special report

Bronchoalveolar Lavage
The Report of an International Conference*

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Washing out a portion of the lung to remove secretions that had accumulated from chronic infection (bronchiectasis) or intractable asthma was first practiced about 50 years ago, and approximately 20 years ago, large-volume, therapeutic pulmonary lavages were performed in patients with alveolar proteinosis through a double-lumen, balloon-anchored endobronchial catheter (Carlen's tube). Soon thereafter, pulmonary washings through smaller catheters (Metras, 19 F size) or during rigid bronchoscopy were obtained from normal individuals and from patients without overt pulmonary disease for analysis of cells and proteins of pulmonary lining secretions. These washings also provided alveolar macrophages for in vitro study of phagocytosis, an important research interest in the late 1960s and early 1970s. Twenty years ago, the flexible fiberoptic bronchoscope was nearing perfection in Japan under the guidance of Dr. Shigeto Ikeda, and the use of this remarkable instrument began to be evaluated in the United States and Europe at the end of the 1960s. Use of the fiberoptic bronchoscope became widespread in the early 1970s, and since then, its use has had a tremendous impact on the practice of pulmonary medicine. Because of its ease and safety and the benign nature of lavaging a subsegment of a pulmonary lobe, fiberoptic bronchoscopy provided pulmonary washings from normal volunteers and from patients with a great variety of pulmonary diseases. For the past ten years or so, the analysis of cells and proteins or enzymes obtained from the alveolar spaces and the in vitro study of cells recovered from the respiratory tract have spawned a prominent direction for pulmonary research, helping to elucidate pathogenetic mechanisms in many pulmonary diseases. Simultaneously, clinical application of analyses of bronchoalveolar lavage fluid was fostered, perhaps prematurely at times, in the hopes that diagnosis of patients could be made more accurately and less invasively than by resorting to open lung biopsy. It was also hoped that sensitive parameters might be found for judging clinical response to therapy and for assessing the natural history of disease. At present the following rather impressive list can be assembled of primary pulmonary diseases that have been investigated using specimens from bronchoalveolar lavage to elucidate pathogenesis, to diagnose patients, or to clinically stage the activity of the pulmonary disease:

- Cigarette-smoke disease and protease-antiprotease balance
- Interstitial/alveolar inflammatory diseases
  - Sarcoidosis
  - Hypersensitivity pneumonitis
  - Eosinophilic granuloma
  - Idiopathic pulmonary fibrosis (cryptogenic fibrosing alveolitis)
  - Scleroderma
- Adult respiratory distress syndrome and oxygen toxicity
- Asthma
- Cystic fibrosis
- Acquired immunodeficiency syndrome
- Pulmonary alveolar proteinosis
- Lung cancer
- Occupational or environmental exposure to inorganic and organic dusts and certain drugs

In each instance, some helpful information has accrued, either about the host's immune responses or the nature of the pulmonary injury, that has contributed to understanding the pathogenesis of these afflictions.

As literature accumulated about the use of bronchoalveolar lavage for recovery of air space cells and secretions in normal individuals and in those with some of the diseases specified earlier, controversy arose. This was inevitable when such issues as standardization of methodology, reproducibility, sensitivity, expression of data, ability to monitor disease activity, usefulness in diagnosis, cost, and availability of analyses for clinical application were considered. Thus, some uncertainty, perhaps confusion, presently exists about the current applications of the procedure for bronchoalveolar lavage.

As a result, the Division of Lung Diseases of the National Heart, Lung, and Blood Institute (NHLBI), in consultation with its Pulmonary Diseases Advisory Committee, decided in 1983 to host an international conference on the topic of "Bronchoalveolar Lavage." This would be the second international conference on this topic, for in November 1978 in Lille, France, the Institute National de la Santé et de la Recherche Medicale sponsored a comprehensive review of the subject.1 Organization of this conference was undertaken by the cochairmen, Dr. Ronald G. Crystal and Dr. Herbert Y. Reynolds, and the staff of the division, Dr. Anthony R. Kalica, Dr. Carol E. Vreim, and Dr. Suzanne S. Hurd. Much planning and work culminated in a successful conference held May 16

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to 18, 1984, at the Columbia Inn in Columbia, Md. This was truly an international conference, with 280 total participants, 91 from foreign countries and 189 from the United States. As all scientists were invited specifically to speak or to present a poster, there were no spectators, and everyone was considered a true participant. The audience’s discussion of papers, critiques of posters, and informal talk was lively and open. This reflected in part the fact that so many of the principal investigators who have worked with bronchoalveolar lavage were present at the meeting and that interchange between the more established investigators and younger colleagues seemed to be relaxed and spontaneous. We are very indebted to many foreign colleagues who made the effort to come and agreeably struggled with the English language. Abstracts were published and may be obtained from the Division of Lung Diseases.

The conference was divided into seven sessions and covered most of the facets of bronchoalveolar lavage, its performance and methodologic use in disease. Our review covers each session, beginning with a few of the questions the session was supposed to evoke, an assessment of what was said, and any consensus on research ideas that seemed to emerge. Eighty presentations were made and about 82 posters exhibited. We regret that not everyone could be acknowledged in this summary; hopefully, those selected for comment are representative and will convey the direction and enthusiasm generated by the conference.

Methods of Bronchoalveolar Lavage

The intent of bronchoalveolar lavage is to sample the cells and extracellular molecules present in the epithelial lining fluid of the human lower respiratory tract. The overall method used is rather standard: physiologic saline solution is infused through a channel of a fiberoptic bronchoscope which has been gently wedged into a midsize bronchus; the saline solution “washes out” the material lining the epithelial surface of the bronchial tree and alveoli distal to the bronchoscope; and the mixture of saline solution and epithelial lining fluid is recovered by gentle suction. The section on “Methodology” revealed what might be expected. While everyone who uses bronchoalveolar lavage agrees with this general method, there are many variations relating to the actual procedure itself and the way in which the recovered material is processed. At the end of this session, it was apparent that for some applications of bronchoalveolar lavage, these variations were irrelevant, while for others, they might be critical.

The Procedure for Lavage

Although no systematic evaluation was made of the various methods used, our impression was that the basic approach to bronchoalveolar lavage is similar around the world. In general, (1) bronchoalveolar lavage is carried out using a fiberoptic bronchoscope with an adult-sized diameter; (2) the amount of topical anesthesia applied is not excessive; (3) a “seal” is achieved by gently wedging the bronchoscope; (4) the site lavaged is usually the right middle lobe or lingula; the lower lobes are much less commonly used, and except for specific purposes, the upper lobes are never used; (5) the fluid used for lavage is sterile, 0.9 percent saline solution at room temperature or warmed to 37°C; (6) the total volume used per site is almost always 100 to 150 ml, and it is usually infused as five 20-ml aliquots or two to three 50-ml aliquots; (7) the maximum total volume used (all sites combined) is usually no more than 300 ml; and (8) after each aliquot is infused, gentle suction is applied, similar to that used in routine bronchoscopy, recovering the mixture of saline solution and epithelial lining fluid in a trap or syringe.

The greatest technical variation in carrying out bronchoalveolar lavage relates to the volume of fluid infused at each site. Several investigators (for example, Babal and colleagues [Paris]), carried out cell differential analyses on the sequential aliquots of recovered lavage fluid and concluded that the first “wash” is different from all subsequent “washes,” for the initial site lavaged is likely to be the distal bronchi and not the alveoli. This concept was emphasized by Voisin et al (Lille, France) who noted more neutrophils in the first aliquot, compared to subsequent aliquots, in patients with idiopathic pulmonary fibrosis, sarcoidosis and hypersensitivity pneumonitis. Similarly, Lam and colleagues (Vancouver, British Columbia) showed that an initial lavage with 5 to 10 ml yields a more “bronchial” lavage with more epithelial cells and neutrophils and proportionally fewer macrophages and lymphocytes. This situation was echoed by Yasuoka and Tsbura (Tokushima, Japan), who performed bronchial and bronchoalveolar lavages in normal subjects and found neutrophils in the former but rarely in the latter; however, there was no uniformity of opinion as to whether all of the returned material should be “pooled” or the initial lavage discarded. For most applications, it was our impression that this does not matter very much, because while the initial 20 ml or so of fluid likely lavages mostly airways, the percentage of return of this volume is sufficiently low so that the mass of cells and acellular components contributed by this initial aliquot to the total volume is small and thus has little influence on the overall average; however, in this context, it was generally agreed that if an individual has inflammatory disease of the airways obvious at bronchoscopy, then the analysis will be heavily influenced by the contribution of the bronchial airways.

One of the basic assumptions of bronchoalveolar lavage is that the cells and acellular components recovered are similar throughout the lung, ie, that a single analysis of lavage is “representative.” While this concept is supported by reports in the literature, it has been systematically evaluated in only a few circumstances (eg, normal subjects, idiopathic pulmonary fibrosis, and sarcoidosis), and it was recognized that exceptions do occur; for example, Staton et al (Atlanta) compared right middle lobe and lingula lymphocyte counts in sarcoidosis. While these investigators found a good correlation between the lobes, the right middle lobe had a higher average lymphocyte count. Similarly, Hunninghake et al (Iowa City) noted that in sarcoid, while lymphocyte proportions in bilateral lavage sites correlated well (r = 0.7), in 25 percent of those studied, one site had significantly more lymphocytes than the other. These investigators also found

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discrepancies in bilateral lavage analyses from patients with idiopathic pulmonary fibrosis and the collagen vascular disorders and suggested that for evaluation of disease activity in the interstitial pulmonary disorders, at least two sites be lavaged and the analyses be carried out separately. Alternatively, many investigators circumvent this potential problem altogether by routinely lavaging two to three sites and pooling the recovered material, thus "averaging" the analysis.

The general conclusion from the literature is that bronchoalveolar lavage is safe, and as long as reasonable guidelines are chosen for the selection of patients, that the mortality is zero and the morbidity very low. Without question, the major complication of lavage is fever. From our experience and our impression from the meeting, as long as the total volume for lavage is kept below 300 ml, the frequency of this complication is acceptably low (in the range of approximately 10 percent). The safety of bronchoalveolar lavage was emphasized by Goldenheim et al (Boston), who compared the functional consequences of large-volume lavage (average, 540 ml) to small-volume lavage (average, 161 ml). Those receiving the small volumes had only a minor reduction in the vital capacity (12 percent), which returned to baseline within 24 hours; all other parameters of pulmonary function did not change. In contrast, there was significant (but also reversible) reduction in several functional parameters within 30 minutes of the large-volume lavage. Further evidence for the safety of bronchoalveolar lavage was presented by Rankin and colleagues (New Haven, Conn), who evaluated individuals with mild asthma (forced expiratory volume in one second greater than 65 percent of predicted). Using intravenous pretreatment with aminophylline, these investigators demonstrated no significant problems in lavaging subjects with mild asthma with a total of 300 ml of saline solution.

Analysis of Recovered Cells

The initial analysis of cells recovered by bronchoalveolar lavage is to determine the number and type of cells recovered. The usual method is to count the cells using a hemacytometer and to determine the cell types on a cytocentrifuged preparation stained with either Wright-Giesma or an analogous stain (such as "Diff-Quick"); however, it is now recognized that while this approach is still adequate for routine analysis, there are several artifacts associated with it that are important for certain applications; for example, Saltini et al (Bethesda, Md) and Babal et al (Paris) demonstrated that the cytocentrifuged preparations underestimate the actual number of lymphocytes recovered by lavage. To circumvent this problem, Saltini and co-workers developed a method using Millipore filters in a fashion similar to that used for cytologic studies in many hospital laboratories. By using a known mixture of lymphocytes and macrophages, they demonstrated that the filtering method accurately assessed the proportions of lymphocytes present, but the cytocentrifugation method underestimated lymphocytes by up to 50 percent. Moreover, the loss of lymphocytes is not consistent or proportional, for there is no "correction" factor that can be applied to determine the true value; however, as was demonstrated by Alfarroba et al (Lisbon), when the percentage of lymphocytes is small, the method used is probably irrelevant. For routine screening purposes, any method is probably acceptable, but for research purposes or in circumstances in which decisions are made on the basis of the proportion of lymphocytes present, as in sarcoidosis, then special attention must be paid to the method of analysis used.

Problems in the conventional approach to determining the numbers of cells recovered in bronchoalveolar lavage were addressed. Usually, cell number is quantified by pooling the lavage material, pelleting the cells, resuspending the pellet in a small volume, and counting the cells using a hemacytometer. Several groups, including Saltini et al (Bethesda, Md) and Mordelet-Dambrine et al (Paris) pointed out that each time cells from bronchoalveolar lavage are centrifuged, there is a loss of cells, and such losses can be large, ie, as much as 5 to 50 percent per centrifugation. Caution was also advised when using an automated cell counter such as a Coulter counter instead of a hemacytometer. While faster and less labor intensive, the Coulter counter may significantly underestimate the number of cells because the broad range of cell sizes present in lavage material may exceed the window settings.

Although cell analyses on cytocentrifuged preparations from bronchoalveolar lavage are usually carried out using rapid stains (such as "Diff-Quick"), Soler et al (Paris), as well as other investigators, pointed out that special techniques may be needed to confirm the identity of certain cell types; for example, mast cells are better seen with a Wright-Giesma stain than Diff-Quick, the peroxidase and esterase methods are more useful for confirming the identity of macrophages, and special stains are needed to accurately search for microorganisms.

A great deal of attention focused on the use of monoclonal antibodies in assessing the subpopulations of cells recovered by lavage. The participants generally agreed that while monoclonal antibodies can be used to accurately assess pulmonary lymphocyte and macrophage populations, the application of these methods to cells from bronchoalveolar lavage is not trivial and that multiple obstacles, not encountered in analyzing blood cells, are encountered with pulmonary cells. The major problem comes from the alveolar macrophage which, particularly in smokers (Edelson et al, Toronto), but to some degree in all individuals, exhibit a broad autofluorescence that complicates fluorescent-activated cell analysis. Hance et al (Bethesda, Md) reviewed this topic in detail, pointing out, at least for now, that the "gold standard" for using monoclonal antibodies to identify cell types in samples from bronchoalveolar lavage is high-powered (>1,000) phase microscopy together with single or double immunofluorescence labeling; however, other methods are in use, including FACS analysis (Morreux et al, Lyon) and immunoperoxidase staining of histologic sections (Paradis et al, Pittsburgh).

Analysis of Recovered Extracellular Molecules

Identification of the various extracellular molecules present in epithelial lining fluid is a major objective of bronchoalveolar lavage. Because retrieval of epithelial lining fluid necessitates the infusion of saline solution, the epithelial lining fluid is diluted by a variable amount of saline solution, with the result that the investigator is faced with the problem of how to express the relative amounts of the recovered

Bronchoalveolar Lavage (Crysal, Reynolds, Kallos)
molecules. In general, three approaches have been used to circumvent what is referred to as the "denominator problem:" (1) presenting the total amount of the molecule recovered; (2) expressing the concentration of a given molecule per volume of lavage fluid returned; and (3) relating the amount of a given molecule to the amount of albumin recovered. Moreover, the volume of saline solution used to recover the epithelial lining fluid significantly dilutes it, such that measuring the amounts of some of the molecules in bronchoalveolar lavage fluid becomes difficult, particularly if they are present in very small quantities in situ. To circumvent this problem, most investigators concentrate the fluid, usually with pressure filtration through a membrane with a molecular weight cutoff of 2 to 10 kilodaltons (eg, Amicon UM 10 membrane). It was of interest, therefore, that Lam et al (Vancouver, British Columbia) noted that some membranes (eg, CX10) resulted in the loss of more protein than others (eg, UM10), and that all filtering methods resulted in significant losses of some protein. Several groups are now using enzyme-linked immunoassays (ELISA) to quantify directly the amount of protein in bronchoalveolar lavage fluid without concentrating it; ie, the enzyme-linked immunoassay method is sufficiently sensitive so that the approximately 100-fold dilution of the epithelial lining fluid by saline solution does not prevent accurate quantification. An example of this approach was presented by Rennard and colleagues (New Haven, Conn), who established values for the total protein, albumin, and immunoglobulins in the lower respiratory tract by evaluating a population of 177 healthy individuals and symptomatic cigarette and marijuana smokers.

It was apparent to all of those who analyze bronchoalveolar lavage fluid that some direct measurement of the volume of epithelial lining fluid recovered would be very useful and would permit expression of the data in molar amounts, thus allowing direct comparisons with other body fluids, such as plasma. This would further avoid problems in interpretation caused by indirect methods. Two approaches were presented that attempted to circumvent this problem. First, Rennard and colleagues (Bethesda, Md) used a method based on the concept that urea is freely diffusible in the body. As urea can be measured easily in plasma, quantification of the total amount of urea recovered by bronchoalveolar lavage fluid permitted calculation by simple dilution principles of the volume of epithelial lining fluid from which the urea was derived. Secondly, Dohn et al (Cincinnati) used methylene blue as an external marker of the dilution of epithelial lining fluid by saline solution. Alternatively, Dohn and colleagues evaluated the use of potassium in lavage fluid as an "internal" marker but concluded that it was not useful.

It is widely accepted that bronchoalveolar lavage recovers cellular and extracellular components from the alveolar and bronchiolar epithelial surfaces. For many purposes, it is irrelevant whether or not the recovered materials are representative of the cells and molecules throughout the alveolar and bronchiolar walls; ie, it is sufficient that they are from the epithelial surface of the lower respiratory tract; however, for certain applications, it becomes very important to know how representative they are.

As the pathogenesis of pulmonary sarcoidosis is intimately linked to the types and functions of T-lymphocytes in the lower respiratory tract, it is important to ask whether the findings concerning T-cell populations from bronchoalveolar lavage correlate with findings obtained by histologic studies. This question was evaluated in detail by Semenzato and colleagues (Padua and Verona, Italy), who used a variety of monoclonal antibodies to demonstrate an excellent correlation between lymphocytes obtained by bronchoalveolar lavage and those from tissue. Consistent with these observations, Paradis et al (Pittsburgh) demonstrated that the ratio of LEU3+ (helper) T-cells to LEU2+ (suppressor/cytotoxic) T-cells in pulmonary tissue correlated very well with that assessed by bronchoalveolar lavage. Interestingly, similar results were found for idiopathic pulmonary fibrosis, thus supporting the validity of bronchoalveolar lavage in assessing T-cell subpopulations in the lower respiratory tract.

Succeeding sessions dealt with some specific diseases in which analysis of bronchoalveolar lavage fluid or in vitro study of cells obtained by bronchoalveolar lavage had special application.

Cigarette Smoking and Bronchoalveolar Lavage

Although long-term inhalation of cigarette smoke is an established risk factor in the pathogenesis of many respiratory diseases, only a small percentage of all smokers develop an overt disease such as bronchitis, emphysema, or primary lung cancer; however, reasonably short periods of smoking (one to five pack-year history) by young normal subjects can be associated with striking changes in the quantity of cells, proteins, and enzymes recovered from the air spaces by bronchoalveolar lavage. In a recent review4 the contents of bronchoalveolar lavage fluid from smokers and nonsmokers were contrasted, and at least 20 differences were found. Merrill and colleagues (New Haven, Conn) sampled two bronchial proteins in bronchoalveolar lavage fluid, free secretory component and keratins, both of which might be altered if early metaplastic changes had or were to occur in ciliated cells from asymptomatic and symptomatic smokers. Controls were nonsmokers and patients with primary lung cancer. Free secretory component was decreased in 22 symptomatic smokers; and keratins, which were absent in nonsmokers, were detected in four of these 22 smokers. In patients with lung cancer, bronchoalveolar lavage fluid contained low levels of free secretory component, and keratins were detected in most of these specimens. Resected pulmonary tissue from all patients mirrored the results in bronchoalveolar lavage fluid. Thus, these two proteins have an inverse relationship in bronchoalveolar lavage fluid from young smokers and patients with lung cancer. Although such altered protein values are not specifically linked to airway irritation induced by cigarette smoke, a search to find such biochemical markers that will reflect early epithelial injury before cellular metaplasia develops should be continued.

An increased number of alveolar macrophages is recovered in bronchoalveolar lavage fluid from smokers. These cells are usually in an activated state, so that secretory function and characteristics of the cell membrane are altered. Using an indirect immunofluorescent method, Lawrence and colleagues (Houston) assessed the absence of Ia surface antigen on macrophages derived from smokers and nonsmokers. Whereas about 80 to 90 percent of nonsmokers' cells express Ia surface antigen, only 40 to 50 percent of smokers' cells have this antigen. Also, Ia antigen is reduced.
on macrophages obtained from patients with lung cancer. DeShazo et al (New Orleans) found that alveolar macrophages from patients with lung cancer had reduced ability to stimulate lymphocytic proliferation. When alveolar macrophages were mixed with purified blood lymphocytes to measure lymphocytic proliferation (with phytohemagglutinin), smokers' and nonsmokers' cells were stimulated (lymphocytic proliferation) to a comparable degree, but alveolar macrophages from patients with squamous cell carcinoma of the lung were less effective.

Autofluorescence of smoker-derived alveolar macrophages presents a problem in using fluorescent antibody methods, as discussed by Edelson and co-workers (Toronto). Hance (Bethesda, Md), who reviewed the use of monoclonal antibodies to enumerate immune and inflammatory cells, as well as other speakers, alluded repeatedly to this limitation, which reduces the usefulness of automated flow cytometry for separation of cell populations and subpopulations.

The impact of cigarette smoke and particulates on alteration of antiprotease enzymes and on stimulation of oxidant enzymes in macrophages remains an important issue in the pathogenesis of smoke-related pulmonary injury. Hoidal (Memphis) reported that alveolar macrophages from smokers had increased levels of superoxide dismutase and glutathione peroxidase to compensate for oxidant stress in the airways, but these increases were reversible, since they decreased to normal levels one week after smoking ceased. Although previous reports have recorded that the functional activity of α1-antiprotease in pulmonary lining fluid was diminished after smoking, Abboud and associates (Vancouver, British Columbia) did not find a striking difference in inhibition of pancreatic elastase in lavage fluid from 38 smokers compared to eight nonsmokers. For the smokers an initial lavage was performed after an eight-hour abstinence from smoking, while another was done minutes and up to two hours after smoking two to four cigarettes. A decrease of about 10 percent was found in antiprotease activity one hour after smoking. No difference in antiprotease function was found in lavage fluid obtained from upper vs lower lobe areas in the smokers; however, cells from the upper lobe lavages contained more elastase, so that the ratios of cellular elastase to α1-protease inhibitor were higher. In contrast, in a model in which mice were exposed to 20 percent smoke from reference cigarettes, Chan and co-workers (Stony Brook, NY) found that inhibition of porcine pancreatic elastase was decreased about 40 percent in lavage fluid, a finding consistent with their earlier results in rats.

Different antiprotease inhibitors may exist in the central airways and bronchi, where a small molecular-weight inhibitor is present. This is in comparison to the alveolar spaces where α1-antiprotease is in high quantity. Alpha1-macroglobulin is present in minute amounts in normal bronchoalveolar lavage fluid. Tettey and colleagues (London) also found bronchial mucous protease activity in bronchoalveolar lavage fluid. The origin of α1-antichymotrypsin, which inhibits cathepsin G, was investigated by Burnett et al (Birmingham, England). Alpha1-antichymotrypsin is synthesized by alveolar macrophages, but also was found in ciliated bronchial epithelial cells by immunohistochemical staining. Therefore, the airways and alveolar spaces have various inhibitor enzymes to attack proteolytic enzymes which are released by inflammatory cells or whose production is stimulated by inhaled irritants.

Asthma

Research interest in asthma currently is high because findings have emerged that point to new concepts about the origin of mediators and to potential development of different kinds of drugs to treat bronchospasm. Recent research has delved deeper into mast cell physiology, mucus secretion, altered permeability of the epithelial cell surface, and the role of alveolar macrophages in production of mediators from arachidonic acid metabolism, as well as other chemottractant factors. Pulmonary lavage has been used to recover alveolar cells from asthmatic subjects who are not severely ill and whose condition is stabilized by medication. In this session, two features of asthma were emphasized: (1) cellular characteristics of bronchoalveolar lavage fluid; and (2) mediator secretion by macrophages. As an introduction the overall topic of arachidonic acid metabolism by human alveolar macrophages was reviewed by Dr. Peter Ward (Ann Arbor, Mich).

Cytologic profiles in lavage fluid obtained from asthmatic subjects were presented in several reports. Takeyama and colleagues (Okayama, Japan, and Omaha) compared cell differential percentages from 64 cases of bronchial asthma with those in normal subjects. Eosinophils in lavage were increased in atopic subjects in proportion to the serum IgE levels and blood eosinophils, but neither nonatopic subjects nor those with persistent asthma had an increased number of polymorphonuclear neutrophils in lavage. Godard and co-workers (Montpellier, France) examined two groups of patients, one with a viral-induced exacerbation of asthma and the other with bacterial associated illness. As might be expected, eosinophil counts in bronchoalveolar lavage fluid did not differentiate either of these groups from the control symptom-free asthmatic subjects. In the virus-provoked bronchospasm, air-space cells were not distinctive except for an increased number of ciliated epithelial cells; but with the bacterial illness, both lymphocytes and polymorphonuclear neutrophils were plentiful (mean of 25 percent and 45 percent, respectively, of cell differential counts of bronchoalveolar lavage fluid). In their analysis, Kanawami et al (Tokyo) reported the novel feature of inspection of peripheral airways of asthmatic subjects using a very small (1.8-mm diameter) fiberoptic bronchiroscope. Another attempt to find a stable marker to be used as a denominator in analysis of normal subjects and asthmatic patients by bronchoalveolar lavage was attempted by Lam and colleagues (Vancouver, British Columbia), but they concluded that neither potassium nor calcium was a reliable enough marker for standardizing protein values in bronchoalveolar lavage fluid.

In 36 atopic subjects with perennial asthma, Kay et al (London) performed a randomized trial of placebo vs inhaled cromolyn disodium (disodium cromoglycate) to observe what changes occurred with therapy in cell counts, immunoglobulins, and complement factors in bronchoalveolar lavage fluid. After four weeks of therapy, the percentage of eosinophils, the amount of IgA and house-dust-specific IgE antibodies were decreased compared to the placebo controls. Following up on the mechanism of this drug, if bronchoalveolar lavage cells from normal subjects were reacted
with IgE antibody, the 1 to 3 percent of mast cells present in the cell mixture released histamine; however, Flint and colleagues (London) found that this dose-dependent response was inhibited by in vitro exposure of cells to cromolyn disodium. For contrast with asthma, results of cellular analysis of bronchoalveolar lavage fluid were reported by Yenokida and colleagues (Bethesda, Md) for a group of patients in Tamil Nadu, India (formerly Madras) with tropical pulmonary eosinophilia from microfilarial infection. Bronchoalveolar lavage fluid from untreated patients with acute disease contained over 50 percent eosinophils, but after treatment with diethylcarbamazine, these cells decreased by almost one half.

Prior reports have established that normal alveolar macrophages can secrete lipoxygenase pathway products that form the mediator known as slow-reacting substance (LTC₄, D₄, E₄, and LTB₄) which has chemotactic properties. Godard and co-workers (Montpellier, France) found that macrophages from asthmatic subjects also secrete LTC₄. Using alveolar macrophages from asthmatic patients, Tonnel et al (Lille, France) stimulated these cells with either anti-IgE antibody or a specific allergen that could combine with cytophilic IgE antibody and assayed cell supernatants for chemotactic activity for polymorphonuclear neutrophils and eosinophils. Preliminary characterization implicated LTB₄ as the responsible chemotactic substance present. Arnaux and colleagues (Clamart, France) found that platelet-activating factor, which has bronchoconstrictor effects, was produced in increased amounts by macrophages from atopic subjects, either spontaneously or following various forms of cellular stimulation; however, if the atopic patient had been treated with corticosteroids, the release of platelet-activating factor was negligible.

In summary, considerable work is being done to characterize new sources of mediators in asthma, especially arachidonic acid metabolites secreted by macrophages. It is noteworthy that combining the number of asthmatic subjects from published reports with those specified in abstracts at this conference, almost 200 volunteers with this syndrome have undergone bronchoalveolar lavage without unacceptable side effects. In questioning various authors, it appears that asthmatic subjects are selected based on their disease being mild and stable, and some form of anti-inflammation therapy is used during the bronchoscopy. About 10 percent have some intra-procedural wheezing or symptoms after lavage that can be treated successfully with aerosol drugs. Use of subjects with minimal disease for bronchoalveolar lavage seems to be a safe and acceptable research procedure. Guidelines have been published recently about this.⁴⁴

**INTERSTITIAL PULMONARY DISEASES**

An early application for analysis by bronchoalveolar lavage fluid was found in patients suffering from diffuse forms of interstitial inflammation and fibrosis. Staging the activity of pulmonary disease and retrieving cells and proteins for in vitro studies have contributed clinical and diagnostic information and improved current concepts of the pathogenesis of disease. This was reviewed by Dr. Crystal (Bethesda, Md). Three sessions on interstitial pulmonary diseases, which represented the largest segment of the conference, reflected the interest that this group of diseases has attracted and the relative amount of ongoing research activity. The presentations and posters provided three themes: (1) details about cell counts and identification of cells, especially lymphocytes, and monoclonal antibody staining methods and related techniques; (2) functional interactions of cells, in particular macrophages and lymphocytes; and (3) cell mediators that modulate disease activity. Much of this segment of the conference dealt with sarcoidosis and idiopathic pulmonary fibrosis, but some work was presented on hypersensitivity pneumonitis, collagen vascular associated pulmonary disease (scleroderma lung) and drug hypersensitivity. Occupational exposure was addressed in a separate session.

In terms of identifying cells in bronchoalveolar lavage fluid, sarcoidosis is a popular subject for study because of the recovery of increased numbers of lymphocytes from patients suffering from the disease. The prognostic clinical use of such counts was central to most studies; however, the location sampled within the lung, guided by the intensity of the uptake of gallium isotope, as advocated by Staton et al (Atlanta) and Perrin-Fayolle and colleagues (St. Genis-Laval, Lyon, France) can cause variation in the cell counts and confuse the overall assessment of alveolitis. The possible error introduced by sampling sites must be weighed, and this suggests that several areas of the lung should be lavaged and the fluid analyzed separately from each. Whether to discard the first lavaged aliquot recovered which may be more of a "bronchial" specimen that contains a disproportionate number of polymorphonuclear granulocytes was raised by Voisin and colleagues (Lille, France) and has been discussed already under methods. Paradis et al (Pittsburgh) and Semenzato et al (Padua, Italy) looked for similarity of lymphocyte phenotypes in bronchoalveolar lavage fluid and in specimens from lung biopsy and found a good correlation between the cell types from both sources. It was noted that Tac-positive lymphocytes were present, indicating active T-cell replication. Rossi and colleagues (Genova, Italy) identified an expanded subset of T-helper cells from among lavaged cells recognized by a monoclonal antibody designed 59/5. These T-cells stimulated B-lymphocytes to produce immunoglobulin. Based on OKT-4 stained lymphocyte counts (putative T-helper cells) in bronchoalveolar lavage fluid from patients with active but untreated pulmonary sarcoidosis, Baughman et al (Cincinnati) noted that patients with this kind of acute alveolitis responded to corticosteroid therapy with improved measurements of pulmonary vital capacity. Rust and colleagues (Frankfurt, West Germany) found that sarcoid patients with higher OKT-4 cells were more likely to have progressive pulmonary disease during a two-year period of follow-up observation. Israel-Biet and co-workers (Paris) observed that persistently elevated lymphocyte counts in bronchoalveolar lavage fluid, initially and especially after one year of observation, favored evolution to a chronic form of pulmonary sarcoidosis.

Functionally, the cells from the lungs of patients with sarcoidosis were active in several ways. As noted by Toews et al (Dallas) and Venet and colleagues (Paris and Bethesda, Md), alveolar macrophages, which can stimulate lymphocytes to proliferate through antigen presentation, may be important in attracting lymphocytes to the lung. How they accomplish this is unclear, but perhaps a cytokine mediator produced by macrophages, such as interleukin-1, might be
Japan) found mast cells were elevated in bronchoalveolar lavage fluid from active cases. King and associates (Denver) found an increased percentage of polymorphonuclear leukocytes, Rich and colleagues (Cleveland) assessed the accessory cell function of macrophages. They found that the ratio of presenting cells to responding cells was crucial for the production of a cytokine such as IL-1 and for blastogenic responses. Furthermore, they found that the macrophage, depending upon its activation status, can have either a stimulatory or suppressive influence. Subpopulations of alveolar macrophages vary also in their cellular activity, as found by Sandron et al (Paris), who examined cells recovered from normal subjects and patients with sarcoidosis. Carrying over functional activity, as represented by release of interleukin-2 from active T-helper cells in bronchoalveolar lavage fluid, to the realm of therapy and clinical assessment, Pinkston and colleagues (Bethesda, Md) examined the effect of corticosteroid treatment on the spontaneous secretion of IL-2 by T-cells and amount of T-cell replication. After patients were treated with corticosteroids for one to three months, lymphocytes from bronchoalveolar lavage, compared with baseline cellular activity before treatment, no longer produced IL-2, and T-cell replication was normal. This approach suggests that parameters of cellular function, not just cell counts or cell phenotypes, will provide alternative and perhaps more sensitive ways for monitoring changes in bronchoalveolar lavage fluid in patients. As reported by Mornex and colleagues (Lyon, France), identification of activated lymphocytes within their cycle of replication can be done with immunofluorescent staining and flow cytometry, and this promises to be another useful method for assessing cell function.

For idiopathic pulmonary fibrosis, interest continues in finding the best marker in bronchoalveolar lavage fluid that might predict prognosis in patients. Takishima et al (Sendai, Japan) found mast cells were elevated in bronchoalveolar lavage fluid from active cases. King and associates (Denver) found that an increased percentage of polymorphonuclear neutrophils reflected more advanced idiopathic pulmonary fibrosis than patients who responded to the challenge with fever, leukocytosis, and pulmonary functional changes, the lavage fluid from patients with established hypersensitivity pneumonitis, very few observations have been made about the character of alveolar pulmonary cells in the acute phase of illnesses following recent exposure to an inhaled antigen. Fournier et al (Lille, France) evaluated five patients with established hypersensitivity to bird-derived antigens who had high baseline percentages of lymphocytes in bronchoalveolar lavage fluid. After aerosol challenge with antigen, repeat lavages were done 24 hours later. In the three volunteer patients who responded to the challenge with fever, leukocytosis, and pulmonary functional changes, the lavage fluid from patients with established hypersensitivity pneumonitis revealed a substantial increase in polymorphonuclear neutrophils (mean, 38 percent cells); evidence of a neutrophilic chemotactic factor and increased serum C5a were found in their serum. These interesting studies, which may be difficult to do in a larger group of patients, suggest that an acute neutrophilic alveolitis reminiscent of an Arthus-type reaction occurs. Finally, a variety of other patients with diffuse pulmonary disease were studied by lavage and included amiodarone-induced pneumonitis by Dennewald et al (Paris), alveolar proteinosis by Wasserman and Mason (Torrance, Calif) and amiodarone-induced pneumonitis by...
Israel-Biet et al (Paris). Ultrastructural study of multilamellar structures that accumulate in cells and alveolar fluid was undertaken by Hook and Gilmore (Research Triangle Park, NC) and by Takemura and colleagues (Bethesda, Md) and may provide insight into differences between normal type-2 cell bodies and abnormal phospholipid secretion in pneumonitis. In the amiodarone-associated pulmonary disease, cell counts in nine patients revealed changes very similar to those obtained in ten patients with hypersensitivity pneumonitis. Lavage cells from amiodarone-affected lungs featured a very high percentage of total lymphocytes and a relative increase in T-suppressor cells (OKT-8 staining cells); foamy alveolar macrophages, plasma cells, and mast cells were increased slightly. Also, for patients with histiocytosis X, or eosinophilic granuloma of lung, Chollet-Martin and Israel-Biet et al (Paris). Ultrastructural study of the exudate with high neutrophilic elastase content; the elastase was complexed with α1-proteinase inhibitor; however, about 30 percent of the total elastase inhibitory activity present in the pulmonary fluids could not be accounted for by the available amounts of α1-proteinase inhibitor or α1-macroglobulin, suggesting that other as yet unidentified inhibitors may exist in the lung. Spragg and colleagues (San Diego) also found neutrophilic elastase in all bronchoalveolar lavage specimens from 24 patients with ARDS, but the functional activity of α1-proteinase inhibitor was variable and often inactive, thus permitting them to detect some free elastolytic activity. Gadek et al (Columbus, Ohio) prospectively studied eight patients with ARDS and four comparable patients with cardiogenic pulmonary edema for the potential role of polymorphonuclear neutrophilic mediators as the cause of pulmonary injury. In the patients with ARDS, polymorphonuclear neutrophils accounted for about 65 percent of cells in bronchoalveolar lavage fluid, compared to about 5 percent in control subjects, and the presence of increased polymorphonuclear neutrophils correlated with the severity of the gas exchange abnormality. Functional elastase was not present in any of these bronchoalveolar lavage specimens, but collagenase and myeloperoxidase were; these latter enzymes produced a high cytotoxic index when reacted with chromium-labelled rat pulmonary explants. To explain the polymorphonuclear neutrophilic influx that occurs in the lungs in ARDS, Robbins and co-workers (Omaha) assessed complement fragment activation (C3, C5, and properdin factor B) in bronchoalveolar lavage fluids. Cleavage products of C3 and factor B as well as C5a were found in most ARDS-derived bronchoalveolar lavage specimens. In plasma, only C3 cleavage products were found in half of the specimens. C5a appears to be an important alveolar chemotactic substance in this disease. Davis et al (Columbus, Ohio) turned their attention to some of the larger proteins that may leak into the air spaces of patients with ARDS (>150,000 daltons molecular weight) and found that IgM was prominent. In three patients, serial bronchoalveolar lavage was used to document the resolution of the protein leak which correlated with decreasing pulmonary inflammation (polymorphonuclear neutrophils in lavage) and improved clinical condition. In summary, bronchoalveolar lavage seems to be a well-tolerated procedure for patients with ARDS, even if they are intubated and require assisted ventilation. Often four to six serial aliquots of 25 to 50 ml are used for lavage. Analysis of lavage fluid has focused on the proteolytic and inhibitor enzyme mechanisms and as yet has not been used in a...
prognostic way to monitor clinical outcome or to predict which patients might progress to fibrotic pulmonary complications.

**Occupational Pulmonary Diseases**

Bignon and colleagues (Creteil, France) enumerated asbestos bodies in lavage fluid and in pulmonary tissue of 60 patients with asbestosis. Five bodies per milliliter of bronchoalveolar lavage fluid correlated with more than 1,000 bodies found per gram of pulmonary tissue which is considered indicative of occupational exposure. Elevated percentages of polymorphonuclear neutrophils were found in some of these patients with asbestos-related disease. In a similar study, Xaubet-Mir (Barcelona, Spain) found that workers with asbestosis had a mild polymorphonuclear neutrophilic alveolitis which correlated with pulmonary rales and hypoxemia.

Berylliosis has resurfaced as an immunologic disease, and findings from pulmonary lavage were reported for a group of these patients by Rossman and colleagues (Philadelphia and New Haven, Conn). The salient feature of this granulomatous pulmonary response was a great increase in the bronchoalveolar lavage fluid's lymphocytes (45 to 80 percent of total cells) and a high ratio (>4.0) of T-helper to T-suppressor lymphocytes (usual is about 1.8 for the alveolar lavage cells). These lymphocytes proliferated in culture when exposed to beryllium salt. Silicosis is another example in which bronchoalveolar lavage has been applied to an occupational pulmonary disease. Davis and co-workers (Burlington, VT) performed bronchoalveolar lavage on granite workers and analyzed the recovered cells and proteins. They submitted alveolar macrophages to scanning electron microscopy to identify specific intracytoplasmic particles. Occupational exposure to granite dust was reflected by silica particles in macrophages and an increase in number of lymphocytes in bronchoalveolar lavage (about 16 percent vs 6 percent in asymptomatic, nonsmoking, control granite workers). Albumin and especially immunoglobulins were also present in high amounts in bronchoalveolar lavage fluid from these individuals.

Ozone exposure is associated with enhanced bronchial reactivity, but the inflammatory condition of the airways that might contribute to this was not known until Seltzer and Boushey (San Francisco) examined bronchoalveolar lavage fluid from exposed, but previously healthy subjects. In four of five subjects with airway hyperresponsiveness in whom specific airway resistance was increased significantly, cells from bronchoalveolar lavage recovered three hours after exposure contained a high percentage of polymorphonuclear neutrophils (13 to 45 percent of cells). Preliminary analysis indicated that several arachidonic acid metabolites were present in the fluid, suggesting their participation in the hyperreactivity and inflammatory responses in the airways. Similar findings were noted in a canine model of ozone exposure by Fabbri et al (San Francisco). A number of animal models of acute pulmonary injury (endotoxin, phorbol myristate acetate, and paraquat) or inhaled substances (asbestos, cadmium nitrogen dioxide, and diesel exhaust) were described in which inflammatory mechanisms were dissected with the aid of analysis of bronchoalveolar lavage fluid.

The prevalence of inhalational pulmonary diseases caused by many occupational and environmental exposures to organic and nonorganic substances appears to be increasing and may be underestimated because in many instances, these diseases may be insidious and the host's response in the lung protracted and smoldering. The correct diagnosis can be missed. The application of bronchoalveolar lavage analysis in patients with some of these diseases may help establish a specific cause, especially the use of particle analysis in phagocytic cells. Also, more information may be gained about the host's immunologic response to particles that cause inflammation. Finally, the question of the therapeutic use of bronchoalveolar lavage arose and was discussed once again as a way to reduce the lung's burden of inhaled particles or toxic substances, thus conceivably lessening parenchymal injury and decreasing the fibrogenic response. This approach has been tried in some animal models of occupational disease (Muggenburg et al, Albuquerque, NM).

**Pulmonary Infections in Immunocompromised Hosts**

Of course, fiberoptic bronchoscopy with airway brushing, swabbing, various protected catheters, lavage, and transbronchial biopsies has been an important procedure used in the diagnosis of pulmonary infections since the instrument's inception. The microbial accuracy of these fiberoptic specimens, which invariably are contaminated with some oropharyngeal flora, is questionable, and the usefulness of such cultures can be debated. Grimfeld et al (Paris and Creteil, France) found bronchoalveolar lavage to be a safe and helpful procedure to use in infants and children, including those immunosuppressed, to recover viruses when severe pneumonitis was present. In patients with bone marrow transplants who developed pneumonia while receiving immunosuppressive drugs, Beraudin and colleagues (Creteil) reported bronchoalveolar lavage fluid analysis and cultures yielded a specific diagnosis in 29 of 54 patients (54 percent). This is a figure which is representative of the experience found in other series of patients.

Nevertheless, in one recently described affliction, acquired immunodeficiency syndrome (AIDS), the accuracy of cultures of bronchoalveolar lavage fluid is quite good for the identification of Pneumocystis carinii and other opportunistic organisms that plague these patients. Venet and colleagues (Paris) analyzed cultures from bronchoalveolar lavage of 28 patients with AIDS who were suspected of having an opportunistic infection. Eighty-two percent of the lavages provided an appropriate infectious agent; most had Pneumocystis or cytomegalovirus (or both) or mycobacteria. Lymphocyte percentages in bronchoalveolar lavage fluid from patients with AIDS were increased (mean, 27 percent), with a reversed ratio from the normal of T-helper and T-suppressor cells. Three other reports (Boylan et al, Los Angeles; Rust et al, Frankfurt, West Germany; and Garay and colleagues, New York) dealing with the use of bronchoalveolar lavage or other bronchoscopic means to diagnose Pneumocystis infection in an aggregate group of almost 200 patients with AIDS gave about a 70 percent success rate in culturing an organism from large specimens, although even higher percentages have been published. Why pulmonary infections occur with microorganisms that are often found in pulmonary macrophages or are known to be difficult for these

Bronchoalveolar Lavage (Crystal, Reynolds, Kalica)
phagocytes to ingest is unclear, except that cellular immune mechanisms within the lung seem to be impaired. Little information about the microbial activity of alveolar macrophages from patients with AIDS exists. Clavel and colleagues (Paris) did find that phagocytic activity (for yeasts) and intracellular killing of Staphylococcus aureus were similar in the macrophages from patients with AIDS, as compared to control cells, thus ruling out an overt deficiency in macrophagic function. We anticipate that more immunologic data will come from the study of the airways of patients with AIDS or perhaps from patients with prodromal stages of AIDS-related disease. It is possible that macrophage-lymphocyte interactions may not be regulated appropriately in this disease.

**Summary**

The application of the method of bronchoalveolar lavage to an increasing array of pulmonary diseases was evident, and the use of sophisticated technology to study cells and measure minute amounts of protein and other components in bronchoalveolar lavage fluid indicated that more meaningful information may be gained about diseased airways and alveolar spaces than suspected. That new techniques are being developed to make assays more sensitive and specific was evident. The popularity of this research approach was underscored by the interest and participation of colleagues in the United States and especially in Europe (notably France, England, Italy, and West Germany) and in Japan. This meeting was an opportune time to reflect on what bronchoalveolar lavage analysis has contributed to date and to focus attention on new applications that will be forthcoming and will improve our understanding of immunopathogenic mechanisms in an expanding number of pulmonary diseases.

**References**