatory airway disease), the fluid is strained through one layer of surgical gauze. A small portion is taken for a cell count; this is accomplished with a hemocytometer or a Coulter Counter (Model FN, Coulter Electronics, Hialeah, Fla). The fluid is then removed from the cells by centrifugation (500g, 15 minutes) and the cell pellet resuspended in Hanks' balanced salt solution (without $Ca^{++}$ or $Mg^{++}$) at the desired cell density.

At this stage, the fluid is frozen and kept at liquid nitrogen vapor temperatures until subsequent use. A differential count is made of the cell suspension by the use of previously described techniques.[10–16] Usually we employ a Wright–Giemsa–stained cytocentrifuge preparation (Cytospin, Shanden Southern Instruments, Sewickley, Pa), and a total of 200 cells is counted. The major problem encountered by those inexperienced with the morphology of lung inflammatory and immune effector cells is how to distinguish epithelial cells from macrophages and small macrophages from large lymphocytes; in this situation, it is best to expose a small portion of the cells to a phagocytic stimulus such as oil red O, neutral red dye, or latex beads, and then determine the percentage of phagocytic cells by the use of both phase optics and routine light microscopy. With experience, the difference between these cells becomes apparent, and the cytocentrifuge Wright–Giemsa stain can be relied upon without the need for other methods.

After the sample is taken for the differential count, the remaining cells are available for a variety of morphologic and functional studies that will be described subsequently. When the fluid is eventually analyzed, the sample is thawed and concentrated by one of several different techniques. Usually we employ pressure filtration with an Amicon apparatus (Amicon EC-20, Amicon Corporation, Lexington, Mass) and a UM2 membrane (molecular weight cutoff, 2000 daltons). In some instances, when the component to be analyzed is less than 2000 daltons, or if it may adhere to the Amicon membrane, the fluid is concentrated by oncotic methods (Aquacide, Calbiochem, San Diego, Calif) or by lyophilization and resuspension. Because the noncellular constituents of the epithelial fluid are diluted with a variable amount of saline used in their recovery, quantitation of these constituents can present problems; how much investigators circumvent this problem will be dealt with in a later section.




TEXT-FIGURE 3—Characteristics of bronchoalveolar lavage of normal, nonsmoking individuals. Between 40 and 60 ml of the 100 ml of saline infused into the lung is recovered. The cell yield varies from 5–10 × $10^6$ cells per lavage; the viability of these cells is greater than 95%. The bronchoalveolar cells are composed of 93% ± 3% alveolar macrophages and 7% ± 1% lymphocytes. Polymorphonuclear leukocytes (neutrophils, eosinophils, and basophils) are rarely found in lavage fluid of normal, nonsmoking individuals. In addition to the cells, the lavage fluid also contains 1–10 mg protein.

## Inflammatory and Immune Processes in Normal Lung

With the use of the techniques described above, lavage of a normal adult human with 100 ml saline yields 40–60 ml of fluid containing 5–10 × $10^6$ cells and 1–10 mg protein (Text-figure 3).[10–16] It is not known how many alveoli are "washed out" during this process, but since normal lung contains a small number of inflammatory and immune effector cells per alveolus,[17] a significant number of alveoli are necessary to yield 5–10 × $10^6$ cells per 100 ml lavage. It is not surprising, therefore, that lavage of multiple sites within the same subject reveals a remarkable consistency from lavage to lavage.[13] Apparently enough alveoli are washed out that local differences from alveoli to alveoli can be averaged and a sample can be obtained that is "representative" of the lung as a whole. Even 25 ml is adequate to give a representative sample; sequential 25-ml lavages of the same site within minutes yield cell differentials within 3% of each other.[13] It is not known, however, whether volumes smaller than 25 ml will suffice. Although studies have been performed in adults with the use of as little as 10 ml,[4] there is always the danger that most of this small volume of fluid will never reach the alveoli (ie, sampling only small bronchi) [18] or that the relatively few alveoli sampled are not "representative" of the overall inflammatory and immune processes operating within the alveolar structures. Another problem in using small volumes is the theoretic possibility that lavage fluid will be contaminated with sufficient local anesthetic to impair the function of the inflammatory and immune effector cells recovered.[19] This is unlikely, however, since the effect of local anesthetics on cell function is reversible and can be removed by washing of the cells.[20] Thus, volumes of 100 to 300 ml seem to be a reasonable compromise, enough to be "representative" and avoid procedure-induced artifacts, yet low enough to keep the risk of the procedure at a minimum.

**General Classification of Inflammatory and Immune Effector Cells in the Normal Alveolar Structures**

A differential cell count of the return of an average lavage of a normal adult reveals 93% ± 5% macrophages, 7% ± 1% lymphocytes, and less than 1% neutrophils, eosinophils, or basophils (Text-figure 3; Figures 4–6).[13-16,21] Earlier studies [10-12, 22-24] often gave values of the proportion of lymphocytes in the lavage fluid of normal individuals as up to 20%. However, it is likely that this was an overestimate, as macrophages vary greatly in diameter (10–40 μ) and the smaller macrophages are difficult to distinguish from lymphocytes, even in the Wright–Giemsa–stained preparation.[10] At present, the high normal percentage of lymphocytes in lavage fluid is considered to be in the 10–15% range, with most individuals falling under 10%.

Earlier reports also probably overestimated the proportion of polymorphonuclear leukocytes in normal nonsmoking individuals [12]; it is now thought that such cells are very rare in the alveoli of normal individuals [13-16,17,21] and represent less than 1% of the total. In support of this concept, direct analysis of lung biopsies of normal individuals has demonstrated that less than 1% of the total inflammatory and immune effector cells within the alveolar structure are polymorphonuclear leukocytes.[25-26] In addition, histologic evaluation of normal lung has demonstrated only 1–2 basophils or mast cells per square millimeter of tissue,[27] and analysis of the histamine content of bronchoalveolar lavage cells (as a measure of the number of basophils and/or mast cells) is consistent with that of such cells comprising less than 1% of the total bronchoalveolar inflammatory and immune effector cells.[28] When polymorphonuclear leukocytes (particularly neutrophils) are found in lavage fluid, the following is suggested: 1) there is contamination with blood secondary to bronchoscopic trauma (an unusual occurrence); 2) the subject is a smoker (see section on smoking be low); 3) the subject has a chronic lung disease associated with the accumulation of polymorphonuclear leukocytes in the alveolar structures (see section on interstitial lung disease below); or 4) inflammatory airway disease is present (ie, the polymorphonuclear leukocytes are washed off bronchi rather than alveoli). The problem of cells from inflammatory airway disease "contaminating" bronchoalveolar lavage fluid cannot be overemphasized. The usual fiberoptic bronchoscope is 8 mm in diameter and when wedged is placed only in a fourth- to sixth-order bronchus. Lavage fluid has to travel approximately 5–10 cm to reach the alveolar structures and thus passes over (in two directions) a large bronchial epithelial surface that may contain a variety of inflammatory and immune effector cells.[29]

Table 1—Properties and Functions of Normal Human Alveolar Macrophages

- Origin
  - Derived from bone marrow[34,15]
  - Capable of self-replication[35]
- Surface receptors
  - Fc-IgG[40–42]
  - C3b[40–42]
  - C3d[41]
  - Proteases–antiproteases[52]
- Interactions with microorganisms
  - Bacteria[6,8,15,44,53,54]
  - Fungi[8,15,44,54]
  - Viruses [55]
- Interactions with noninfectious particulates
  - Phagocytosis of particulates[44,58]
  - Pinocytosis[57]
  - Metabolism of ingested compounds[58,65–72]
- Effector and accessory cell in inflammatory and immune reactions
  - Responds to lymphokines[103,104]
  - Responds to chemotactic stimuli[105,106]
  - Regulates lymphocyte response to mitogens and antigens[100–102]
  - Secretes
    - Colony-stimulating factor[74]
    - Chemotactic factor for neutrophils[44,76–78]
    - Superoxide anion[53]
    - Platelet-activating factor[75]
    - Elastase[87]
    - Collagenase[86]
    - Neutral protease[84,90]
  - Mediates antibody-dependent cellular cytotoxicity [15]
- Protection of alveolar structures from protease attack
  - Contains $\alpha_1$-antiproteinase[7,108]
  - Cytosol inhibits human neutrophil elastase[110]
  - Ingests neutrophil elastase[52]

**Properties and Functions of Normal Human Alveolar Macrophages (Table 1)**

Alveolar macrophages are a population of cells that are a part of the so-called mononuclear phagocyte system.* In this classification tissue macrophages, monocytes, and promonocytes are considered part of a continuum in which alveolar macrophages, Kupffer cells, splenic macrophages, connective tissue "histiocytes," lymph-node macrophages, osteoclasts, and microglial cells are thought to be ultimately derived from promonocytes in the bone marrow. Perhaps the most convincing data for this concept derive from chimeric experiments in animals in which marrow with a unique genetic marker is placed into an animal of a different genetic type and the unique marker is later observed in various tissue macrophages.[31–33] Similar data have been obtained for alveolar macrophages in man; the chimeric experiment has been to evaluate the sex chromosomes of alveo-

*Originally termed the "reticuloendothelial system" or "reticulohistocytic system," macrophages, monocytes, and their precursor cells were reclassified in 1970 as the "mononuclear phagocyte system" in a World Health Organization study.[30]

lar macrophages in individuals receiving a bone marrow transplant from a histocompatible donor of the opposite sex. Such individuals are prepared for transplantation by the destruction of all of the bone marrow of host origin by cytotoxic drugs and radiation. Therefore, following a successful transplantation with a donor marrow, all of their circulating blood monocytes are of donor origin (ie, of the opposite sex). In a classic study of the origin of the human alveolar macrophage, Thomas et al,[34] using the techniques described above, showed that at least some of the alveolar macrophage population is derived from marrow precursors; three months after transplantation, a significant number of these cells were of donor origin (ie, of the sex opposite that of the recipient).

In such "chimeric" individuals, populations of alveolar macrophages of host origin (ie, of the same sex as the recipient of the marrow transplant) can remain within the lung for long periods of time. Studies in our laboratory, in collaboration with Drs. Albert Diesseroth and Jacqueline Weng-Ping, have demonstrated that 35% of the alveolar macrophages of a man who had received female marrow were still male 4.5 years following transplantation.[15] Thus, although it is reasonable to assume that originally all alveolar macrophages are derived from marrow, it is not clear that all alveolar macrophages present in lung at any one time are directly derived from bone marrow precursors. In this regard, Golde et al [35] have shown that human alveolar macrophages are capable of proliferating; 0.35–1.25% of these cells will incorporate $^{3}$H-thymidine in 30 minutes *in vitro*. Thus, it is likely that the population of alveolar macrophages can be sustained by two mechanisms: by recruitment from peripheral blood monocytes and by local proliferation.

If local proliferation is an important mechanism for sustaining the alveolar macrophage population in humans, it is likely that these cells replicate within the alveolar interstitium. This concept accrues from the recent morphometric studies by Barry et al [36] showing that approximately 6% of the cells within the alveolar interstitium are macrophages and the studies of Adamson and Bowden [37,38] showing that in animals macrophages proliferate in the interstitium and then migrate to the epithelial surface. However, the mechanism and route by which macrophages migrate from interstitium to alveoli has not been defined in man; presumably, these cells migrate through epithelial junctions, but this migration has not been carefully defined.[39] Likewise, the eventual fate of the human alveolar macrophage is not clear. Animal studies, however, suggest they either are swept up the tracheal–brochial tree by the so-called "mucociliary ladder" or move back into the interstitium and are taken via the lymphatics to the regional lymph nodes.[39]

### Surface Receptors

One way in which cells interact with their environment is through receptors on their external surface. Human alveolar macrophages are no exception; receptors for C3b, C3d, and the Fc portion of IgG have been identified.[40–42] Human alveolar macrophages, as well as other phagocytic cells, do not have receptors for IgM; however, they may indirectly interact with this immunoglobulin class through C3b generated by IgM antibody–antigen complexes.[41] Macrophages also lack receptors for IgA, and IgA immune complexes (such as secretory IgA-coating microorganisms) are apparently not recognized by these cells.[43]

In general, interaction of the alveolar macrophage with a particulate via the macrophage's IgG Fc or C3b receptor will lead to phagocytosis of the particulate. However, in some instances, particulates coated with C3b are not ingested by these cells; for example, human alveolar macrophages will not phagocytize erythrocytes coated with IgM and C3b but will ingest IgG-coated erythrocytes.[41,44] Such experiments also suggest that macrophage IgG and C3b receptors may act independently. This concept is supported by studies in which rabbit alveolar macrophages "loaded" with latex particles were shown to have decreased IgG, but not C3b, receptors.[40]

As with other cells of the mononuclear phagocytic system, it is likely that alveolar macrophages possess receptors for glucocorticoids,[45] $\beta$-adrenergic agonists,[46] antiprotease–protease complexes,[47,48] lysosomal glycosidases,[49] and lactoferrin.[50,51] As yet, there are few data concerning such receptors on human alveolar macrophages, but such information should be forthcoming. In this regard, Campbell et al [52] have recently shown that human macrophages have membrane receptors for human neutrophil elastase and for $\alpha_2$-macroglobulin–elastase complexes, suggesting that at least some of the elastase activity found in macrophages may be ingested rather than formed *de novo* (see below).

### Interactions With Microorganisms

One of the classic functions ascribed to alveolar macrophages is keeping the lower respiratory tract sterile by engulfing and killing microorganisms. Although most of these studies have been carried out using animal alveolar macrophages, it appears from the human data available that little, if any, species difference exists. Human alveolar macrophages can ingest bacteria, fungi,[8,15,44,54] and viruses.[55] These cells are also capable of inactivating viable staphylococci [8,15] and inhibiting the growth of certain viruses (eg, herpes simplex) but not others (eg, cytomegalovirus).[55]

Optimal ingestion of microorganisms by macrophages is mediated by

opsonins such as immunoglobulins or complement.[6,41,43] As with other mononuclear phagocytes, IgG acts as an opsonin by attaching to the microorganism through its antigen-combining site and to the alveolar macrophage through its Fc receptor.[41,43] C3b can also act as an opsonin for human macrophages, either by coating the microorganism alone or by associating with IgM that has already interacted with the infectious agent.[41]

When the human alveolar macrophage ingests microorganisms, it becomes activated, as manifested by increased oxygen uptake,[53] more rapid glucose utilization,[8,53] increased production of superoxide,[53] and release of the alveolar macrophage chemotactic factor for neutrophils.[44] For optimal function as phagocytic cells, human alveolar macrophages depend on both glycolysis and cytochrome electron transport.[6] Oxygen is not a mandatory requirement; macrophages cultured in a nitrogen atmosphere for 1 hour will still ingest microorganisms, as will macrophages incubated in a low-oxygen environment.[6] In comparison, the partial pressure of $CO_2$ in the environment (with constant pH) seems to have little effect on macrophage function.[6]

#### Interactions With Noninfectious Agents

Macrophages interact with a variety of noninfectious agents; in many instances, these agents are endocytosed by phagocytosis [15,44,56] or pinocytosis.[57] As with infectious materials, optimal phagocytosis of noninfectious agents is medicated by opsonins. However, macrophages can attach and phagocytize without opsonins; presumably, such attachment is medicated by charge interactions between the particle and the outer surface of the macrophage.[56] The majority of information in this area is derived from animal studies. *In vitro*, human alveolar macrophages interact with inorganic particulates such as asbestos [58] or sepharose 4B.[44] There is also evidence that such interactions occur *in vivo*, because individuals who have been exposed to various inorganic dusts have these same particulates within their alveolar macrophages.[59–62]

As with the ingestion of microorganisms, phagocytosis of nonfectious particulates causes a general activation of macrophages.[31] In addition, ingested particulates may injure the macrophage and eventually cause cell death. This process has been widely studied in animal macrophages in relation to silica [56] but has also been shown to be true for human alveolar macrophages that have ingested large concentrations of asbestos.[58]

Human alveolar macrophages also can ingest materials by pinocytosis.[57] This process is energy-dependent and does not require serum factors, at least in normal individuals. Presumably, pinocytosis is used by macro-

phages to engulf soluble substances or very small (< 0.1 $\mu$) particles. For example, alveolar proteinosis is a disorder associated with an accumulation of lipids and proteins within the alveoli; macrophages lavaged from individuals who have this disorder show large vacuoles containing lipid and other materials,[63,64] probably endocytosed by pinocytosis.

The knowledge that alveolar macrophages are capable of ingesting compounds in their environment has led to several studies concerned with the interactions of macrophages and carcinogens and putative precursor carcinogens. It is known, for example, that human alveolar macrophages contain aryl hydrocarbon hydroxylase, an enzyme capable of transforming aromatic polycyclic hydrocarbons (eg, benzo(a)pyrene and benzanthracene) present in cigarette smoke to compounds with altered carcinogenic properties.[58,65–72] Although enzymes such as aryl hydrocarbon hydroxylase may give the macrophage ways to deactivate potential carcinogens, there is some evidence that macrophages can metabolize carcinogens such as benzo(a)pyrene to more mutagenic compounds [71] and thus may potentiate the injury caused by inhaled materials.

### Effector and Accessory Cells in Inflammatory and Immune Reactions

There is increasing evidence that the alveolar macrophage plays a central role in pulmonary inflammatory and immune processes.[73] Animal studies have clearly identified a direct effector role for the macrophage as well as a role accessory to other immune effector cells.

Human alveolar macrophages can amplify an inflammatory response within the lung by producing colony-stimulating factor,[74] platelet activating factor,[75] and chemotactic factor for neutrophils.[44,76–78]

Colony-stimulating factor probably stimulates marrow to produce granulocytes and monocytes,[79] platelet-activating factor induces platelets to release their mediators,[80] and the alveolar macrophage chemotactic factor for neutrophils (AMCF) recruits neutrophils to the alveolar structures.[44,81–82] Like other chemotactic factors,[83] AMCF probably plays an additional amplification role by also activating neutrophils to release their preformed mediators.[84,85] Other human alveolar macrophage effector functions include mediation of antibody-dependent cytotoxicity [15]; production of superoxide anion [53]; and secretion of collagenase,[86] elastase,[87–89] and neutral protease.[84,90] While each of these effector processes plays a valuable role in normal lung homeostasis (eg, superoxide anion may participate in destroying microorganisms,[91] antibody-dependent cytotoxicity may destroy tumor cells,[92] and proteases may mediate connective tissue homeostasis [93]), these processes may also secondarily cause damage to normal alveolar structures. Thus, as in the case of aryl hydrocarbon hydroxy-

lase, the effector functions of the human alveolar macrophage may be a double-edged sword, protective in some instances but injurious in others.

In addition to those effector functions described above, the human alveolar macrophage possesses and probably secretes a variety of other enzymes, including acid phosphatase,[94-96] β-glucuronidase,[94,96] esterases,[90,94,95,97] and lysozyme.[96,98] Although each of these enzymes is thought to serve some effector role in inflammation, the exact nature of these roles is unclear.

Although it is clear that cells of the mononuclear phagocyte system play a critical accessory role in the immune response by virtue of their ability to "process" and "present" antigens to lymphocytes,[99] very few studies have been carried out with alveolar macrophages.[73] There is evidence, however, that human alveolar macrophages physically interact with lymphocytes [100] and are capable of functioning as accessory cells for optimal lymphocyte responses to antigens and mitogens and in mixed lymphocyte cultures.[100-102] In addition, human alveolar macrophages are responsive to lymphokines, such as migration inhibition factor,[103-104] as well as to chemotactic stimuli, such as casein [105] and activated serum.[106]

Further evidence for the involvement of alveolar macrophages in the immune response comes from studies of individuals immunized with influenza virus.[9] Alveolar macrophages recovered from individuals immunized by the aerosol route have elevated levels of lysosomal enzymes, suggesting that these alveolar macrophages are activated. In comparison, no macrophage activation is seen in individuals immunized by the subcutaneous route. Thus, human alveolar macrophages probably participate in immune responses involving the lung but not necessarily in systemic immune processes that do not involve the lung.

### Protection of Alveolar Structures From Protease Attack

With the introduction of the protease–antiprotease theory of emphysema,[107] attention has been focused on the various mechanisms that protect the alveolar structures from proteolytic attack. In this concept, the exact role of the alveolar macrophage is not known, but human macrophages clearly contain $\alpha_1$-antiproteinase.[7,108] Although much of this $\alpha_1$-antiproteinase is in the form of a complex with protease,[109] at least some functional antiproteases must be present within the macrophages, since cytosol of human alveolar macrophages will inhibit human neutrophil elastase.[110] In addition, as discussed above, it is known that human alveolar macrophages can associate directly with human neutrophil elastase through a defined receptor on the macrophage surface,[52] thus giving this cell an additional protective role.

**Classification and Function of Alveolar Lymphocytes**

Morphologic observations of normal human lung suggest that, within the alveolar structures, lymphocytes are found within the interstitium and on the epithelial surface. There is no information concerning lymphocyte traffic to and from the human lung, but it is likely that there is movement of lymphocytes to the alveolar structures from peripheral blood, bronchus-associated lymphoid tissue, and regional lymph nodes.[29,111] However, even though such lymphocyte movement is likely, it is clear that in certain instances lung lymphocytes can be "compartmentalized" from the peripheral circulation. For example, evaluation of lung and blood lymphocytes of normal individuals with positive skin tests for *Mycobacterium tuberculosis* demonstrated that cells from both sites are sensitized to *M tuberculosis* antigens. In comparison, influenza virus preferentially sensitizes lung lymphocytes when administered via an aerosol, whereas immunization via the subcutaneous route preferentially sensitizes blood lymphocytes.[9]

In normals, the types of lymphocytes found within the alveolar structures are similar to those of blood [12,14-16,23,24,112-115] (Table 2). Using surface marker criteria, approximately 73% of alveolar lymphocytes are T cells and 7% are B cells.[14,16] The remaining 19% of alveolar lymphocytes do not react with conventional reagents and hence are classified as "null" cells. Earlier studies [12,24,112] probably overestimated the proportion of null cells within the lung; with more sensitive techniques [14,16] a significant number of such cells have now been identified as T lymphocytes. It is also likely that the early estimates of the proportion of B lymphocytes were high, as it is now known that some lymphocytes thought to be B lymphocytes by virtue of their reaction with complement-coated erythrocytes or fluorescein-labeled intact immunoglobulin were actually other mononuclear cells.[116,117]

The subtypes of alveolar T and B lymphocytes are also similar to those found in peripheral blood [14,16] (Table 2). For T lymphocytes, approximately half have surface Fc receptors for IgM, and 5–10% have Fc receptors for IgG. Although the significance of so-called T$\mu$ and T$\delta$ lymphocytes is controversial,[118] lymphocytes with Fc receptors for IgG are considered to be relatively "activated" T lymphocytes.[119,120] Evidence for an additional population of "activated" T lymphocytes in normal lung comes from the demonstration that 5–12% of lung T lymphocytes rosette with sheep erythrocytes at 37 C.[14,16,121]

The majority of lung B lymphocytes have stable surface immunoglobulins of the IgM and IgD classes, while a much lower proportion have IgG or IgA on their surface [16,112] (Table 2). In addition, lung B lymphocytes

Table 2—Classification of Lymphocytes Present in Normal Human Alveolar Structures

| | Lung | | Blood | |
|---|---|---|---|---|
| | Percentage of total lymphocytes (range) | | Percentage of total lymphocytes (range) | |
| Lymphocyte type | | | | |
| T lymphocyte* | 73 ± 4 (65–80) | | 74 ± 5 (65–80) | |
| B lymphocyte† | 8 ± 3 (4–12) | | 7 ± 4 (4–11) | |
| Null lymphocytes‡ | 19 ± 3 (8–28) | | 19 ± 5 (7–26) | |
| | | Percentage of total T lymphocytes (range) | | Percentage of total T lymphocytes (range) |
| T lymphocyte subtypes | | | | |
| Rosetted with SRBC at 37C§ | 6 ± 2 (3–9) | 8 ± 3 (5–12) | 5 ± 2 (3–8) | 7 ± 2 (4–11) |
| Tγ (Fc-IgG) ‖ | 5 ± 2 (3–8) | 7 ± 2 (5–10) | 6 ± 2 (3–9) | 8 ± 3 (4–12) |
| Tμ (Fc-IgM)¶ | 37 ± 4 (32–44) | 51 ± 5 (45–60) | 41 ± 6 (31–48) | 55 ± 7 (42–65) |
| | | Percentage of total B lymphocytes (range) | | Percentage of total B lymphocytes (range) |
| B lymphocyte subtypes | | | | |
| Surface Immunoglobulin# | | | | |
| IgG | 1.0 ± 0.1 (0–3) | 13 ± 6 (0–38) | 1.0 ± 0.1 (0–2) | 14 ± 5 (0–28) |
| IgA | 1.0 ± 0.1 (0–3) | 13 ± 8 (0–38) | 0.5 ± 0.1 (0–2) | 7 ± 3 (0–28) |
| IgM | 7.0 ± 0.8 (6–8) | 88 ± 7 (75–100) | 6.5 ± 0.9 (5–7) | 93 ± 8 (79–100) |
| IgD | 5.0 ± 0.8 (4–7) | 63 ± 12 (50–88) | 5.0 ± 1.0 (4–6) | 71 ± 9 (57–86) |
| Immunoglobulin secretion** | | | | |
| IgG | 0.019 ± 0.004 (0.012–0.028) | 0.23 ± 0.04 (0.15–0.35) | 0.022 ± 0.004 (0.010–0.031) | 0.28 ± 0.06 (0.14–0.44) |
| IgA | 0.025 ± 0.004 (0.016–0.034) | 0.31 ± 0.06 (0.20–0.43) | 0.016 ± 0.003 (0.008–0.024) | 0.20 ± 0.04 (0.11–0.34) |
| IgM | 0.008 ± 0.002 (0.004–0.012) | 0.10 ± 0.02 (0.05–0.15) | 0.008 ± 0.001 (0.005–0.011) | 0.11 ± 0.03 (0.07–0.16) |

* Proportion of total lymphocytes capable of rosetting with neuraminidase-treated sheep red blood cells (SRBC) at 4C.[16]

† Proportion of total lymphocytes with stable surface immunoglobulin; detected with a $F(ab')_2$ goat anti-human immunoglobulin reagent.[16]

‡ Proportion of total lymphocytes not detected as T or B lymphocytes.[16]

§ Proportion of T lymphocytes rosetting with sheep red blood cells (not neuraminidase-treated) at 37C.[16]

‖ Proportion of T lymphocytes rosetting with ox red blood cells (ORBC) opsonized with rabbit IgG anti-ORBC antibody.[16]

¶ Proportion of T lymphocytes rosetting with ox red blood cells (ORBC) opsonized with rabbit IgM anti-ORBC antibody.[16]

# Proportion of B lymphocytes with stable surface immunoglobulin; detected with $F(ab')_2$ goat antihuman IgG, IgA, IgM, or IgD reagents.[16]

** Proportion of B lymphocytes spontaneously secreting immunoglobulin; detected with a reverse hemolytic plaque assay.[123,124]

have receptors for complement, probably the C3b fragment.[12,24,112] These findings are consistent with observations made on peripheral blood lymphocytes.[122]

Normally, approximately 0.1 to 0.3% of B lymphocytes within the lung actively secrete immunoglobulins, including IgG, IgM, IgA, and IgE [123,124] (Table 2). A higher proportion of lung B cells secrete IgA than either IgG or IgM. In addition, a higher percentage of lung B lymphocytes secrete IgA than do blood B cells. These observations are consistent with the concept that IgA is a secretory immunoglobulin localized to the respiratory tract, gut, and glandular structures.[125]

There is increasing evidence that human lung lymphocytes function in a way similar to that of peripheral blood lymphocytes. Lung lymphocytes respond to mitogens,[23,101,112,126,127] antigens,[101,126] and in the mixed lymphocyte reaction.[101] Human lung lymphocytes have also been shown to produce lymphokines when activated, including migration inhibition factor, leukocyte inhibitory factor, and monocyte chemotactic factor.[9,16,128,129] There is some evidence that lung lymphocytes respond less intensely to mitogens than do peripheral blood lymphocytes,[112] but it is not clear whether this is an intrinsic property of lung lymphocytes or whether it is secondary to the relative proportions of various mononuclear-cell subtypes isolated from lung versus those from blood.

#### Protein Components

The existence of an epithelial fluid lining layer within the alveolar structures has been known for many years.[130] Initially, most attention was focused on the surfactant system, a lipid-protein complex that plays a critical role in regulating alveolar surface tension.[130] However, it soon became obvious that the alveolar epithelial fluid contained a wide variety of nonsurfactant molecules, many of which play an important role in inflammatory and immune processes within the lung.

There are two sources of alveolar epithelial protein: production by cells within the alveolar structures and transudation from serum. Since many of the proteins involved in the pulmonary inflammatory and immune system (eg, immunoglobulins, complement, antiproteases) are also found in the serum, the latter must be considered a primary source for many of these molecules. Studies with animals have clearly shown that albumin, a serum protein (molecular weight, 69,000 daltons) produced by hepatocytes, will pass from serum to alveolar interstitial lymph and the alveolar epithelial surface as well as from the alveolar epithelial surface in the opposite direction.[131] A number of studies have shown that albumin is found in hu-

man alveolar epithelial fluid.[1,3,11,21,108,132–134] However, not all of the proteins present in serum are free to pass to the epithelial surface of the alveoli. As might be expected, the alveolar structures are more permeable to molecules of low molecular weight (eg, potassium, urea, and glucose are all found in lavage fluid[13,21,135,136]) and are relatively impermeable to very large molecules (eg, $\alpha_2$-macroglobulins at 820,000 daltons and $\beta$-lipoprotein at 3,200,000 daltons are not found in normal lavage fluid [10–13,137]) It is likely, therefore, that the relative concentration of serum proteins in epithelial fluid is governed, in part, by their molecular weight. Proteins of less than 150,000 daltons are fairly diffusible, but those of larger molecular weight are progressively less able to cross the normal barriers.[137] This molecular-weight–dependent gradient of serum molecules found in alveolar epithelial fluid strongly argues against bronchoscope-induced trauma as a source of serum proteins in the alveoli. Another argument against trauma as a source of serum proteins in recovered epithelial fluid is the fact that erythrocytes are rare in lavage fluid, as is hemoglobin.[11]

There is a large body of evidence that shows that the alveolar epithelial fluid of normal individuals contains most of the protein components involved in normal inflammatory and immune processes (Table 3). Of the major immunoglobulins, IgG [3,11,12,21,28,132,133–141] IgA[3,11,12,21,132,140,142,143] and IgE [12,28] are all present. In normal individuals, IgM is not routinely detected, and thus it is not present or is present in very low amounts.[3,11,12,21,132,133,135,137,138] Most investigators who have looked for IgD in lung have not found it,[10,133,137,138] but this immunoglobulin has received little attention in lung. In addition to the immunoglobulins, it is clear that both complement pathways [11,12,136,137,146] are functional in normal lung, as is a significant proportion of the antiprotease system.[108,132–134,136,137]

It is likely that the majority of IgG found in alveolar epithelial fluid of normal individuals was originally derived from serum. The relatively small molecular weight of IgG (180,000 daltons) permits it to leak from serum to the alveolus. Even though normal alveolar lymphocytes actively secrete IgG [123–124] (Table 2), there are relatively few immunoglobulin-secreting cells per normal alveolus, and thus it is unlikely that local production would significantly contribute to the epithelial-fluid IgG. It is not suprising that IgM is not usually detected in alveolar epithelial fluid; this immunoglobulin has a molecular weight of $10^6$ daltons and thus is not likely to move from serum to the alveolar surface. In addition, there are even fewer IgM-secreting lymphocytes per alveolus than IgG-secreting cells (Table 2), making local production even less likely.

In normal human lung, the IgA is found as both a dimer and a monomer.[10,11,139,140,143] Most of the IgA in lavage is in the dimeric form, with the secretory piece attached.[10,11,142] This is opposite to the form of IgA in

Table 3—Inflammatory and Immune Related Protein Constituents of Normal Human Alveolar Epithelial Fluid

| Components | Status in Normal Lung |
|---|---|
| Immunoglobulins | |
| IgG | Present*[3,11,12,21,28,132,133–141] |
| IgM | Absent†[3,11,12,21,132,133,135,137,138] |
| IgA | Present[3,11,12,21,132–140,142–143] |
| Monomeric | 9% of total[10,11,139,140,143] |
| Dimeric | 91% of total[10,11,139,140,143] |
| Free secretory piece | Present[10,11,144,145] |
| IgE | Present‡[12,28,133,137,138] |
| IgD | ?§[10,133,137,138] |
| Complement | |
| Classical pathway | Functional[11] |
| Clq | ?‖[11] |
| C4 | Present[11,12,136] |
| Alternate pathway | Functional[146] |
| Factor B | Present[146] |
| Common pathway | Functional[11] |
| C3 | Present[11,136,137] |
| C6 | Present[11,12] |
| Antiproteases | |
| $\alpha_1$-antiproteinase | Present[108,132–134,136,147] |
| $\alpha_2$-macroglobulin | Very low if present¶[133,137,11,21] |
| Anti-chymotrypsin | ?#[4] |
| Low-molecular-weight bronchial inhibitor | ?#[4] |
| Enzymes thought to be capable of deranging alveolar structures | |
| Collagenase | Absent[140,148] |
| Elastase** | Absent[140,148] |
| Neutral protease†† | Present[90,148] |
| Other enzymes‡‡ | |
| Lysozyme | Present[90] |
| $\beta$-glucuronidase | Present[148] |
| Esterase | Present[90,149] |
| Acid protease | Present[90] |
| Phospholipase A | Present[150] |
| Glycosidase | Present[140] |

* $IgG_1$ is always present; initial studies suggest that other IgG subclasses are variably present.[11]

† In most studies evaluating normal alveolar epithelial fluid for the presence of IgM, lavage fluid has been concentrated 30–50-fold and assayed with the use of conventional radial immunodiffusion plates. These systems have a detection limit of approximately 10 μg/ml, suggesting that if IgM is present, it is in very low concentrations.[11]

‡ Some investigators have been unable to detect IgE in lavage fluid[133,137,138]; however, with a radioimmunoassay, it is easily detected.[12,28]

§ IgD has not yet been detected, but this may be a technical problem, as with IgE.

‖ Antigenic Clq has not been found; the presence of functional Clq has not been specifically evaluated.

¶ Most investigators do not find $\alpha_2$-macroglobulin in concentrated lavage fluid[11,21]; however, it has been variably detected by others.[133,137] Thus, if present, it must be in very low amounts.

# Anti-chymotrypsin[4] and a low-molecular-weight bronchial inhibitor[4] have been detected in "bronchial mucus." In these studies, a rigid bronchoscope was used with very small amounts of lavage fluid; it is doubtful that this represents a valid alveolar epithelial sample.

** Elastase is strictly defined as an enzyme capable of breaking peptide bonds in elastin. As such, elastase has never been detected in lavage fluid of normal individuals.[140,148] Early studies used low-molecular-weight artificial substrates that were "elastin-like."[90,149] The enzymes that attack such substrates are strictly esterases, and the significance of finding these enzymes in lavage fluid is unknown.[151]

†† Assayed with a $^{14}C$-labeled denatured globin substrate[148]; although "nonspecific," this protease is active at neutral pH and is probably capable of attacking a variety of protein components of the alveolar structures.

‡‡ Although functionally active, the relevance of these enzymes to inflammatory and immune processes in the alveolar structures is unknown.

serum, where it exists mainly as a monomer. Alveolar lymphocytes synthesize IgA (Table 2), but the relative contribution of local production versus serum transudation is not known. However, it is reasonable to assume that at least some lung monomeric IgA is derived from serum, since this form of IgA has a molecular weight of 170,000 daltons [152], comparable to other proteins that pass from serum to alveoli.[137] The dimeric form of IgA has a molecular weight of 385,000 daltons [152] and is probably locally produced. In this regard, it is known that the secretory piece of dimeric IgA is produced in lung epithelial cells [125] and is found in lavage fluid [10,11,144,145] but not in serum.[10,11]

The source of IgE in normal lavage fluid is not known. IgE has a molecular weight of approximately 190,000 daltons [152] and thus could be partially derived from serum. The amount of IgE in lavage fluid is approximately that of serum,[12] suggesting that serum may be a major source in normal individuals.

It is surprising that IgD has not been readily detected in lavage fluid,[10,133,137,138] since it is present in serum [153] and has a molecular weight of 170,000–200,000 daltons.[152] This fact, however, may be due to the antiserums used, the sensitivity of the assay, or how the lavage fluid was concentrated.

Both the classical and alternative complement pathways are represented on the alveolar epithelial surface of normal individuals.[11,136,137] Functional levels of C4,[11] factor B,[146] and C6,[11] are easily detected. Like the immunoglobulins, however, the source of the complement components within lung is not known. The molecular weights of many of the proteins of the complement system are less than 200,000 daltons,[154] and thus some alveolar complement components are probably derived from serum. Local production is also possible, because human lung fibroblasts synthesize the C1 components [155] and mononuclear phagocytes synthesize C2, C4, and possibly C1q, C3, C5, and properdin factor B.[156] However, complement production has not yet been demonstrated for human alveolar macrophages.

The alveolar structures are reasonably well protected against proteolytic attack, as there are ample quantities of the major serum antiprotease, $\alpha_1$-antiproteinase, in normal alveolar epithelial fluid.[108,132–134,136,137] Most of the $\alpha_1$-antiproteinase present in lavage fluid is free and thus functional (in contrast to $\alpha_1$-antiproteinase complexed with protease; the latter is the primary form of $\alpha_1$-antiproteinase found in human alveolar macrophages).[147] It is important to note, however, that although $\alpha_1$-antiproteinase is plentiful within the lung, it does not afford the

alveolar structures complete antiprotease protection. Recent studies suggest that although $\alpha_1$-antiproteinase inhibits neutrophil elastase,[157,158] it does not inhibit elastase produced by alveolar macrophages.[89]

Alpha$_2$-macroglobulin is generally not found on the alveolar epithelial surface,[11,21] although some investigators claim it can be detected in some normal individuals.[133,137] If it is present within the alveoli, it is unlikely that it is derived from serum, as it has a very large molecular weight (820,000 daltons). There is evidence, however, that human lung fibroblasts and blood monocytes make $\alpha_2$-macroglobulin,[159,160] but this theory is controversial.[161] Thus, the contribution of locally produced $\alpha_2$-macroglobulin to lung antiprotease defense is unclear. The other general serum antiprotease, anti chymotrypsin, has a molecular weight of 69,000 [4] and thus probably moves from serum to lung. This protein has been found in bronchial mucus [4] but has not been looked for in alveolar epithelial fluid.

In addition to the serum antiproteases, it has been suggested that a bronchial inhibitor of low molecular weight may serve as a major antiprotease in lung.[4,162] This inhibitor has a molecular weight of 10,500 daltons; it is made by airway epithelial cells [4,162] and effectively inhibits granulocyte proteases. This inhibitor may be important as a lung antiprotease, but to date it has only been demonstrated on the surface of large airways and not on the alveolar epithelial surface.

With the reasonable antiprotease protection present within the alveolar structures, it is not surprising that the proteases most likely to damage alveolar structures (ie, collagenase and elastase)[93] are not found in alveolar epithelial fluid of normal individuals [140,148] (Table 3). It is likely, therefore, that the low-level burden of proteases present in normal alveolar structure is adequately balanced by local protective measures. The known exception is neutral protease, a nonspecific protease probably capable of attacking a variety of proteins. This enzyme activity is present in low levels in normal lavage fluid,[90,148] suggesting that it may not be inhibited by the normal antiprotease screen.

A number of other enzymes have been found in lavage fluid of normal individuals, including lysozyme,[90] $\beta$-glucuronidase,[148] esterase,[90,149] acid protease,[90] phospholipase A,[50] and glucosidase.[140] However, the relevance of these enzymes to inflammatory and immune processes within the lung is not known, and thus the importance of these findings must await further study.

One of the major problems facing investigators evaluating noncellular constituents of alveolar epithelial fluid is how to express data so that comparisons may be made from lavage to lavage. Optimally, one would like to

know the concentration of related inflammatory and immune protein constituents within the alveolar epithelial fluid *in situ*. Unfortunately, at present, the only way to obtain this fluid in humans is to wash it off the epithelial cells with physiologic saline.[11] Thus, the noncellular material recovered by bronchoalveolar lavage is a variable mixture of epithelial fluid and saline. Since the recovery of bronchoalveolar lavage fluid varies widely from lavage to lavage (and probably the relative proportion of epithelial fluid recovered varies as well [11,12]), there is little meaning in expressing the data as "amount of protein per milliliter fluid recovered."

Early investigators [1,10-12] made the important observation that albumin is always found in lavage fluid. They reasoned that since albumin is made external to lung and is not selectively destroyed within the lung, they could express protein constituents in lavage fluid in relation to the amount of albumin present. In addition, the molecular weight (69,000 daltons) of albumin is approximately in the middle molecular weight range of serum proteins that permeate through the normal endothelial–interstitial–epithelial barrier; and thus, comparison of lavage constituents with albumin gives a reasonable estimate as to whether a particular protein is increased or decreased, in comparison with its concentration in serum. Other standards have been tried, including potassium, glucose, and blood urea nitrogen [13,21,135-137]; but for various reasons, none is as suitable as albumin.[11-13] Thus, although there is still controversy concerning the use of albumin as a lavage standard,[21,135,136] most investigators agree that it is the best standard available.

## Inflammatory and Immune Processes in Human Lung Disorders

The major disorders involving alveolar structures can be classified as destructive (ie, loss of alveolar structures, as in panacinar emphysema), interstitial (ie, derangement and thickening of alveolar structures as in interstitial lung disease), infectious (ie, acute and chronic disorders caused by microorganisms), and neoplastic (ie, primary lung tumors). Although there is a great deal of crossover among these categories, such classification is useful because it implies disparate pathogenetic processes and thus helps us to focus on the mechanisms underlying these disorders. Bronchoalveolar lavage has been an invaluable tool in evaluating the pathogenesis of these disorders and has underscored the critical role played by inflammatory and immune processes in effecting damage to the alveolar structures.

### Destructive Lung Disease

Since the recognition of the association between familial panacinar emphysema and a deficiency of serum $\alpha_1$-antiproteinase,[163] a body of evi-

dence has been gathered in support of the concept that protease–antiprotease inbalance within the alveolar structures is a general mechanism underlying destructive lung disease.[107] Most of the evidence for that concept comes from animal studies (eg, experimental elastase-induced emphysema).[164] The only studies directly evaluating protease–antiprotease balance in the alveolar structures of patients with destructive lung disease are the observations that macrophages of individuals with chronic obstructive disease have normal enzyme contents[97] and that there are decreased levels of $\alpha_1$-antiproteinase in lavage fluid[165] and in alveolar macrophages[7] of patients with PiZ homozygous $\alpha_1$-antitrypsin deficiency. There are, however, a number of observations concerning alveolar inflammatory and immune processes in cigarette smokers, compared with controls (Table 4). Many of these observations are directly relevant to protease–antiprotease processes; taken together with the overwhelming evidence associating cigarette smoking with destructive lung disease,[166–172] these findings provide a compelling argument that inflammatory and immune processes within the alveolar structures of cigarette smokers are biased toward a relative functional excess of proteolytic activity.

### Observations in Cigarette Smokers Suggesting an Increased Protease Burden Within the Alveolar Structures

All investigators performing bronchoalveolar lavage are impressed by the increased number of inflammatory and immune effector cells that are recovered in lavage of smokers, compared with lavage of nonsmokers[8,10–12,14,24,87,90,95,105,106,133,173,174] (Table 4). This observation is consistent with the morphologic observation of Niewoehner et al[71] and Kuhn et al[182] that cigarette smokers have large collections of inflammatory and immune effector cells in respiratory bronchioles and alveoli. Evidence that such collections of cells may be associated with the pathogenesis of emphysema is provided by the study of McLaughlin et al,[183] which showed increased numbers of inflammatory and immune effector cells adjacent to areas of lung destruction.

Not only do smokers have increased numbers of inflammatory and immune effector cells within their alveolar structures, but the distribution of the types of cells is different from that in normals. Most strikingly, smokers have neutrophils in their alveoli, whereas such cells are rarely found in alveoli of nonsmokers.[13–17,21,174] Since both macrophages and neutrophils can secrete proteases capable of destroying alveolar structures,[84–87,93,182,184–187] these findings are consistent with the concept that the lungs of cigarette smokers may carry an increased protease burden.

Table 4—Cigarette-Smoking–Induced Abnormalities in the Inflammatory and Immune Effector Systems Within Human Alveolar Structures

| Parameter | Findings in Smokers |
|---|---|
| Cell types present | |
| Total number of cells | Increased[8,11,21,24,87,90,95,105,106,133,173,174] |
| % polymorphonuclear leukocytes | Increased[13,14,174] |
| % T lymphocytes | Increased,[24] normal[23,113,14] |
| % B lymphocytes | Normal[24,23,113,14] |
| Lymphocyte function | |
| Response to mitogens | Decreased[23,113] |
| Macrophage structure | |
| Diameter | Normal[8,95] |
| Ruffling of cell surface | Decreased[175] |
| Number and size of cytoplasmic structures | Increased[33,69,95,96,174–177] |
| Abnormal cytoplasmic inclusions | Pigmented inclusions,[69,95,96,174,176,177] particulates with plate or needle-like configuration,[33,95,174,176,177] presence of aluminum silicate[176] |
| Macrophage properties and function | |
| Surface receptors | |
| IgG-Fc | Normal[42,54,178] |
| C3b | Decreased[42,54,178] |
| Phagocytosis and killing of microorganisms | |
| Bacteria | Normal,[6,8] decreased[54] |
| Fungi | Normal[8] |
| Effector and accessory cell function | |
| Responsiveness to chemotactic factors | |
| Casein | Increased[105] |
| Activated serum | Normal[106] |
| Function as accessory cell to lymphocytes | Decreased[101] |
| Responsiveness to MIF | Decreased[178] |
| Production of neutrophil chemotactic factor | Increased[174] |
| Secretion of superoxide anion | Increased[53] |
| Secretion of elastase | Increased[87] |
| Miscellaneous properties and function | |
| Glucose utilization | Increased,[8] normal[53] |
| Oxygen consumption | Normal[53] |
| Protein content | Increased[90,95] |
| Content of various enzymes | |
| Elastase | Increased[90] |
| Acid protease | Increased[90] |
| Neutral protease | Normal[90] |
| Esterase | Increased[90] |
| Acid phosphatase | Increased[96] |
| $\beta$-glucuronidase | Increased[96] |
| Lysozyme | Normal,[90] increased[96] |
| Aryl hydrocarbon hydroxylase | Increased[58,65–72] |
| Angiotensin-converting enzyme | Increased[179] |
| Spreading and adherence properties | Increase in presence of serum,[180,181] decreased nylon adherence[175] |
| Pinocytosis | Decreased[57] |
| Content of $\alpha_1$-antiproteinase | Increased[108] |

*Table 4 continued*

| | |
|---|---|
| Epithelial surface fluid composition | |
| Immunoglobulins | |
| IgG | Increased,[10,11] normal[21,133] |
| IgA | Normal[10,11,133] |
| Free secretory component | Decreased[144,145] |
| Proteases | |
| Elastase | Not detectable[140,148] |
| Collagenase | Not detectable[140,148] |
| Neutral protease | Normal[90,149,148] |
| Antiproteases | Decreased function[165] |
| $\alpha_2$-macroglobulin | Rarely detected[10,11,12,13,137,165] |
| $\alpha_1$-antiproteinase | Normal quantity[10,11,90,165,137] |
| Quantity of miscellaneous proteins and enzymes | |
| Albumin | Normal[133] |
| Transferrin | Normal[133] |
| Lysozyme | Increased[90] |
| Esterase | Normal[90] |

Cigarette smokers not only have the potential for alveolar destruction because of the number and type of inflammatory and immune effector cells within their lungs, but also because of the fact that these cells are "activated." This activation is important, because *in vitro* studies demonstrate that activated human alveolar macrophages secrete quantities of their armementarium of inflammatory mediators, including proteases capable of destroying alveolar structures.[84,85] Most of the evidence regarding activation of inflammatory and immune effector cells in smokers' lungs concerns alveolar macrophages (Table 4).

Morphologically, macrophages of smokers have increased numbers of large lysosomes, phagolysosomes, endoplasmic reticulum, ribosomes, and Golgi vesicles,[33,69,95,96,174-177] findings that are generally associated with an "activated" mononuclear phagocyte.[188] It is likely that these macrophages have become activated by the ingestion of particulates present in cigarette smoke.[176] In this regard, it is interesting to note that macrophages of smokers have pigmented inclusions [69,95,96,174,176,177] that appear to have a plate-like or needle-like configuration when seen by electron microscopy.[33,95,174,176,177] Studies of the nature of these inclusions by x-ray analysis suggest they may be, at least in part, particulates of aluminum silicate.[176] These findings, together with *in vitro* studies showing that alveolar macrophages are activated following phagocytosis of particulates,[44] are compatible with the notion that macrophages of smokers are activated *in vivo*. Further evidence for macrophage activation in smokers comes from studies showing that these cells contain

increased levels of a variety of enzymes, including elastase,[90] acid protease,[90] neutral protease,[90] angiotensin-converting enzyme,[179] esterase,[90] acid phosphatase,[96] β-glucuronidase,[96] lysozyme,[96] and aryl hydrocarbon hydroxylase.[58,65-72] In addition, smokers' macrophages secrete superoxide anion,[53] a mediator capable of injuring parenchymal cells.[91] There is also evidence that macrophages of smokers have increased glucose utilization,[8] although this evidence is controversial.[53]

Direct evidence for the concept that alveolar macrophages add to the alveolar protease burden of smokers comes from *in vitro* evaluation of elastase secretion by macrophages recovered from lungs of smokers and nonsmokers.[87] Under the conditions used, smokers' macrophages actively secreted elastase, whereas nonsmokers' macrophages did not. It is not known, however, whether the increased elastase secreted by smokers' macrophages is actually elastase made by macrophages or neutrophil elastase that the macrophages ingested.[52] However, in addition to the proteases they secrete themselves, macrophages amplify the protease burden in cigarette smokers by recruiting neutrophils to the alveolar structures and activating the neutrophils to release their content of lysosomal proteases.[174]

In summary, there is a significant amount of circumstantial evidence suggesting that the lungs of smokers are burdened by an increased load of proteases capable of destroying alveolar structures. It must be kept in mind, however, that no study has actually demonstrated increased levels of active proteases in the alveolar structures of smokers, compared with those of nonsmokers. A neutral protease has been found in human lavage fluid,[90,148,149] but the same levels are present in smokers and nonsmokers.[90,149] Most important for its relevance to destructive lung disease is the fact that free, active elastase has never been recovered from the alveolar structures of smokers or nonsmokers.[165] However, although the lack of a direct demonstration of an increased protease burden in the lungs of smokers is disappointing, it is likely that the chronic inflammation associated with cigarette smoking is at such a low level that the proteases released from macrophages and neutrophils may only have a short time in which to act before they are inhibited by the local antiproteases mechanisms. In this regard, there is evidence that macrophages from smokers have increased levels of $\alpha_1$-antiproteinase.[108] Since the majority of intracellular macrophage $\alpha_1$-antiproteinase is complexed with protease,[147] the finding of increased levels of this antiprotease within macrophages of smokers suggests these cells may have been exposed to (and have ingested [52]) larger quantities of proteases than have the alveolar macrophages of nonsmokers.

#### Observations in Cigarette Smokers Suggesting Decreased Antiprotease Protection in the Alveolar Structures

In addition to the evidence suggesting that the alveolar structures of smokers are exposed to an increased burden of proteases, there also is evidence that their antiproteases, although normal in quantity,[10,11,90,137,165] do not function normally. In studies evaluating the function of smoker versus nonsmoker antiproteases in lavage fluid, Gadek et al [165] have demonstrated that for the same amount of $\alpha_1$-antiproteinase, lavage fluid from smokers has an approximately 40% reduction in inhibitory effectiveness against elastase, compared with lavage fluid from nonsmokers. This finding is consistent with *in vitro* data showing that cigarette smoke interferes with the effectiveness of $\alpha_1$-antiproteinase by oxidizing critical methionine groups near the active site of the antiprotease.[189]

### Infectious Lung Disease

As discussed in the section on normal lung, the inflammatory and immune systems of the alveolar structures have a number of mechanisms for handling inhaled microorganisms. Surprisingly, even though patients with infectious disorders often undergo fiberoptic bronchoscopy, there have been relatively few studies detailing the inflammatory and immune processes in the lungs of individuals with these disorders. The observations that have been made concern changes in the types of inflammatory and immune processes present and the putative loss of protection against infection under different circumstances.

#### Changes in Inflammatory and Immune Processes Associated With Infection

In a sequential study of the cell populations present in the lung during the evolution of bacterial pneumonias, Tonnel et al [18] have shown that neutrophils predominate in the acute phase. Associated with the resolution of the infection, however, the proportion of lymphocytes increases, sometimes remaining at a high level for some time. In contrast, neutrophils are not found in the lungs of patients with chronic pulmonary infections such as tuberculosis. Lavage fluid from patients with this disease reveals an accumulation of lymphocytes within the alveolar structures, particularly T lymphocytes.[190,191]

There is little information concerning inflammatory and immune proteins in lungs of patients with infectious disorders. In a study of bronchoalveolar lavage in "unilateral pneumonitis" (presumably an acute infectious process), IgA was found to be similar in involved and uninvolved regions of lung, while IgG was elevated in the region of the pneumonitis.[135] In contrast, patients with unilateral tuberculosis had

higher immunoglobulin levels in uninvolved regions.[135] The significance of these observations is not clear. They may represent a specific local immune response to the infectious process, or they may be secondary to phenomena such as changes in capillary permeability caused by local inflammation.

### Observations Relevant to Decreased Protection Against Infection

There is clinical evidence that smokers have increased susceptibility to lung infections.[192-194] In this regard, a number of observations have suggested that alveolar macrophages of cigarette smokers may have functional defects that impair their ability to deal with microorganisms. For example, macrophages of smokers do not respond normally to macrophage migration inhibitory factor,[78] suggesting that smoking has altered the ability of the macrophage to respond appropriately to inflammatory stimuli. There is also evidence that smokers' macrophages have decreased pinocytotic capacity, at least *in vitro*.[57] This decreased capacity has been interpreted as representing a loss of macrophage endocytic function, suggesting that these cells have an impaired capacity to deal with microorganisms. Smokers' macrophages can phagocytize fungi normally,[6,54] but there are opposing thoughts on how these cells deal with bacteria.[6,8,54] IgG Fc receptors in smokers' macrophages are considered to be normal,[54] but there are data suggesting that these cells have decreased numbers of C3b receptors,[42,54,178] raising the possibility that macrophages of smokers may not be able to use all available opsonins in dealing with microorganisms.

Alveolar macrophages of smokers do not function normally as accessory cells to lung lymphocytes.[101] It is not clear, however, whether this abnormality has any significant effect on the immune response in the lungs of cigarette smokers. In this regard, there is no evidence of immunoglobulin abnormalities in the lungs of smokers; most reports suggest that epithelial-fluid immunoglobulin levels in the lungs of smokers are either normal or elevated, compared with those of nonsmokers.[10,11,21,133]

In addition to cigarette smoking, several other conditions have been studied in which alveolar inflammatory and immune processes appear to be disturbed and, therefore, are less likely to deal effectively with inhaled microorganisms.

1) Alveolar macrophages from patients with chronic granulomatous disease of childhood appear to have an intrinsic metabolic defect that renders these patients more susceptible to pulmonary infection. These cells exhibit markedly depressed metabolic function in response to

phagocytosis of bacteria or to phorbol myristate[195] (an artificial activator of macrophages). This depressed response could not be explained by a decreased ability of the macrophage to phagocytize the microorganisms.[195]

2) Patients with alveolar proteinosis, a disorder characterized by an accumulation of a lipid-protein material within alveoli, have an increased susceptibility to unusual infectious disorders.[196] Macrophages retrieved from the lungs of these individuals show decreased adherence to glass, diminished responsiveness to chemotactic stimuli, and reduced ability to kill ingested fungi.[64] The mechanism of these functional defects is unknown but is thought to be associated with the known accumulation of lipid-protein materials within the macrophages.

3) Individuals who have experienced acute smoke inhalation often develop life-threatening pulmonary infections.[106] Studies of alveolar macrophages of such individuals show that these cells are less responsive to serum-derived chemotactic factors than are macrophages from normal individuals, suggesting that acute inhalation of toxic products of combustion has a depressive effect on macrophage-mediated lung defense mechanisms.

4) There are scattered reports of patients with recurrent pulmonary infections having selective IgA deficiency detected in bronchoalveolar lavage fluid.[3,142] It is not known whether this deficiency is just a local manifestation of systemic secretory immunoglobulin deficiency, nor is the relationship between the IgA deficiency and the recurrent lung infections completely defined.

#### Neoplastic Lung Disease

Although fiberoptic bronchoscopy with lavage is carried out in almost all individuals with primary lung tumors, there are relatively few studies in which the material obtained by lavage has been evaluated for anything other than the presence of tumor cells.

Mandel et al [135] noted that alveolar IgA and IgG levels were increased in patients with lung tumors, but only in regions of lung with tumor growth. However, it is not clear whether this increase precedes tumor growth or is simply a secondary phenomenon associated with local stimulation of alveolar inflammatory and immune processes by the tumor.

It is known that cigarette tars will stimulate human alveolar macrophages to produce elevated levels of aryl hydrocarbon hydroxylase.[69] This observation, however, does not correlate with the development of lung cancer, as macrophages of smokers with lung neoplasms have the same levels of aryl hydrocarbon hydroxylase as do macrophages of smokers with no lung tumor.[70]

Recently, Merrill et al [144] have reported that individuals with lung cancer have decreased levels of free secretory component in bronchoalveolar lavage fluid, compared with controls. In addition, these investigators have identified a subgroup of cigarette smokers who are free of cancer but have decreased free secretory component in lung.[145] This may be a finding of major importance, as secretory component is a glycoprotein synthesized by the lung epithelial cells, and thus its production may serve as an index of injury to epithelial cells. The finding that certain cigarette smokers have diminished secretory component suggests that this subgroup of individuals may have early epithelial-cell injury and may be at higher risk for the development of lung cancer.

#### Interstitial Lung Disease

The interstitial lung diseases are a group of approximately 130 different disorders, each characterized by a chronic alveolitis, ie, an increase in the number of inflammatory and immune effector cells within the lung parenchyma. In many of these patients, the alveolitis leads to a derangement of alveolar structures, including thickening and fibrosis of the adjacent interstitium. If unchecked, these inflammatory and immune processes will disturb the structure of the lung parenchyma so that alveoli are no longer able to mediate gas exchange.[197,198] It is of major interest, therefore, to evaluate the alveolitis of interstitial lung diseases relative to the inflammatory and immune processes that govern the form and intensity of each disorder.

Bronchoalveolar lavage is ideally suited to this purpose. In general, patients with interstitial lung disease do not have inflammatory airway disease; therefore the cells obtained are reflective of alveolar rather than airway processes. In addition, while morphologic evaluation of lung biopsies shows that the alveolitis of most interstitial lung disease is comprised of large numbers of mononuclear cells,[17,197,199,200] conventional morphologic methods are not suited to accurate quantitation of the types of cells present and give little information about the functional state of alveolar inflammatory and immune processes. By contrast, lavage results are easy to quantitate, and the cells obtained are viable and thus readily available for functional studies. Analyzing the cells and proteins obtained from bronchoalveolar lavage of more than 300 patients with interstitial disease, we have found that examination of lavage fluid has three advantages: 1) it presents classification of interstitial disorders by the types of inflammatory and immune effector cells comprising the alveolitis; 2) it provides insights into the pathogenesis of the different interstitial diseases, particularly dis-

Table 5—Classification of the Interstitial Lung Disorders by the Inflammatory and Immune Effector Cells Present in the Alveolar Structures

| Cell Differential* | | |
|---|---|---|
| Neutrophils | Lymphocytes | Disorder |
| None | Increased† | Hypersensitivity pneumonitis[12,128,201]<br>Sarcoidosis[13,26,14,16,179,202,203,204,121,22,98,115,205,206] |
| High | Normal | Idiopathic pulmonary fibrosis[12,13,26,16]<br>Familial pulmonary fibrosis[207]<br>Asbestosis[132,209] |
| Variable | Normal | ILD‡ associated with rheumatoid arthritis[12,13,207] |
| Low | Normal | ILD associated with PSS‡[12,13,207]<br>ILD associated with overlap syndrome[12,13,207]<br>ILD associated with SLE‡[12,13,207]<br>Histiocytosis-X[13,207] |

* The cellular content of bronchoalveolar lavage fluid gives a valid representation of the status of inflammatory and immune effector cells in alveolar structures only when no inflammatory airway disease is present. The classification given is for nonsmokers; smokers generally will have variably higher proportions of neutrophils in the cell differential.

† Increased lymphocytes have also been noted in patients with pulmonary tuberculosis [190,191,207], and pulmonary lymphormas.[207]

‡ ILD = interstitial lung disease; PSS = progressive systemic sclerosis; SLE = systemic lypus erythematosus.

orders of unknown etiology; and 3) it allows repetitive sampling, thus helping the clinician to stage the disease and make therapy decisions.

### Classification of the Interstitial Disorders Using Bronchoalveolar Lavage Criteria

As expected in disorders where there are large numbers of inflammatory and immune effector cells in alveolar areas, bronchoalveolar lavage of patients with interstitial disease yields large numbers of cells, often two to four times more than in normals.[12,13,173] Analysis of the types of inflammatory and immune effector cells recovered can be used to quantify the alveolitis. In time, this analysis may aid in the classification of the patient. Of the nearly 130 interstitial lung disorders known, only a few have been studied by bronchoalveolar lavage (Tables 5–7). Although all of the patients investigated have had increased numbers of monocytes and macrophages as part of their alveolitis, the classification of the alveolitis rests upon a quantitation of the relative numbers of neutrophils and lymphocytes recovered from the alveolar structures (Table 5)* Basically, of the disorders evaluated to date, alveolitis can be classified as one of two types: those with high and those with normal proportions of lymphocytes (Table 5) (Figures 7 and 8). The disorders with high proportions of

* Presumably other polymorphonuclear leukocytes (such as eosinophils) play an important role in the alveolitis of interstitial disorders such as eosinophilic pneumonia; however, lavage data are not yet available.

lymphocytes include hypersensitivity pneumonitis [12,128,201] and sarcoidosis (Figure 8).[13,15,16,22,98,115,121,179,202–206] Disorders associated with normal numbers and ratios of lymphocytes include idiopathic pulmonary fibrosis,[12,13,16,26] familial pulmonary fibrosis,[207] asbestosis,[132,209] histiocytosis X,[13,207] and several interstitial lung diseases associated with the collagen vascular disorders, including rheumatoid arthritis, progressive systemic sclerosis, overlap syndrome, and systemic lupus erythematosus.[12,13,207]

There is, of course, a striking histologic difference between those disorders in which the alveolitis includes increased numbers of lymphocytes and those disorders with normal numbers of lymphocytes. For instance, the lymphocyte-predominant group indicates granulomatous disease. In accord with this observation, high proportions of lymphocytes have also been observed in tuberculosis.[190,191,207] The only known exceptions to the idea that high numbers of lavage lymphocytes are found only in granulomatous disorders are the findings of high proportions of lymphocytes in T-cell lymphomas involving the lung [207] and the brief report of Tonnel et al [18] suggesting that individuals with lung tumors receiving radiation of the chest have a transient and variable rise in the proportion of lymphocytes in their lavage fluid following irradiation. The latter observation needs to be confirmed; but if valid, it suggests that some interstitial lung disorders are associated with changing patterns of alveolitis.

As might be expected from disorders characterized by alveolar interstitial granulomas, lymphocytes found in the lungs of patients with sarcoidosis and hypersensitivity pneumonitis are predominantly T lymphocytes [12,14,16,26,121] (Table 6). In contrast, those disorders without pulmonary granulomas have normal proportions of T lymphocytes within their alveolar structures.[16,26] The most striking observation concerning lymphocyte subpopulations has been made in sarcoidosis. Even though these patients have increased proportions of T lymphocytes in lavage fluid, they have decreased proportions of these cells in their peripheral blood, suggesting a relative compartmentalization of the immune response in this disease.[16]

Analysis of other inflammatory and immune effector cells found in the alveolitis of those interstitial disorders with normal numbers and ratios of lymphocytes reveals that the alveolitis of these diseases is categorized by the presence of the neutrophil, a cell rarely found within the alveolar structures of normal nonsmoking individuals (Table 5; Figure 7). In this regard, idiopathic pulmonary fibrosis is the most impressive, with average lavage neutrophils counts of 20% ± 5% of the total cells recovered.[12,13,16,26]

Table 6—Subtypes of Lymphocytes Found in Lung and Blood in Various Interstitial Lung Disorders

| Condition | Proportion of lymphocytes in lavage cell differential | T lymphocytes* | | B lymphocytes† | |
|---|---|---|---|---|---|
| | | Lung | Blood | Lung | Blood |
| Normal | 7 ± 1% | 73 ± 4% | 74 ± 5% | 8 ± 3% | 7 ± 4% |
| Sarcoidosis‡ | ↑§ | ↑ | ↓ | ↓ | ↑ |
| Hypersensitivity pneumonitis‡ | ↑ | ↑ | ↔ | ↓ | ↔ |
| Idiopathic pulmonary fibrosis‡ | ↔ | ↔ | ↔ | ↔ | ↔ |
| ILD-CV§ | ↔ | ↔ | ↔ | ↔ | ↔ |
| Histiocytosis-X ‖ | ↔ | ↔ | ↔ | ↔ | ↔ |

* Proportion of total lymphocytes capable of rosetting with neuraminidase-treated sheep red blood cells at 4 C.[16]

† Proportion of total lymphocytes with stable surface immunoglobulin; detected with a $F(ab')_2$ goat antihuman immunoglobulin reagent.[16]

‡ Patients with active disease.

§ ILD-CV = patients with interstitial lung disease associated with the collagen vascular disorders; ↑ = increased compared with normals; ↓ = decreased compared with normals; ↔ = similar with normals.

‖ Patients with stable disease.

Other forms of interstitial disease characterized by the persistent accumulation of high proportions of neutrophils within lavage fluids include familial pulmonary fibrosis (an interstitial disease with a family clustering that is indistinguishable from idiopathic pulmonary fibrosis [197]) and asbestosis.[132,209] The other interstitial disorders with an alveolitis characterized by normal proportions of lymphocytes also have neutrophils in lavage fluids, but the proportions of these cells are variable and usually less than in the disorders described above (Table 5).

In addition to cell differentials at the light-microscopic level, cell differentials of bronchoalveolar lavage fluid seen by transmission electron microscopy or energy-dispersive x-ray microanalysis may prove useful in characterizing the alveolitis of some of the interstitial diseases. One example is the characteristic lipid droplets seen in macrophages of patients with hypersensitivity pneumonitis (Figure 9). Basset et al [209] have convincingly shown that lavage fluid from individuals with histiocytosis-X has cells that contain cytoplasmic structures 42 nm in diameter, termed "X-bodies" (Figure 10). These X-bodies are present in so-called tissue histiocytes, abnormal cells probably derived from the mononuclear phagocytic system.[210] These tissue histiocytes are not specific for histiocytosis-X; they are also found in interstitial disorders such as

idiopathic pulmonary fibrosis.[211] However, these cells, with their X-bodies, are much more abundant in histiocytosis-X and thus might be used as a diagnostic criterion as well as for staging the activity of the disease.

Several investigators have shown interest in the application of energy-dispersive x-ray microanalysis of alveolar macrophages recovered by lavage as a means of identifying interstitial disease due to the inhalation of inorganic dusts.[59–62] However, this approach is in its infancy, a major problem being the development of baseline normal values. Early reports are encouraging, and there appears to be a good correlation between the inorganic particles found in macrophages and a history of inhalation of specific types of dust or fumes.

Whereas analysis of proportions of various types of inflammatory and immune effector cells has obvious application to the classification of the interstitial disorders, application of the data obtained by analysis of the inflammatory and immune related protein constituents of alveolar epithelial fluid is not as clear (Table 7). Most chronic interstitial disorders are characterized by elevated levels of IgG and normal levels of IgA in alveolar structures.[12,13,22,201] Such findings are not specific to interstitial disease: elevated IgG levels are also found in primary lung malignancies.[135] There are some reported specificities in epithelial-fluid immunoglobulins (eg, increased levels of IgA in primary lung malignancies,[135] and increased levels of IgM in hypersensitivity pneumonitis [12,201]); but until more data on alveolar immunoglobulins become available, it is probably best not to consider such findings as specific for certain disorders.

In contrast to the immunoglobulin data, no differences have been noted in the relative activity of complement components in alveolar epithelial fluid from patients with the interstitial disorders [12,22] (Table 7). However, only a few components have been evaluated in only a few disorders, and more data are necessary before a judgment can be made as to the usefulness of quantitating these elements in lavage fluid.

Data are just becoming available concerning the protease–antiprotease balance in the alveolar epithelial fluid in interstitial disorders. In normal individuals there are approximately 50–100 $\mu$g of $\alpha_1$-antiproteinase per milligram albumin in lavage fluid.[11,108,212] Smokers have the same levels of epithelial-fluid $\alpha_1$-antiproteinase as do normal nonsmokers,[108] but homozygous PiZ individuals with destructive lung disease have no detectable $\alpha_1$-antiproteinase in their lower respiratory tract.[165]

Strikingly, most patients with idiopathic pulmonary fibrosis have

Table 7—Inflammatory and Immune Related Protein Components of Alveolar Epithelial Fluid in a Variety of Chronic Parenchymal Lung Disorders

| Condition | Immunoglobulins | | | | | Complement | | Antiproteases | | Proteases | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | IgG | IgM** | IgA†† | SP | IgE | C4 | C6 | $\alpha_1$AT | $\alpha_2$M§§ | E'ase | C'ase |
| Normal† | +‡ | 0 | + | + | + | + | + | + | 0 | 0 | 0 |
| Idiopathic pulmonary fibrosis | ↑ | 0 | ↔ | X | ↔ | ↔ | ↔ | ↔ | 0 | 0 | + |
| Interstitial lung disease associated with the collagen-vascular disorders§ | ↑ | 0 | ↔ | X | X | X | X | X | X | X | X |
| Sarcoidosis | ↑ | 0 | ↔ | X | ↔ | ↔ | ↔ | ↔ | 0 | 0 | 0 |
| Hypersensitivity pneumonitis ‖ | ↑‡‡ | ↑ | ↔ | X | ↔ | ↔ | ↔ | X | X | X | X |
| Histiocytosis-x¶ | ↑ | 0 | ↔ | X | X | X | X | X | X | X | X |
| Primary lung malignancy | ↑ | X | ↑ | ↓ | X | X | X | X | X | X | X |
| $\alpha_1$-antitrypsin deficiency# | X | X | X | X | X | X | X | 0 | X | X | X |
| Tuberculosis | ↓ | X | ↓ | X | X | X | X | X | X | X | X |

* SP = secretory piece for dimeric IgA; $\alpha_1$AT = $\alpha_1$-antiproteinase; $\alpha_2$M = $\alpha_2$-macroglobulin; E'ase = elastase assayed using a labeled insoluble elastin substrate; C'ase = collagenase assayed using a labeled Type I soluble lung collagen substrate. See text for references.

† Normal values are listed in the references as are the methods used for detection; + = present; 0 = not detectable using currently availble methods. See Table 3.

‡ Symbols used refer to value as compared to normal individuals; ↑ = increased compared to normal; 0 = evaluated but absent; ↔ = same as normal; ↓ = decreased compared to normal; x = not evaluated; + = present.

§ This data includes patients with rheumatoid arthritis, progressive systemic sclerosis, systemic lupus erythematosus, and the overlap syndrome.

‖ Includes patients with hypersensitivity to *M faeni, T vulgaris, A pullulans,* and pigeon serum.

¶ Data from patients with stable histiocytosis-X.

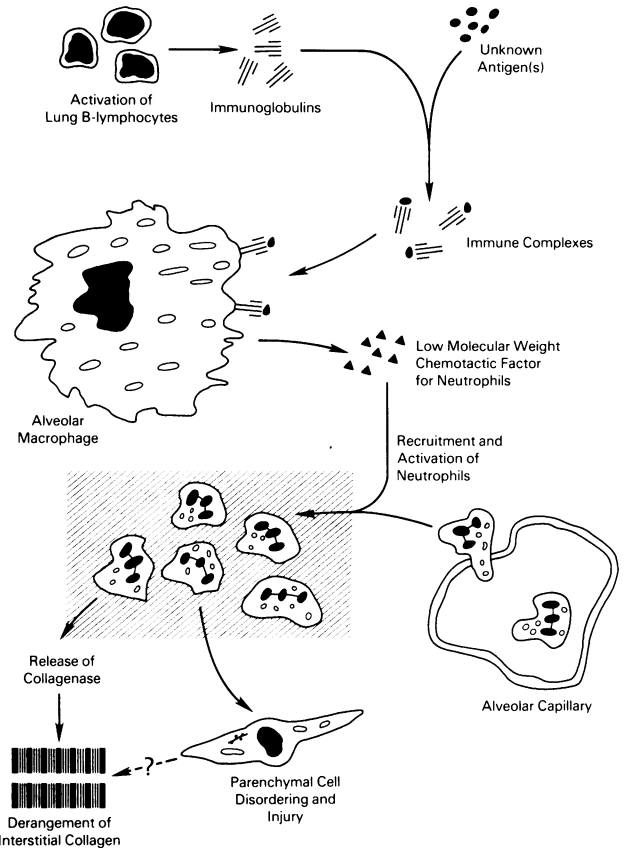
# PiZ homozygous individuals with panacinar emphysema.

** IgM is rarely found in normal individuals (<5%); in patients with idiopathic pulmonary fibrosis it is detected in 15%.

†† In normal individuals, epithelial fluid IgA is 91% dimeric, 9% monomeric; in patients with idiopathic pulmonary fibrosis 75% is dimeric and 25% monomeric.

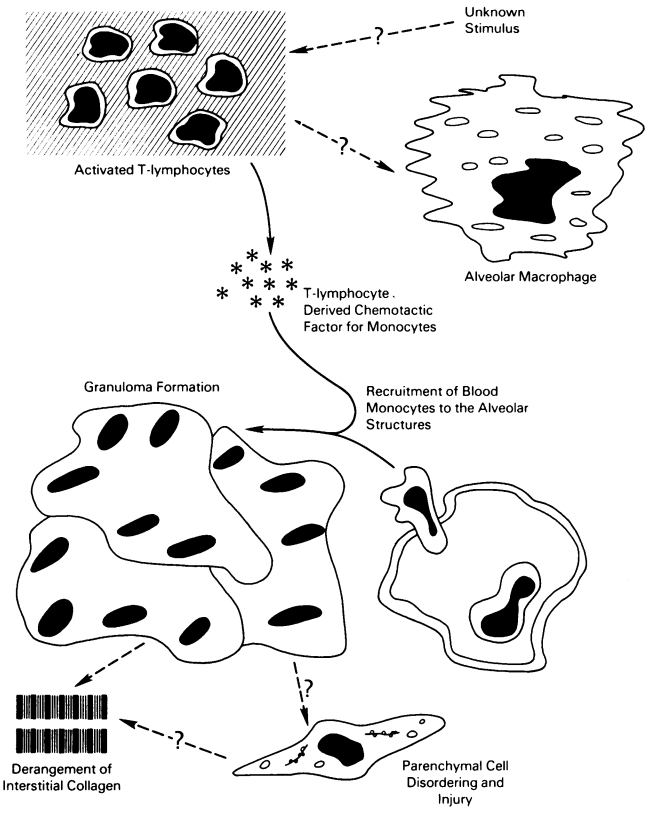
‡‡ In some individuals with hypersensitivity pneumonitis, IgG directed against a specific antigen has been detected in epithelial fluid.

§§ Only a few individuals (<5%) have $\alpha_2$-macroglobulin present in lavage fluid.




TEXT-FIGURE 4—Current concepts of the pathogenesis of idiopathic pulmonary fibrosis. Antigens, at present unknown, activate lung B lymphocytes to produce immunoglobulins. The antibody and antigen combine to form immune complexes, which stimulate alveolar macrophages, via their Fc receptors for IgG, to release a low-molecular-weight chemotactic factor for neutrophils. The chemotactic factor attracts neutrophils to migrate from alveolar capillaries to the alveoli; these neutrophils are the characteristic cell of the alveolitis of this disease (shaded area). Once the neutrophils are present within the alveolar structures, the chemotactic factor also activates these cells to release collagenase and to injure and disorder lung parenchymal cells. The presence of free collagenase within the lung also results in a derangement of interstitial collagen.

detectable levels of active collagenase in their bronchoalveolar lavage fluid.[148,213] It is extremely rare to find this enzyme in a biologic fluid, and it is not detectable in lavage fluid of normal individuals (smokers as well as nonsmokers) or patients with sarcoidosis.[214] However, as with many of the other findings concerned with inflammatory and immune protein components of alveolar epithelial fluid in the interstitial disorders, it is too early to determine whether this finding is a specific characteristic of only idiopathic pulmonary fibrosis.




TEXT-FIGURE 5—Current concepts of the pathogenesis of sarcoidosis. A stimulus that at present is unknown results in the activation of lung T lymphocytes; these T lymphocytes are the characteristic cell of the alveolitis of this disease (shaded area). The activated T lymphocytes release a chemotactic factor that stimulates blood monocytes to migrate into the alveolar structures. Once the monocytes are present within the lung, they may differentiate into epithelioid cells and multinucleated giant cells, which are critical components of granulomas. It is likely that the granulomas secrete various enzymes that derange interstitial collagen and also mediate the disordering and injury of lung parenchymal cells.

### Pathogensis of Various Interstitial Lung Disorders

Analysis of the cells and proteins recovered by bronchoalveolar lavage of patients with interstitial lung disease has provided remarkable insights into the pathogenesis of these disorders. Most of this information concerns idiopathic pulmonary fibrosis (Text-figure 4) and sarcoidosis (Text-figure 5).

As described above, analysis of the inflammatory and immune effector

cells contained in bronchoalveolar lavage fluid of patients with idiopathic pulmonary fibrosis has clearly shown that this disorder is associated with a chronic accumulation of neutrophils in the alveolar structures. These neutrophils are not only on the epithelial surface; analysis of of open lung biopsies of these patients have shown that 18% ± 6% of the total inflammatory and immune effector cells within the biopsy are neutrophils,[25,26] a finding confirmed by morphologic evaluations.[213] The presence of these cells within the alveolar structures provides a firm basis for understanding the derangements in lung parenchyma that are characteristic of the disease.

Neutrophils carry a variety of preformed proteolytic enzymes that have the potential to cause significant injury to the alveolar structures.[182,187] Most important is the enzyme collagenase, a proteolytic enzyme capable of deranging interstitial collagen.[185] Unlike neutrophil elastase, which is inhibited by $\alpha_1$-antiproteinase (an antiprotease plentiful in the lung of patients with idiopathic pulmonary fibrosis), collagenase is inhibited primarily by $\alpha_2$-macroglobulin (an antiprotease not found in epithelial fluid).[215] As described previously, most patients with idiopathic pulmonary fibrosis have collagenase detectable in their epithelial fluid, probably explaining the mechanism for the derangement of their interstitial collagen.

Neutrophil proteases may also be the cause of some of the disordering of parenchymal cells noted in this disease. McDonald et al [186] have found that fibronectin, a large, adhesive glycoprotein found on the surface of many cells (including lung fibroblasts), is very susceptible to neutrophil proteases. Since fibronectin is thought to mediate cell–cell and cell–matrix interactions, destruction of this macromolecule by neutrophil proteases may be an important step in the pathogenesis of idiopathic pulmonary fibrosis.

Since neutrophils are so central to the pathogenesis of idiopathic pulmonary fibrosis, it is important to consider the mechanisms that attract these cells to the alveolar structures. Recent studies have shown that patients with this disease have immune complexes within their alveolar structures [78,216]; it is also known that pulmonary B lymphocytes of these patients are activated to the extent of secreting 100 times more immunoglobulin than do normal lung lymphocytes.[124] Neither the specificity of these immunoglobulins nor the antigen constituting the immune complexes are known; however, it is reasonable to hypothesize that they are directed, at least in part, against alveolar components uncovered by some type of injury. In this regard, it is known that circulating lymphocytes of these individuals are activated in the presence

of Type I collagen,[217] an interstitial macromolecule known to be deranged in this disorder.[213]

Independent of the causes of these immune complexes, their presence within the alveolar structures gives a likely explanation for the chronic accumulation of parenchymal neutrophils that is so characteristic of idiopathic pulmonary fibrosis. Studies of the alveolar macrophages of these patients demonstrate that these cells are activated such that they are producing the alveolar macrophage chemotactic factor (AMCF) for neutrophils.[48,77,78] This low-molecular-weight chemotactic factor is known to be heterogeneous, made up, at least in part, of lipid.[44] Not only does AMCF attract neutrophils, but it activates them as well,[84,85] thus explaining the elevated levels of collagenase in the epithelial fluid as well as the propensity of these neutrophils to injure parenchymal cells.[218] Several observations suggest that the stimulus activating the macrophages in idiopathic pulmonary fibrosis is probably immune complexes [44,77,78]: 1) normal human alveolar macrophages can be activated by immune complexes; 2) lavage fluid of these patients contains immune complexes, and this lavage fluid will activate normal macrophages; 3) macrophages from these patients have immunoglobulin and complement on their surfaces; and 4) there is a strong correlation between the presence of immune complexes in idiopathic pulmonary fibrosis and the proportion of neutrophils found in the lungs of patients with this disease.

In contrast to idiopathic pulmonary fibrosis, analysis of bronchoalveolar lavage of patients with sarcoidosis has shown the alveolitis to be free of neutrophils but characterized by increased proportions of T lymphocytes. Analysis of these cells, as well as of T lymphocytes obtained directly from open lung biopsy specimens from these patients, has shown that many of the T lymphocytes are activated [14,16,121,129] (ie, form rosettes with sheep erythrocytes at 37 C or have Fc receptors for IgG) and are spontaneously secreting chemotactic factors, including monocyte chemotactic factor.[129] Thus, even though the stimulus activating the T lymphocytes in the sarcoid lung is unknown, it is likely that T lymphocytes play a central role in recruiting blood monocytes to the alveolar structures and hence probably modulate granuloma formation. Although unproven, it is likely that it is the granulomas that cause injury to the alveolar structures, either by their physical bulk or by their secretion of mediators.[219]

The role of the alveolar macrophage in the pathogenesis of sarcoidosis is unknown. Some investigators have suggested that the sarcoid macrophage is activated [98,179] by virtue of increased levels of lysozyme and angiotensin-converting enzyme within the cell, but the latter observation has been questioned.[202] In an interesting series of experiments, Yeager et

al [204,220,221] have shown that macrophages of patients with sarcoidosis will spontaneously interact with lymphocytes and that such interactions may be modulated with Kveim antigen. Whether this process represents specific macrophage–lymphocyte interactions or nonspecific macrophage binding of activated T lymphocytes will have to await further study.

Bronchoalveolar lavage has also given us useful insights into the pathogenesis of hypersensitivity pneumonitis. In many ways, the pathogenesis of hypersensitivity pneumonitis and that of sarcoidosis are similar, although in the former the antigen is known. Both are granulomatous disorders, and both are associated with an alveolitis involving high proportions of T lymphocytes.[12,14,16,121,129] In bird breeder's disease, Schuyler et al [128] have shown that lung lymphocytes, but not peripheral blood lymphocytes, are sensitized to pigeon serum, so that the lung lymphocytes produce lymphokines when exposed to pigeon serum *in vitro*.

In inorganic dust disease, analysis of the alveolitis of asbestosis has shown it to be remarkably similar to that of idiopathic pulmonary fibrosis, in that it is characterized by a large accumulation of neutrophils.[132,209] Although it has not yet been examined, a likely mechanism of neutrophil accumulation in the lung affected by asbestosis is through the production of alveolar macrophage chemotactic factor by macrophages "activated" through their attempts to phagocytize asbestos particles. Interestingly, there is also evidence suggesting that macrophage–asbestos interactions may be important in carcinogen handling by the asbestosis lung; when asbestos fibers are preincubated with cigarette tars or benzanthracene, macrophages exposed to these fibers are induced to have large quantities of aryl hydrocarbon hydroxylase in their cytoplasm.[58]

### Staging and Following the Alveolitis of Interstitial Lung Disease

In addition to classifying the interstitial disorders and yielding insights into their pathogenesis, bronchoalveolar lavage appears to be an ideal clinical tool for staging and following the status of inflammatory and immune processes within the lungs of patients with these disorders, especially those with idiopathic pulmonary fibrosis and sarcoidosis.

In studies of idiopathic pulmonary fibrosis, a 1-year prospective comparison of bronchoalveolar lavage findings with pulmonary function tests suggests that the intensity of the alveolitis may be a useful predictor of functional deterioration.[222] Although these studies are still continuing, it appears that a neutrophil level of about 10% of the total inflammatory and immune effector cells in lavage fluid is the highest level that can be tolerated without progressive functional derangement. Thus, if the patient

has a relatively less intense neutrophil load in the alveolar structures, the prognosis is good. If the neutrophil load is higher, however, the chances of functional deterioration are greater.

In sarcoidosis, the intensity of the alveolitis can be measured in terms of the proportion of T lymphocytes within the alveolar structures. Preliminary studies by Keogh et al [208] have shown that evaluation of lavage T lymphocytes together with the 67-gallium scan (another, possibly independent, measure of the alveolitis) may predict which patients with pulmonary sarcoidosis are likely to deteriorate and thus are candidates for therapy.

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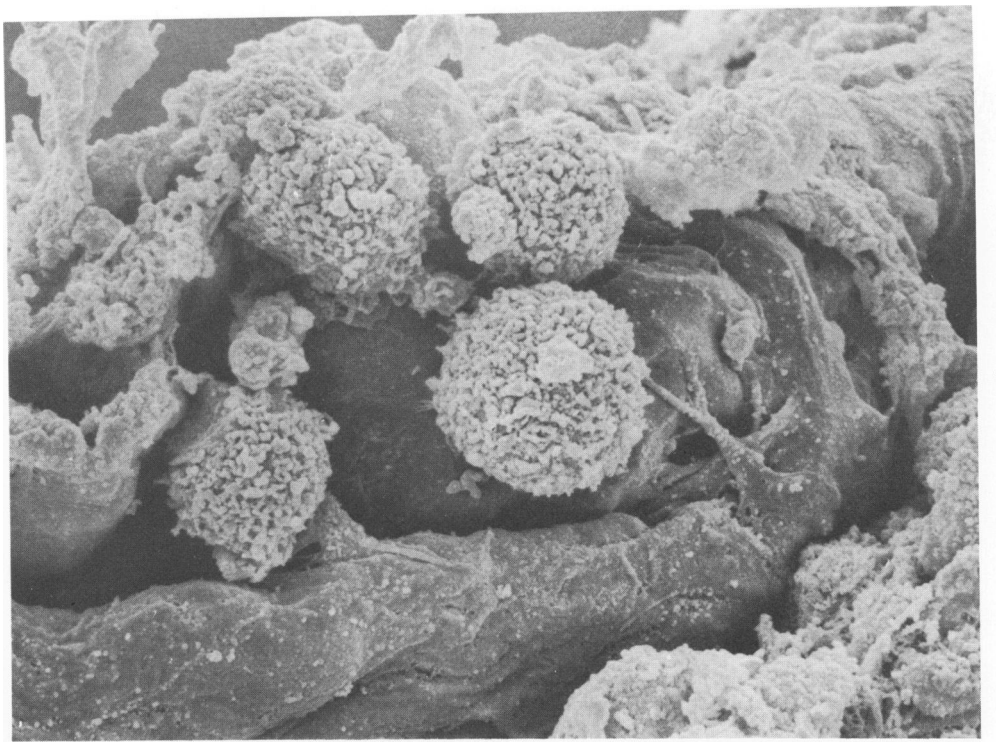

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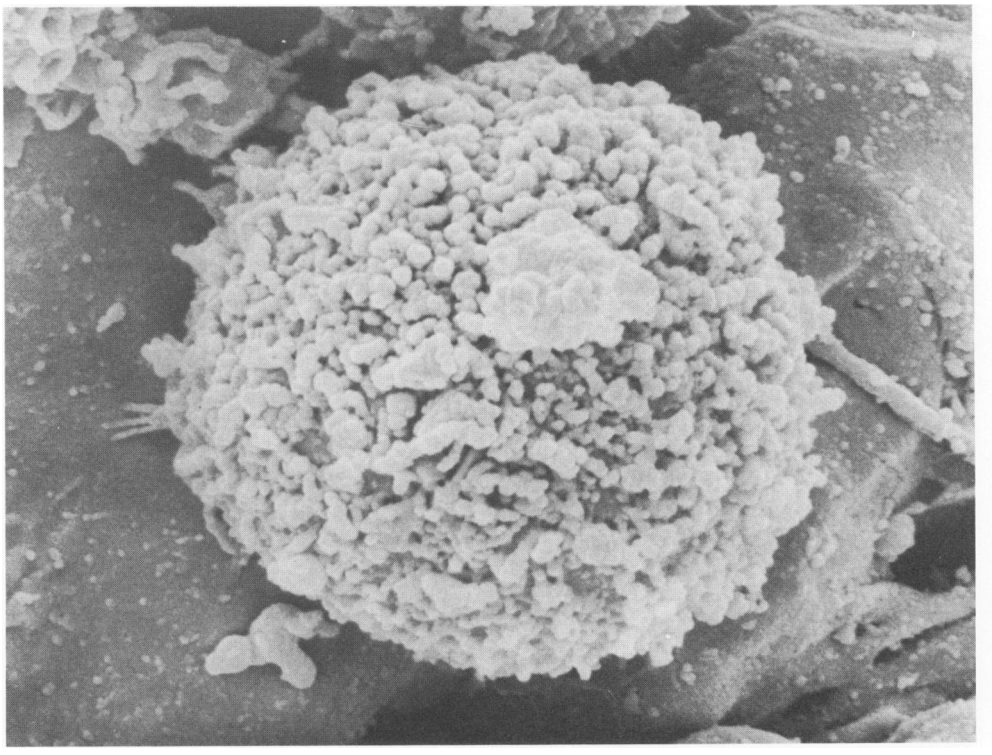

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**Figure 1**—A low-magnification scanning electron micrograph of a lung biopsy specimen from a patient with idiopathic pulmonary fibrosis. Several macrophages occupy the lumen of the alveolus and overlie Type I alveolar epithelial cells. (×2800) **Figure 2**—A high-magnification view of macrophage shown in the central portion of Figure 1. The cell surface is extremely irregular and shows numerous cytoplasmic processes. (×8500) (Both with a photographic reduction of 3%)

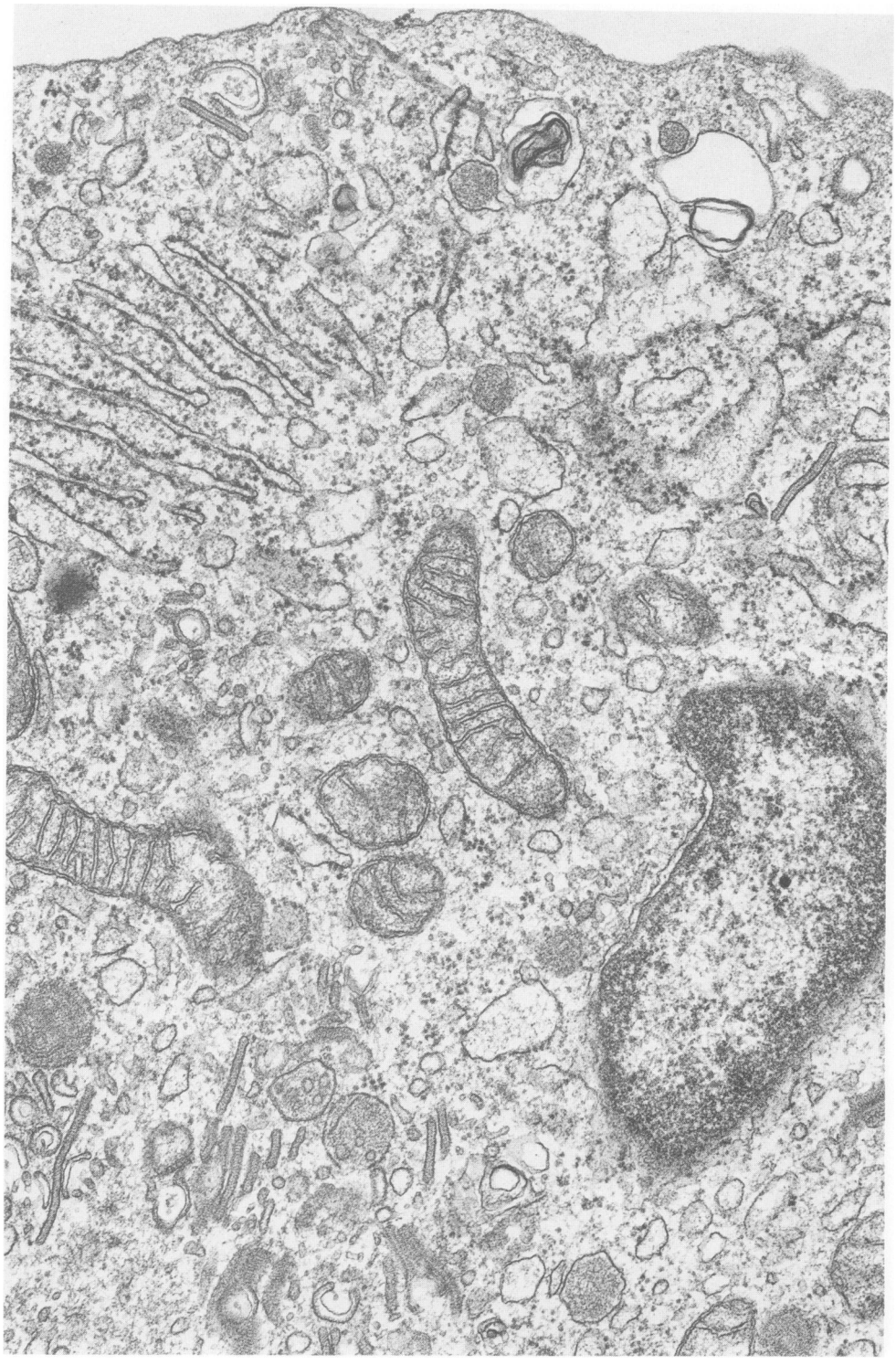
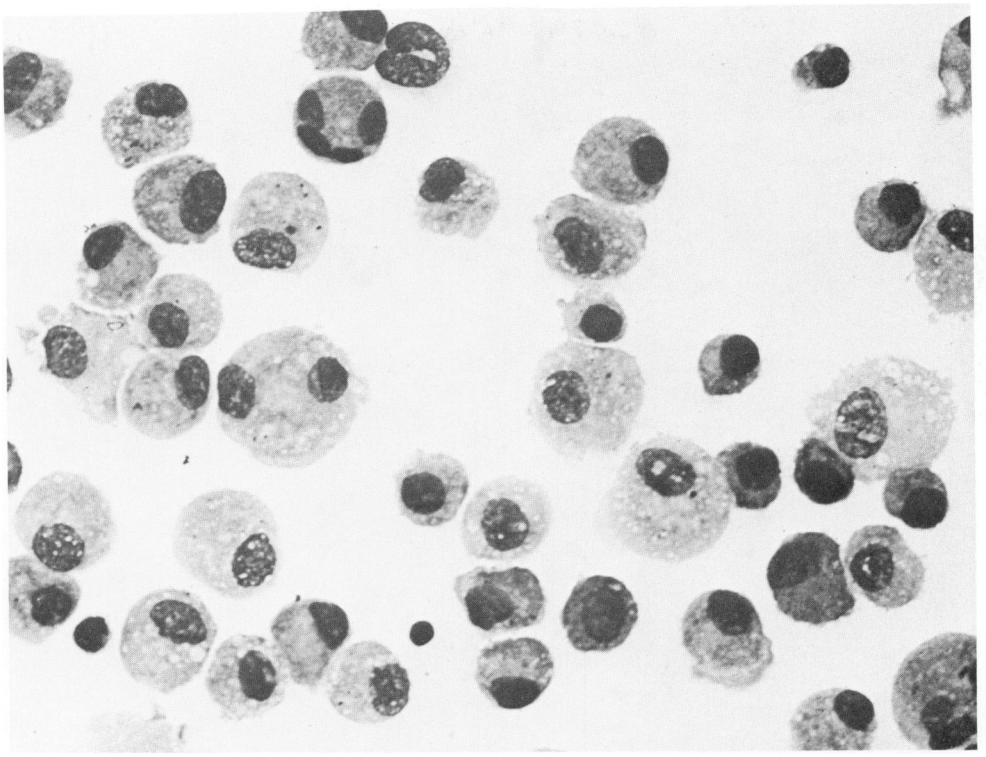


**Figure 3**—Several macrophages and a lymphocyte (*top center*) are present in the lumen of the alveolus from a patient with idiopathic pulmonary fibrosis. Note the numerous filopodia and electron-dense bodies in the macrophages. (×10,000) (With a photographic reduction of 3%)



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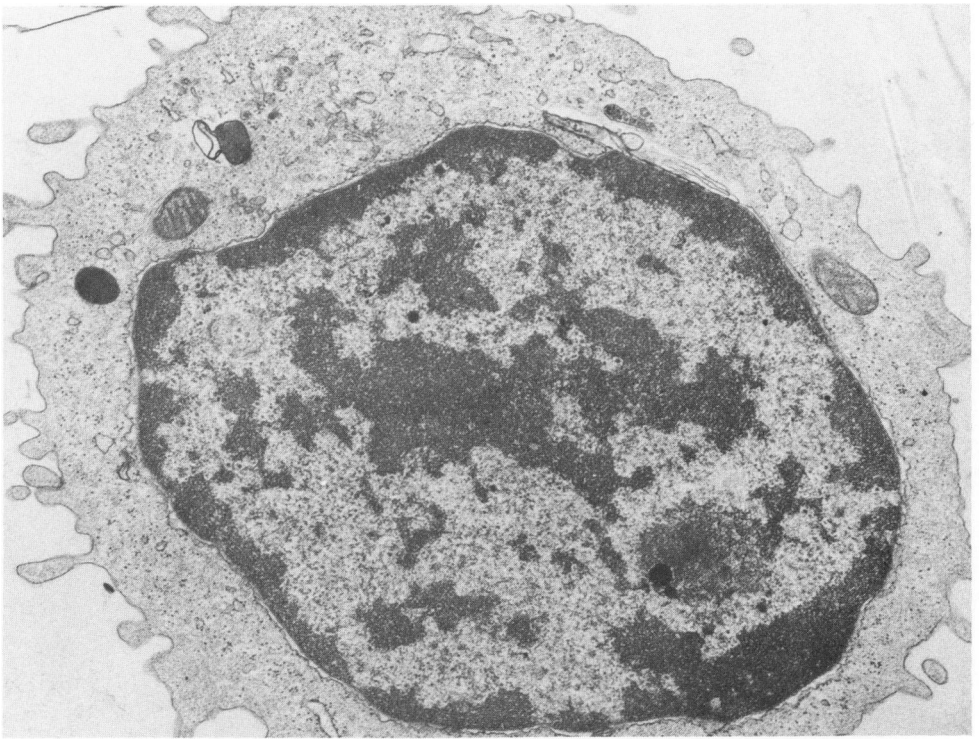

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**Figure 4**—A cytocentrifuge preparation of cells obtained by bronchoalveolar lavage from a normal individual. The majority of these cells are alveolar macrophages; however, a few lymphocytes are also present. (Modified Wright–Giemsa stain, ×500) **Figure 5**—An electron micrograph of a normal lymphocyte in bronchoalveolar lavage fluid. (×18,000) (Both with a photographic reduction of 3%)

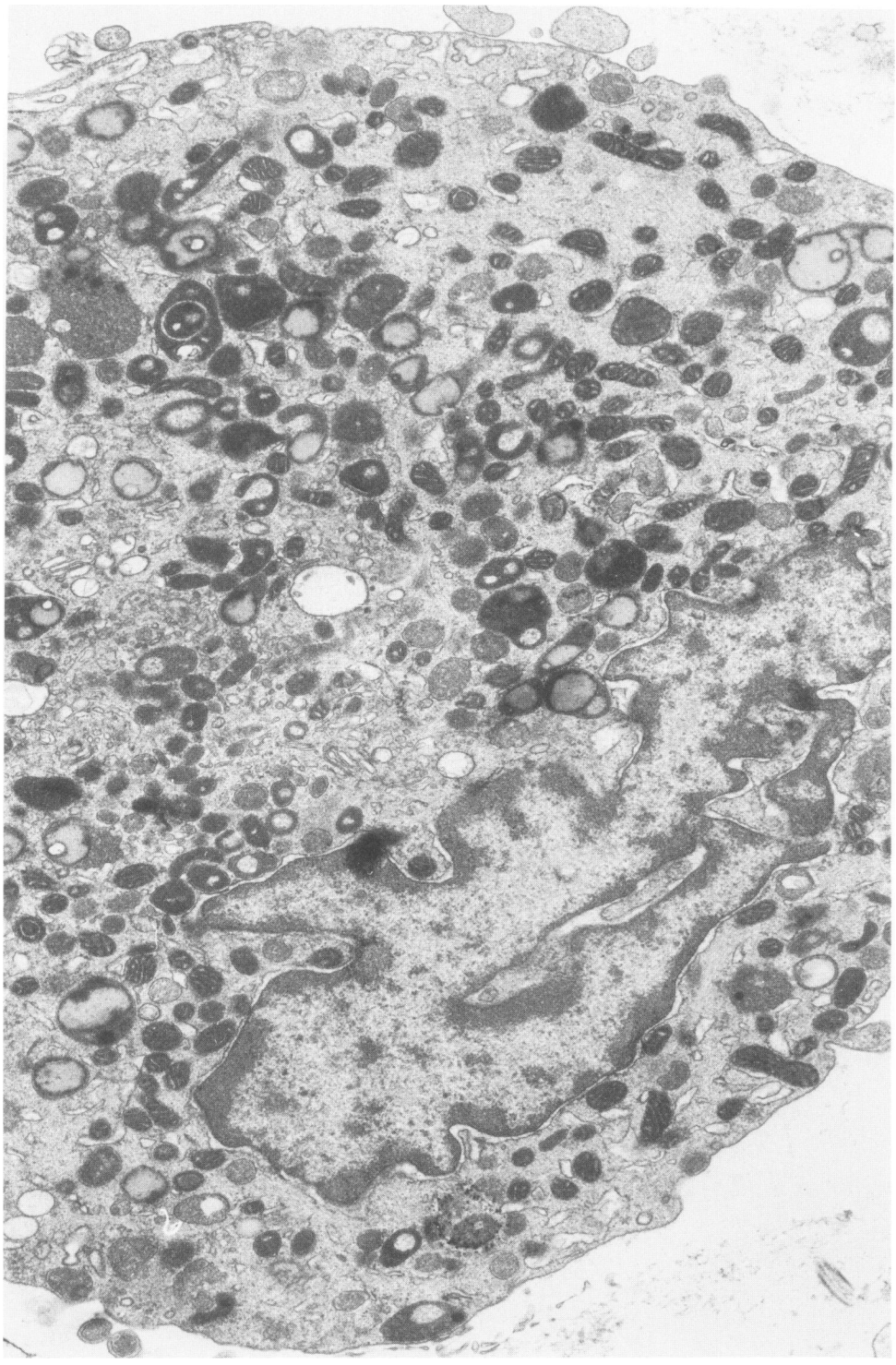
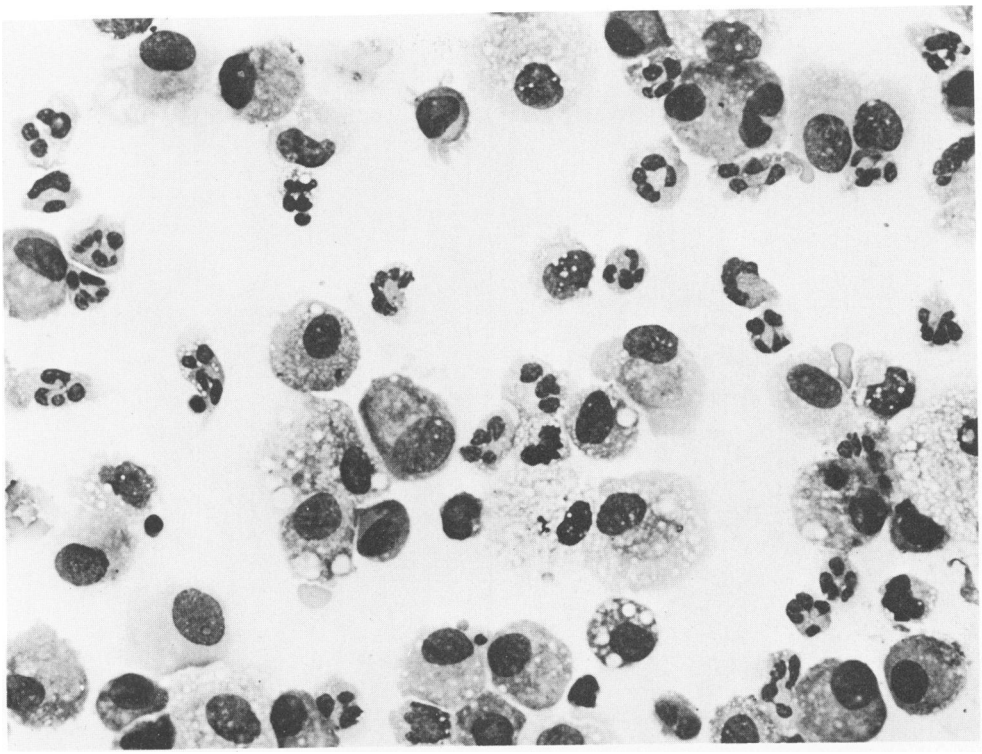


**Figure 6**—A normal macrophage in bronchoalveolar lavage fluid contains numerous dense bodies and an irregularly shaped nucleus. (×16,900) (With a photographic reduction of 3%)



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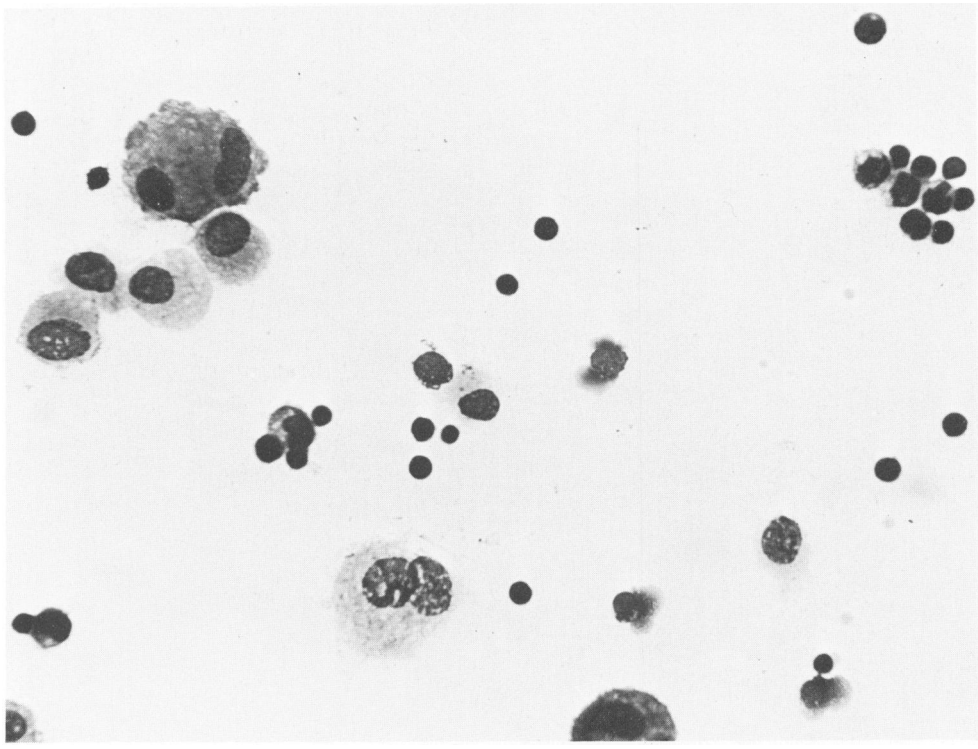

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**Figure 7**—A cytocentrifuge preparation of cells obtained by bronchoalveolar lavage from a patient with idiopathic pulmonary fibrosis. In contrast to the normal cells population, a large proportion of these cells are neutrophils. (Modified Wright–Giemsa stain, ×500) **Figure 8**—A cytocentrifuge preparation of cells obtained by bronchoalveolar lavage from a patient with pulmonary sarcoidosis. A large proportion of these cells are lymphocytes. (Modified Wright–Giemsa stain, ×500) (Both with a photographic reduction of 3%)

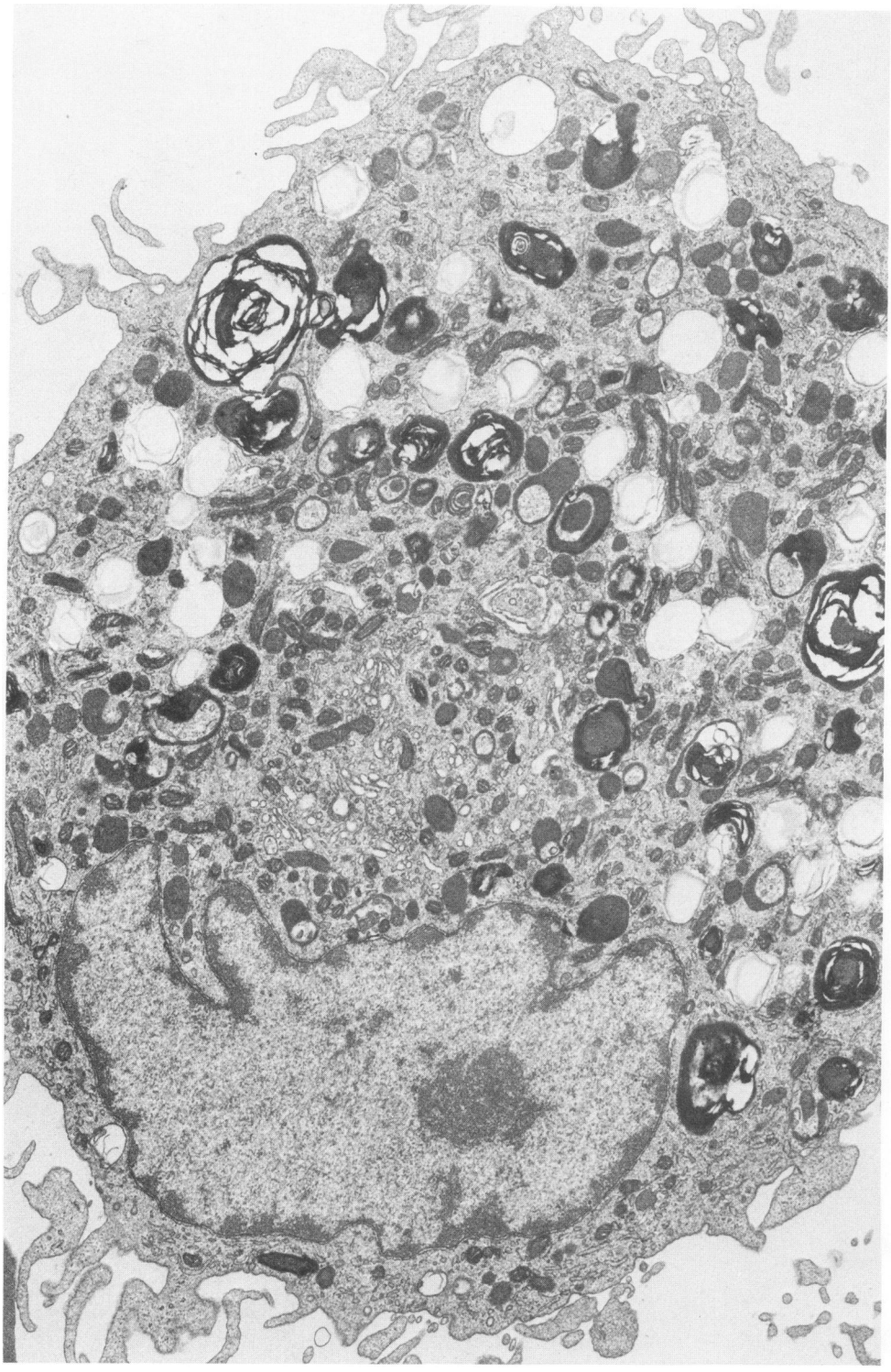

**Figure 9**—A macrophage in broncholveolar lavage fluid from a patient with hypersensitivity pneumonitis (farmer's lung) contains numerous lipid droplets and dense bodies, as well as a few concentric lamellae. (×12,400) (With a photographic reduction of 3%)

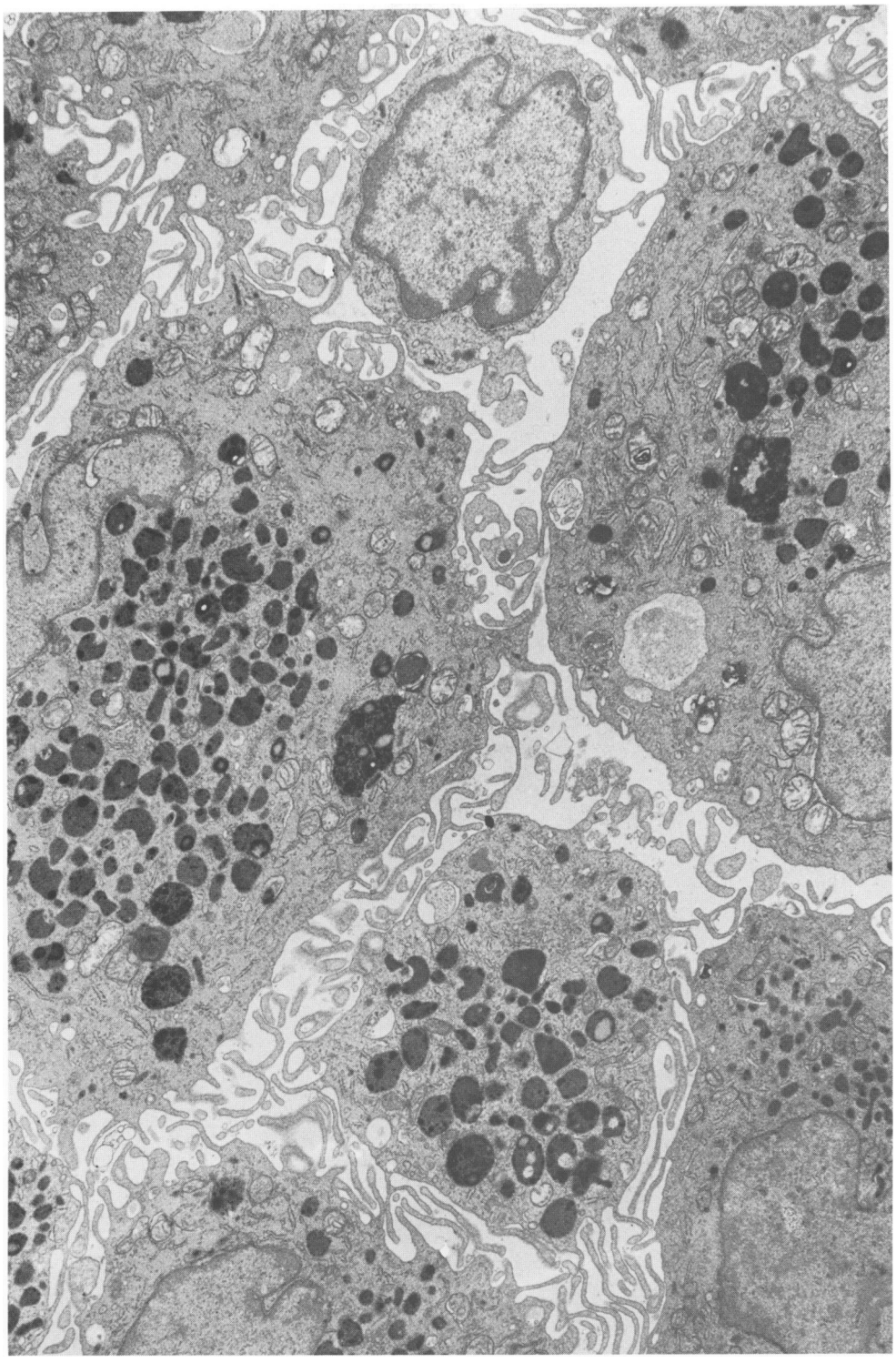

**Figure 10**—Characteristic X-bodies are present in the cytoplasm of a histiocytic cell in bronchoalveolar lavage fluid from a patient with histiocytosis-X and pulmonary involvement. (Courtesy of Dr. F. Basset) (×35,000) (With a photographic reduction of 3%)

[*End of Article*]