Lung (2000) 178:271–293
DOI: 10.1007/s004080000032

Lung

Use of Bronchoalveolar Lavage in Humans—Past Necessity and Future Imperative

H. Y. Reynolds

Department of Medicine, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, PA

Abstract. Limited bronchoalveolar lavage (BAL) as an extension of fiberoptic bronchoscopy has permitted the recovery of airway-alveolar space cells and soluble substances in the extracellular lining fluid that have been used diagnostically and as research specimens in patients with a variety of lung diseases and in normal subjects for the study of lung host defenses. During the past three decades, use of BAL specimens has stimulated immunologic and cellular research of pulmonary diseases, which has provided significant insight into local host immunity, inflammation, fibrogenesis, asthma mechanisms, and infections. From this research new methods of antifibrotic therapy of interstitial pulmonary fibrosis, for example, have followed. Moreover, BAL applications have greatly enhanced professional interest in the field of pulmonary medicine. This review attempts to analyze the history and impact of BAL, appraise its current status, and assess its future usefulness.

Understanding the immunopathogenesis of many lung diseases is predicated on obtaining *in situ* specimens from affected lung tissue and airways. BAL provides a direct sample that can be compared with an endobronchial or transbronchial biopsy tissue specimen and with cellular and immunologic components in the vascular circulation. Thus, the recovery of BAL fluid and its components involved directly with a disease process or continguous with interstitial tissue permits a much more detailed assessment of new cellular mediators and cytokines participating in the pathologic process. Furthermore, subjecting BAL cells to microarrays of DNA to discern what genes are activated will be one step closer to identifying intracellular processes involved or deranged. Identification of causative factors may solve questions of causation, so that preventive strategies or definitive therapy can be used.

Key words: Bronchoalveolar lavage—Fiberoptic bronchoscopy—Diffuse interstitial lung disease

Offprint requests to: Herbert Y. Reynolds, MD, The Milton S. Hershey Medical Center, The Pennsylvania State University, P.O. Box 850, Hershey, PA 17033-0850.

Introduction

Through the rigid bronchoscope, optimally designed almost a century ago by Dr. Chevalier Jackson (1904) [92], washing a portion of the lungs to remove secretions could be done therapeutically. More extensive lung lavage became treatment for patients with alveolar proteinosis [82] and other respiratory illnesses that featured extensive accumulation of purulent secretions as found with cystic fibrosis, chronic asthmatic bronchitis, and bacterial pneumonia [104]. This method of using a large volume bronchopulmonary lavage (BAL), termed "bronchioloalveolar debridement" [82], was performed through a double-lumen bronchospirometry tube (Carlens tube) [7] with saline fluid and was the established treatment for alveolar proteinosis [104].

To study the physiologic effects of small volume bronchopulmonary lavage, Finley and colleagues [29] lavaged seven healthy volunteers (average age, 27 years) and four patients with obstructive lung disease. A Métras catheter [69], 19F size, was passed into the locally anesthesized airways of awake subjects and anchored in a segmental bronchus under fluoroscopic control. The lung segment was lavaged with 300 mL (100-mL aliquots) of normal saline, after which lung function, arterial oxygenation, and chest film changes were monitored. This study illustrated the feasibility and safety of doing small volume bronchopulmonary lavage in normal subjects. Pratt and colleagues [80] extended the study of endobronchial lavage through a Métras catheter in healthy volunteers, enlisting 16 subjects, equally divided as smokers and nonsmokers. Thereafter, more studies followed [11, 37, 60], using smoker and nonsmoker normal subjects, to compare the cellular function of alveolar macrophages in these groups and the yield of surfactant material [28]. Endobronchial lavage was performed through a Métras catheter in these studies. Bronchial washings from intubated surgical patients were aspirated and analyzed for immunoglobulins as well [49, 64].

Clearly, cellular immunology of the lower airways was beginning to be investigated in humans, perhaps stimulated by the innovative work of Myrvik and colleagues [72] to obtain alveolar macrophages with lung lavage in rabbits. This review will summarize first (Fig. 1) past methods that provided access to human airway materials, how these became important in studying respiratory tract host defenses in the context of newly evolving immunologic insights, and how BAL fluid (BALF) and its analysis was first applied to a group of important diseases of the lungs. Then future applications for BAL will be considered.

Retrospectascope—A Look at the Past

A confluence of several research streams occurred about 30 to 35 years ago: new insights were numerous about cellular and humoral immunity; research emphasis shifted to understanding the host's response, especially local defense mechanisms of the respiratory tract, as emphasized by Green [36], but that were still based largely on animal model research; and procurement of human respiratory cells was now feasible with safe methods. Thus, explanation of human respiratory immunology in normal subjects was underway, and investigators were poised to take advantage of a powerful technologic advancement—bronchofiberscopy developed by Dr. S. Ikeda and collabo-

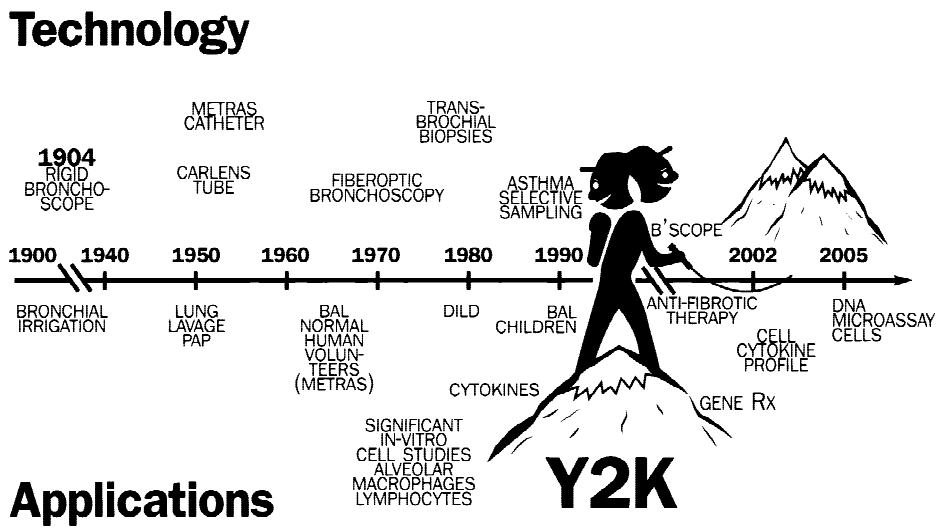

Fig. 1. From the viewpoint of the millennium, the development and application of BAL can be traced from its initial use of therapeutic lavage to a clinical research method for normal subjects and patients, especially with DILD, that has provided considerable insight into local lung host responses and derangements found with illness; a scorecard of this success can be evaluated. However, a considerable foundation of immunologic infrastructure has been developed that should be the basis for dissecting cellular interactions and discovery of etiologic agents to definitively solve and treat heretofore perplexing lung diseases. Although some approaches in the immediate future look promising with molecular genetic techniques, other horizons (mountains) are certain to emerge as hurdles.

rators in Japan [47]. This new instrument would permit routine sampling of cells and secretions from the airways and alveoli of normal research subjects and the affected airway surfaces of patients with various lung diseases.

After introduction of the flexible bronchofiberscope into US and Western European medical centers around 1970 [46, 47], the practice of fiberoptic bronchoscopy (FOB) grew quickly after initial guidelines were described [108, 113]. For research, BAL was used initially to retrieve macrophages from normal volunteers [6, 134] for functional studies, but its use expanded to sample other cellular and soluble components in the fluid [18, 99, 127]. Using volunteers, the impact of cigarette smoking to alter BALF components was examined [129], and the origin of individual substances in BALF such as immunoglobulins, began to be investigated [53]. Analysis of other soluble components was added [58]. As a personal example of exploiting this new technique to readily obtain human BAL cells and protein materials, we had completed an assessment of respiratory host immune responses in a rabbit model [100, 101] and were able to shift immediately into human immunology [95, 98, 99].

Our preliminary study [99] established baseline values for both cells and immunologic components in a specimen of BALF obtained from the lingula or lower lobes of young normal smokers and nonsmokers and from middle-aged smokers and nonsmokers undergoing diagnostic evaluation for an isolated upper lobe lesion. Then, we applied this systematic analysis to groups of patients being evaluated for diffuse in-

terstitial lung disease (DILD), especially idiopathic pulmonary fibrosis (IPF) and chronic hypersensitivity pneumonitis (CHP) [16, 97]. For 29 patients with IPF, 19 patients had BALF analysis; for 17 of these 19 patients with IPF, the diagnosis had been established by histopathologic examination of lung biopsy specimens [16, 97, 122]. Of these patients, 7 were untreated and 12 were receiving oral corticosteroids when BAL was performed; 7 were current cigarette smokers. Among BAL cells, a characteristic pattern was noted in patients on corticosteroid treatment of an increased percentage of polymorphonuclear neutrophils (PMN) (mean about 14% with range of 3–40%) and of eosinophils approximately 4%. For untreated patients with IPF, the mean PMN percentage was even greater, but eosinophils were about the same. Low-grade eosinophilia in BALF persisted despite prednisone therapy. The combined finding of more PMNs and eosinophils in BALF from IPF patients was even more striking when compared with values from control smokers and nonsmokers. Other immunologic changes such as a higher ratio of IgG to albumin and more monomeric form of IgA were noted [97].

This pattern of BAL cells with an increased percentage of PMNs and an even higher percentage eosinophils in BALF was also found by Haslam and colleagues [41] in patients with cryptogenic fibrosing alveolitis (CFA). A small number of these patients had a lymphocytosis in BALF. Also, Davis and colleagues [24] described cellular profiles in 16 patients with diffuse interstitial lung disease; they described changes of lymphocytosis in these patients. This abstract appears to have been the first published report of BAL cell profiles in a group of patients with DILD.

In contrast to IPF, BAL cells and protein in fluid from seven patients with chronic hypersensitivity pneumonitis (CHP) [97] disclosed distinctive “foamy” cytoplasm-appearing alveolar macrophages, a very high percentage of lymphocytes, about 60% of BAL cells, which were predominantly T-cells, a high IgG/albumin ratio, and IgM, an immunoglobulin not detected in BAL fluid of normals or from IPF patients. IgG precipitin antibodies against relevant etiologic microbial antigens could be detected in some patients. In BAL samples from CHP patients, eosinophils and IgE values were not different from controls, nor were values of two components of complement, C4 and C6. Therefore, an active process of cellular and humoral immunity in CHP was evident, which did not include reagin-mediated type I factors found with atopic-allergic respiratory disease [90]. Thus, BAL components provided a distinctive profile of cells and immune factors that helped with disease diagnosis and gave insight into local airway immunopathogenic mechanisms. Whether lavage analysis would “prove useful in following the effect of therapy on these disorders” (IPF, CHP) [97], we were not certain, and this has required critical studies from others, which will be addressed [41, 50, 107, 125, 130].

Although diffuse interstitial pulmonary fibrosis had been well described and often discussed in pathologic and physiologic terms [9, 56, 110], these diseases began to be freshly re-examined by investigators in the Pulmonary Branch, National Heart, Lung, Blood Institute of the National Institutes of Health, Bethesda, Maryland [16] and also at other academic centers [24, 133] with new immunologic and biochemical methods, seeking an origin by dissecting the immunopathology of these (still) enigmatic lung diseases. BAL became part of the diagnostic approach and a means for obtaining

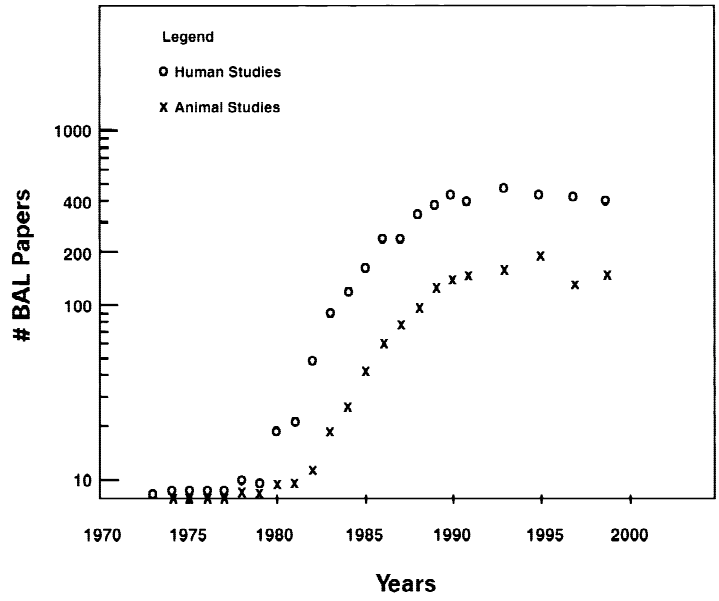

Fig. 2. One indicator of the interest and popularity generated for the BAL approach to studying lung diseases is the number of research publications that have appeared after the initial descriptions of the procedure in human volunteers (early 1970s) and patients, especially with DILD in the mid 1970s. A review of titles was produced from the MEDLINE database containing bronchoalveolar lavage in the title or papers that would use the BAL method to retrieve respiratory cells for *in vitro* research, primarily alveolar macrophages, lymphocytes, and inflammatory cells. Symbols used distinguish between human studies (○) and animal model research (X) over a 25-y span.

airway-alveolar substances to study *in vitro.* Fiberoptic bronchoscopy with BAL plus the capability of performing transbronchial biopsies [55] contributed several things:

1. With the reasonably minimally invasive method of fiberoptic bronchoscopy available to visually inspect, sample by BAL, and biopsy endobronchial and parenchymal tissue of patients undergoing diagnostic evaluation, initial and repeated studies could be done for longitudinal monitoring of disease activity. Excellent safety of bronchoscopy and BAL was documented for patients with forms of ILD [120] and mild asthma [85].
2. BAL-retrieved cells and fluid were used for research studies that provided insight into the immunopathogenesis and inflammatory processes of DILD.
3. This combination of an improved method potentially for diagnosis and the recovery of clinical specimens for investigation obtained directly from affected lung airways literally ignited widespread interest worldwide in the study of diffuse interstitial lung diseases. This was evident in the late 1970s and decade of the 1980s from the proliferation of BAL-related research publications (Fig. 2) from medical centers across the United States; in Europe from England, France, Italy, and Germany; and from Japan. As expressed [4], BALF was “a new material in pulmonology.”

The laboratory of Dr. Ronald G. Crystal in the Pulmonary Branch, NHLBI of NIH

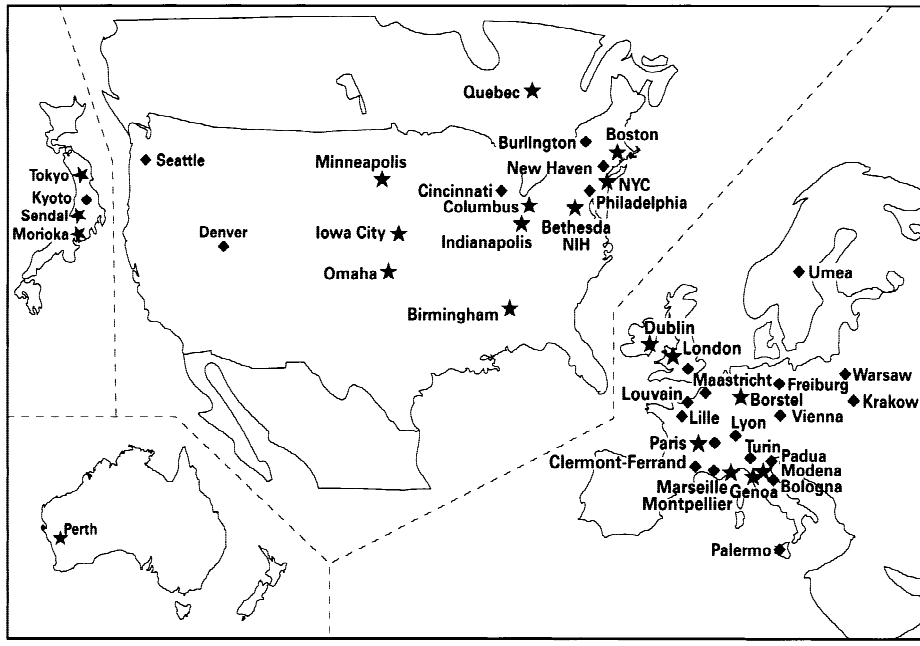

Fig. 3. One of the original locations for BAL-oriented investigation of lung diseases was at the National Institutes of Health in Bethesda, Maryland, where patient-directed BALF studies were begun in 1974. This research approach was picked up or adapted, or evolved concomitantly, in many places (◆) or was disseminated with members of the Pulmonary Branch, NHLBI, who left to relocate in other medical centers (✯) in the decade that followed the clinical application of BAL.

was an original site for lung studies using BAL [16, 97] and contributed to a ripple or radiating effect across the "pulmonary" world. This is documented by the medical staff and trainees who were attracted to his laboratory and subsequently returned to their original center or relocated in other pulmonary groups in the decade that followed BAL's clinical description and popularization (Fig. 3, location designated by ✯ symbol). Many of these pulmonary physicians are readily identified as contemporary leaders in lung medicine worldwide (Appendix I). Others did not come to NIH in Bethesda but were located in other centers that had derived their research methods from the original NIH group, or were made up of other senior scientists who adapted the BAL approach (Fig. 3, ◆ symbol) (Appendix II). This BAL phenomenon was illustrated by the organization of the first international conference on BAL in 1979 to discuss research and clinical applications [54]. This scientific gathering in Lille, France, featured presentations by investigators from France, Italy, The Netherlands, Canada, Sweden, and the United States.

Critique of BALF as a Diagnostic Pulmonary Test

Aside from important research insights derived from the study of BAL components, the analysis of BALF seemed to indicate that certain cellular profiles correlated with

Appendix I
Relocation of original/initial BAL investigators from Pulmonary Branch, NHLBI, NIH

Jack D. Fulmer, MD (deceased 1996)	University of Alabama Birmingham, AL
Steven E. Weinberger, MD Paula Pinkston, MD	Beth Israel Hospital, Harvard Medical School Boston, MA
Gary W. Hunninghake, MD	University of Iowa Medical Center Iowa City, IA
James E. Gadek, MD Mark D. Wewers, MD	Ohio State University Columbus, OH
William J. Martin, MD	Indiana University Medical Center Indianapolis, IN
Peter B. Bitterman, MD	University of Minnesota Medical Center Minneapolis, MN
Steven I. Rennard, MD	University of Nebraska Medical Center Omaha, NE
Mark L. Brantly, MD	University of Florida College of Medicine Gainesville, FL
William N. Rom, MD	New York University School of Medicine New York, NY
Bruce W. S. Robinson, MD	University of Western Australia Perth, Australia
Joachim Müller-Quernheim, MD	Institut für Biologie und Medizin Borstel, Germany
Oichi Kawanami, MD	Nippon Medical School Tokyo, Japan
Allan J. Hance, MD	Hopitat Bichat Paris, France
Jean-Francois Mornex, MD	Hopital Louis Pradel Lyon, France
Roland du Bois, MD	Brompton-National Heart and Lung Hospital London, England
Brendan A. Keogh, MD	Mater Hospital Dublin, Ireland
Giovanni A. Rossi, MD	Instituto Giannina Gaslini Genova, Italy
Cesare Saltini, MD	Universita Degli Studi di Modena Modena, Italy
André Cantin, MD	Hospitalier Universitaire deSherbrooke Sherbrooke, Quebec, Canada

several types of lung disease that might be especially helpful in clinically differentiating between forms of DILD. This suggested that a new diagnostic test might be incorporated into the bronchoscopy procedure. Thereafter, several articles presented a methodologic approach for BAL analysis, giving characteristic findings associated with several diseases [22, 44, 131].

Appendix II
Other initial investigators/proponents BAL

Canada	Yvon Cormier, MD	Québec, Canada
Austria	Heinrich H. Klech, MD	Vienna, Austria
England	Margaret Turner-Warwick, MD, PhD Patricia L. Haslam, PhD	London, England
France	M. Perrin-Fayolle, MD	Lyon, France
	Claude Molina, MD	Clermont-Ferrand, France
	C. Voisin, MD Andre-Bernard Tonnel, MD	Lille, France
	Jean Bousquet, MD F. B. Michel, MD Phillippe P. Godard, MD	Montpellier, France
	J. Bignon, MD	Créteil, France
	Gerard J. Huchon, MD F. Basset, MD Jacques Chretien, MD	Paris, France
Germany	Ulrich Costabel, MD	Freiberg, Germany
Italy	Carlos Albera, MD	Torino, Italy
	Bruno Balbi, MD	Veruno, Italy
	Venerino Poletti, MD	Bologna, Italy
	Gianpietro Semenzato, MD	Padua, Italy
Japan	Takateru Izumi, MD Sonoko Nagai, MD	Kyoto, Japan
Sweden	Leif H. Bjermer, MD	Umea, Sweden
USA	Herbert Y. Reynolds, MD	Laboratory of Clinical Investigation, National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD
	Harold H. Newball, MD	Baltimore, MD
	William W. Merrill, MD John A. Rankin, MD J. Bernard L. Gee, MD Richard A. Matthay, MD Yves Sibille, MD Allan Cooper, MD Randy K. Young, MD Robert B. Fick, Jr., MD	New Haven, CT
	Gerald S. Davis, MD Robert B. Low, PhD	Burlington, VT
	Ronald P. Daniele, MD Milton D. Rossman, MD James H. Dauber, MD	Philadelphia, PA
	Robert P. Baughman, MD	Cincinnati, OH
	Talmadge E. King, MD	Denver, CO
	Thomas R. Martin, MD Ganesh Raghu, MD	Seattle, WA
	Henry M. Yeager, MD	Washington, DC

From enumeration of cells in BALF, seemingly characteristic patterns were evident, for example, in IPF [16, 40], of more PMN and eosinophils, in hypersensitivity pneumonitis of a very high proportion of T-lymphocytes [16, 97], especially the suppressor subset CD_8 [53A]; in sarcoidosis [20, 43, 133] of increased T-helper cells and a high CD_4/CD_8 ratio, and in both "lone" CFA and collagen-vascular disease with associated lung involvement, a high lymphocyte percentage [41]. All these studies suggested that cellular analysis might be reasonably diagnostic and possibly could supplant other more invasive biopsy procedures.

Although soluble components in BALF seemed distinctive for certain diseases, this aspect of an immediate clinical analysis was not emphasized because measurement of immunologic and biochemical components would be delayed and not as quickly reportable as cell pellet differential counts. Concentration of the speciman and the problem of not having a reliable denominator substance against which to express values for soluble components made interpretation more difficult in the clinical context. In normal subjects or in the absence of tissue inflammation, the concentration of albumin was a fairly reproducible value in BALF [99], but less so when alveolitis and airway inflammation existed. This prompted investigation for a better marker that would estimate the volume of extracellular fluid, accounting for the dilution by the instilled lavage fluid. Many substances have been proposed, and urea [88] was promising, but even its use became problematic [48, 61].

Over the decade after these initial and generally more descriptive diagnostic uses of BALF analysis in the spectrum of interstitial lung diseases [22, 44, 131], an impressive number of subsequent editorial or review-type publications about BAL appeared [13–15, 17, 19, 21, 30–33, 38, 42, 45, 52, 62, 63, 66, 68, 71, 83, 87, 91, 96, 103, 105, 106, 115, 117, 123, 124]. The use of BAL analysis was extended to some rarer lung diseases [38]. Statements from task forces representing the major respiratory societies were published [35, 51]. A book about BAL soon followed [5].

In retrospect, the initial enthusiasm to use BAL analysis as a diagnostic clinical test was probably premature. Certainly, expectations become too great without an agreed approach for standardization and additional clinical research that would make the "test" more reliable, simpler, and adaptable for general use. As discussed [92], on the basis of preliminary use, BALF analysis was considered at one extreme to represent a liquid biopsy of the lung and at the other end, more modestly, the equivalent of a complete blood count or cerebrospinal fluid analysis obtained from venous blood or a lumbar spinal puncture. Actually, BALF analysis more nearly approximated a microscopic analysis of a random urine or stool specimen. Although both a urine and stool specimen are affected by dilution and/or concentration factors, neither has the complexity of an infused volume being added directly to the potential specimen before being collected or retrieved, as is the case with BALF.

As a "clinical test," BALF analysis introduced several questions that would need to be resolved before wide-scale application could occur or succeed: First, was BAL sampling reproducible and of sufficient specificity that analysis would be an accurate or sensitive indicator of pathologic events occurring throughout the airways? Restated, would BAL cells, putatively reflecting alveolitis, necessarily correlate with contiguous tissue histopathology contained in transbronchial biopsy specimens taken during the same procedure in areas sampled by lavage, or with tissue obtained later by open lung

biopsy? Second, could laboratory processing of BALF, an unwieldly, large-volume specimen, be simplified by a hospital's clinical pathology laboratory? Third, what costs were realistically attributable to the analysis, and how much would these increase the technical bill for bronchoscopy? Related also was how much professional charge could be added to the fiberoptic bronchoscopy procedure, and what was a reasonable fee for clinical interpretation of BALF results, such as applied to pulmonary function testing? The issue of costs for BALF analysis was addressed in only a few of the review or editorial articles about BAL [21, 92, 123, 124]. Fourth, did serial BALF results correlate with other indicators of the patient's response to treatment? For example, the first serial study of BAL in patients with CFA was not published until 1987 [125]. These issues have been variously addressed in the series of perspectives on BALF just listed and by another Task Force report from the European Respiratory Society, published in 1989 [121].

Technical improvements in the procedure of BAL or in processing cells and fluid continued to be offered, and some made lavage more reproducible. Several were significant, such as optimal conditions for the fluid to be infused [79] and a better understanding of the kinetics of cells and soluble substances recovered in sequential lavage fluid aliquots [23, 67, 89], thus how to use (or exclude) the first lavage return specimen. Likewise, the need to standardize the loss of lymphocytes in the distribution of cells during cytocentrifuge preparation was reported [109]. Although many technical suggestions have been made in the review articles, these were particularly well collected and critiqued in the Task Force report edited by Drs. Klech and Pohl [121].

It was important to determine whether the profile of BAL cells did resemble the *in situ* alveolitis or parenchymal changes found in histopathologic examination of contiguous lung biopsy specimens taken from areas adjacent to the lavage sites. Haslam and colleagues [41] reported 18 patients with CFA, considered to have UIP, who had a BAL in the right lung 2 weeks before an open lung biopsy in the right lower or right middle lobes, areas similar to the site lavaged. Three other patients had asbestos lung disease. A portion of the biopsy specimen was fixed for pathologic review, and in 12 patients with the remaining specimen, cell extraction studies were done with a tissue chopping and pipette dispersion method (no enzyme digestion was used) to recover inflammatory cells. Comparisons between cells in lung washings and biopsy extractions in 12 patients reached statistical significance for neutrophils, and were reasonably good for eosinophils and lymphocytes. Between the extraction cell results and biopsy histologic scores, a significant correlation was found only for lymphocytes and not for inflammatory cells. In an expanded series of 20 patients (12 with lone CFA and 8 with collagen-vascular disease) [38], percentage counts of neutophils and eosinophils were clearly higher in BALF than biopsy cell extracts; whereas, lymphocytes were higher in the cell extracts (Figure 4). In another study, Haslam [38A] used immunocytochemical staining to identify B and T lymphocytes in CFA biopsies which were of different proportions in tissue infiltrates and BAL; five CFA patients with elevated B-cells in tissue did poorly, clinically.

Hunninghake and colleagues [45] performed a similar kind of analysis in nine patients with IPF, six patients with sarcoidosis, and six nonsmoker control subjects undergoing lung surgery for a solitary lung nodule. To identify inflammatory and immune effector cells, BAL cells were compared with lung biopsy material dispersed

into cell suspensions with a teasing and filtering method. They concluded that the alveolitis of these lung diseases sampled by BAL did reflect the inflammatory and immune effector cells in lung parenchyma. Perhaps, a more conclusive approach to determine whether BAL cells reflected the histologic changes in lung tissue (transbronchial biopsies) was performed by Semenzato and colleagues [112] in patients with sarcoidosis ($n = 26$) and hypersensitivity pneumonitis ($n = 7$). BAL cells were compared with immunohistochemical staining of tissue sections with specific monoclonal antibodies. This method avoided the tissue maceration problem and permitted a topographic assessment of immune and inflammatory cells. Generally, the correlation between the percentage of lymphocytes and macrophages in lung biopsy specimens with their respective cell types in BALF was quite good.

Because the BAL technique washes down conducting airways distal to the tip of the wedged bronchoscope into the alveolar units, BALF in the aspirated sample is a composite mixture collected from both distal conducting airways mucosa and alveolar spaces [92]. It has been surprising to me, but reassuring nevertheless, that BAL cellular analysis reflected cellular changes in adjacent tissue as well [40, 45, 112]. This was an important correlation to establish that added more validity to the diagnostic potential of BALF for diffuse interstitial lung disease but also demonstrated a difference between alveolitis and adjacent interstitial tissue cells in some situations.

Because of widespread interest among pulmonary clinicians to include BAL analysis and its interpretation as part of diagnostic FOB, we undertook to determine a fair price for the technical analysis and to evaluate the logistics of providing this laboratory service [84]. BALF was an unwieldy sample to process and one that was obtained infrequently at most hospitals or surgical outpatient centers where elective FOB was done. Moreover, it required some urgency in processing and did not lend itself to automated measurements. There remained a secondary benefit that cells or other lavage components could be used for research if processed readily.

As a service for pulmonologists located within a geographically contained area (ie, about 50 miles to the most distant hospital or a one hour maximum transport time), who were also associated through an affiliated hospital network around New Haven, Connecticut, a central laboratory processing and analysis protocol was established [84]. The objective was to use a standard approach to BAL analysis that would provide reliable information in a timely manner to clinicians (results communicated within 2–24 h after the lavage sample received) that would help with subsequent diagnostic decisions for patients with an unknown form of DILD. The impact of this analysis on the pulmonologists's subsequent management of the patient was also assessed [119].

The logistics of transporting the BAL specimens and standard analysis of them that followed revealed several interesting things about lung cells in the aspirated BAL fluid samples. BAL cells were hardy and survived well, even ingesting the few nasopharyngeal-contaminating microbes that inevitably are in lavage fluid [99], so that adding an antibiotic to the specimen was not necessary for bacteriostasis if processed within a reasonable period (4 h). Enough glucose was washed off/out of the airways and concentrated in BAL to provide sufficient nutrients for cells (approximately 40 mg/mL glucose in unconcentrated lavage fluid), such that good viability of cells was maintained for up to 4 h. This facilitated subsequent use of the cells for *in vitro* research cell cultures. The initial cost for cell counts, cellular analysis of T-lymphocyte subsets, and

soluble liquid phase components (protein, immunoglobulins, and lipids) was about $110 plus labor costs of about $275. This total of just less than $400, reflecting costs of reagents and technical labor in the early to mid 1980s [84], would be more now. There was no billable professional fee for the lavage procedure added to bronchoscopy. At present, this fee for basic FOB is $598 at The Milton S. Hershey Medical Center, and an additional $60 can be added for bronchial lavage; for transbronchial biopsies another $113 can be charged. The technical charge for a diagnostic bronchoscopy is $422. Thus, added costs for lavage and the BALF analysis are within reason and justifiable if the results would contribute to better diagnostic accuracy and more cost-effective patient management [21]. An important outcome of BALF analysis would be if it helped with clinical diagnostic reasoning and with monitoring disease activity or progression.

The impact of BALF analysis on pulmonary clinicians' diagnostic evaluation of patients with DILD was assessed by Stoller and colleagues [119]. For pulmonologists submitting BAL specimens to a central laboratory for processing [84], 93 of these specimens were accompanied by a questionnaire completed by the referring physician when the BALF was initially submitted to the laboratory, and another questionnaire was completed immediately after results were returned, which was within a 2- to 24-h period. Results of other diagnostic tests done at FOB, such as microbial cultures and pathologic interpretation of transbronchial biopsy specimens, would not yet have returned; thus, a specific final diagnosis would rarely be available on the basis of other laboratory data before this second questionnaire was completed by the pulmonologist. The objective of the study was to judge whether the clinician's diagnostic reasoning was affected or subsequent approach modified. How would a test such as BALF analysis affect the relative likelihood of several contending diagnoses that the clinician had generated in his/her differential diagnosis? Admittedly, BALF differential cell counts alone do not identify conclusively a specific DILD, because most single laboratory tests are not pathognomonic unless a specific cytologic or serologic value is present that definitively defines a disease as in some rarer diseases [38]. However, would the analysis of differential cell counts alter or reinforce diagnostic impressions or add certainty? Seventy-eight of 93 paired questionnaires (84%) were evaluated and three findings were noted [119]: (1) when the first and second questionnaires were compared, in 59% ($n = 48/78$ patients) at least one diagnostic change had occurred; (2) the type of diagnostic change made was in the level of confidence given to a particular diagnosis (38 of 77 or 49%); and (3) the diagnostic change recorded was usually considered appropriate in relation to the final diagnosis. In 24 patients in whom the diagnostic change was considered to be appropriate, several changes were significant, especially in patients suspected of having sarcoidosis. In two patients BALF results precluded the need for planned surgical lung biopsy procedures, and another patient with presumed sarcoidosis actually had acquired immunodeficiency syndrome and an opportunistic lung infection that gave a diffuse interstitial appearance on chest imaging studies. This was a small and perhaps modest study but was an example of the kind of prospective impact BALF assessment might have on clinicians who were evaluating patients with unknown forms of DILD [117].

Two contemporary studies [73, 81] that assessed the accuracy of clinicians' diagnosis of patients with DILD, using results of high-resolution lung scans and expert

clinical acumen, indicated that clinical diagnosis is still not very good. Reliance on high-resolution computed tomography (HRCT) for a correct diagnosis among the top three choices overall was about 60% [73]. BAL analysis was available to help ascertain the diagnosis for only 22 of 134 cases (16%) [73]. Combining expert clinical evaluation and HRCT, approximately 30% of patients were not accurately diagnosed with new-onset IPF and still had to undergo a selective open lung biopsy [81]—still a very invasive procedure. BAL analysis was not included in the assessment with this protocol [81], because these investigators believed that the usefulness of a BALF cellular profile was controversial as a diagnostic tool and was not done routinely in their geographic area; this was based on their published studies also. But could the use of BAL results have added confidence to clinicians' reasoning, as found by Stoller [119]? Future clinical studies that include BALF analysis with new diagnostic modalities such as HRCT scan might decrease the necessity for mandatory lung biopsies.

Apart from its value as an aide to diagnosis, BAL analysis was anticipated to help with management as a prognostic indicator or to monitor a response to treatment. However, these issues are controversal and unresolved. What bedeviled BAL cellular analysis early on and singey had the most impact on largely discrediting it as a clinical laboratory test for monitoring patients' responsiveness to corticosteroid therapy for IPF (or CFA) was the enumeration of PMNs in an initial or serial BALF samples. Originally, the distinctive cellular findings in BALF from IPF (lone CFA) patients were the combination of an elevated percentage of PMNs and of eosinophils noted in several studies [16, 39A, 41, 97]. Of all the cells to be identified readily and counted on the stained cell (cytocentrifuged) sample, PMNs are the easiest to recognize; this has been realized and reported as the neutrophilic alveolitis characteristic of IPF [15]. The counterpart in the blood cell differential of an elevated PMN count or leukocytosis, especially with a left shift to more immature cell forms, is ingrained as a sensitive parameter to monitor for systemic or local organ infection and for subsequent change with antibiotic or other therapy. So it was with the BALF cell PMN percentage. Certainly, patients already on oral prednisone as therapy for IPF at the time of an initial BAL did have lower PMN percentages than some untreated patients and a higher percentage than control smokers (Fig. 1, Ref. 97); the percentage of eosinophils was unaffected by corticosteroids. Likewise, for 36 patients with CFA (most with "lone" fibrosing alveolitis among the group receiving treatment), 24 patients received BAL before treatment. The neutrophil counts (as percentages) tended to be lower in the responder patients, especially in those receiving prednisolone, although not attaining statistical significance (Fig. 3, Ref. 107). However, the most important findings were that lymphocyte counts were significantly higher in patients responding to prednisolone, while eosinophils in BALF were much higher in the non-responders, confirming earlier findings [41].

Keogh and colleagues [50] treated the alveolitis in a group of mid-stage IPF patients for 6 months with a low dose of oral prednisolone (0.25 mg/kg daily, $n = 8$ patients) contrasted with high-dose therapy (oral regimen plus 2 g/weekly of methylprednisolone intravenously, $n = 5$ patients). Results indicated that four of the patients receiving high-dose treatment had a reduction in the BALF percentage of PMN, a mean of 46% below their baseline values. Other cells, lymphocytes and macrophages, and total cells did not change significantly between the groups. Eosinophils were not

reported. Thus, the neutrophil component of the alveolitis responded to a high dose of corticosteroid treatment that included oral and parenteral administration.

These prior studies were point in time [41, 97] or seemed to be preliminary [50], and a more detailed longitudinal study was required to clarify the issue whether inflammatory cells, especially neutrophils, in the BAL cellular profile would decrease as clinical improvement occurred, and if the initial lavage cell counts would predict patient progress or response to therapy. Drs. Turner-Warwick and Haslam [125] prepared a valuable report about serial BAL analyses and clinical progress in 32 patients with CFA (27 patients had lung biopsy confirmation), of whom 26 had "lone" fibrosing alveolitis and no connective tissue disorder. The patient treatment regimens were with prednisolone at a high dose, orally 60 mg/day, or a low-dose 20 mg orally on alternate days plus cyclophosphamide, 100 to 120 mg daily. Importantly, 23 of 26 patients with "lone" fibrosing alveolitis were untreated at the time of the first lavage; follow-up lavages could occur at 3 to 6 months and at 12 to 18 months, but a mean of three lavages were done (range, 2–5) for each of the 32 patients. Patient improvement, classified into four groups at 1 year follow-up, was assessed clinically and by a breathlessness questionnaire, chest radiographs, and lung function tests. For predictability of BAL cellular results, Group 1 of the responders with lone CFA to prednisolone ($n = 4$) or cyclosphosphamide ($n = 4$) therapy were of note, between an initial lavage and follow-up. For those receiving prednisolone, the total percentage of all inflammatory cells (lymphocytes, neutrophils, and eosinophils) decreased from an initial percentage of 48 to 14.5, with selective reductions in the individual percentages of lymphocytes (13.5–4.0%), neutrophils (23.5–6.0%), and eosinophils (4.0–1.0%). These cellular changes did not occur for the cyclophosphamide group. Overall, the conclusions reached for the predictive value of BAL cellular profiles and patient response were conservatively presented and are repeated briefly. Characteristics of the alveolitis were confirmed, as in prior findings [41, 107], that some patients had an initial increase in lymphocytes (8 of 32 patients) and most of these (7 of 8 patients) responded or had an initial response to therapy. The cellular pattern of an increase of both neutrophils and eosinophils (17 of 32 patients) was found, and the therapeutic response was less good, but better with cyclophosphamide. As a comment, the role of the eosinophil is perhaps a more important ingredient in the alveolar inflammatory response than originally considered. After the serial changes in inflammatory cell profiles from lavages were analyzed in several different ways, conclusions showed "a trend of return towards normal in those improving compared with those in the other groups" [125]. However, many patients with an increased percentage of neutrophils failed to respond to therapy so "the raised initial neutrophil count does not clearly distinguish patients who will respond well to treatment from those who will not" [125].

Another well-conducted study by Watters and colleagues [130] that involved 26 patients with IPF made comparisons between pretreatment BAL cellular analysis sampled from the right middle lung lobe, histologic interpretation in open lung biopsy specimens obtained 3 weeks after lavage from upper and lower lobes of the same lung, and subsequent response to prednisone therapy. Lung biopsy specimens were analyzed from stained tissue sections and not by a cellular extraction method [40, 45]. Results indicated that the neutrophilia among BALF cells did not correlate with the histologic changes nor was this a predictor of clinical improvement. Lymphocytes could be increased in some patient's BAL cells, and this increase did correlate with moderate to

severe alveolar septal inflammation. For five of seven patients with BAL lymphocytosis before treatment, clinical improvement was found after 6 months of prednisone therapy, whereas only three of nine patients with BAL neutrophilia improved with therapy. Eosinophils were increased in BALF of patients with more severe overall clinical impairment. A general BALF pattern of more neutrophils (mean 23% in count), more eosinophils (mean 17%), and low lymphocytes (6% mean) was found in patients with the most severe clinical impairment (highest clinical-radiographic-physiologic score). In contrast, BALF lymphocytosis was associated with alveolar septal inflammation but little honeycombing change and more likely indicated improvement from corticosteroids, perhaps reflecting an earlier, cellular stage of IPF. This study also confirmed earlier results [47, 107]. Clearly, the lavage neutrophil alveolitis pattern reported by Keogh [50] was not found.

Thus, in summary, reliance on a single type or limited number of cells in a profile of BALF-recovered cells was too simplistic, or perhaps too good to be true, because this single laboratory parameter did not necessarily reflect the complexity of the alveolitis among this group of poorly understood idiopathic diseases, DILD. This as much as any factor torpedoed widespread acceptance of BAL analysis as a routine, helpful laboratory test in clinical pulmonology, and rightly so, relegating BAL to a clinical research status. A secondary impact was not to have BAL qualify for a professional fee charge or the cellular analysis to generate an interpretative fee such that the procedure would be used routinely in clinical practice. Where did this leave the status of BAL about a decade ago at the end of the 1980s?

Scorecard on BAL

Unquestionably, BAL permitted recovery of normal human airway-alveolar cells and soluble substances in the epithelial lining fluid for *in vitro* research that has helped explain local host responses such as antibody formation and immunologic components involved with the induction and regulation of inflammation, fibrogenesis, surfactant production and clearance, and the cellular kinetics of certain cytokine driven T-lymphocyte responses, characterized as TH_1 and TH_2 [1, 39]. This research has greatly improved concepts of immunopathogenesis and provided a basis for investigating many forms of lung disease. Clinically, BAL has been used extensively to sample affected airways–alveolar spaces of patients with various conducting airway diseases, such as asthma and bronchitis, with lower tract infection resulting from disease-related immunosuppression, such as HIV, or from medically induced immunosuppression related to organ transplantation or cytotoxic chemotherapy, and with air exchange surface abnormalities reflecting alveolitis and diffuse interstitial inflammation. Acute lung injury and occupational lung diseases have been studied. Even a minimal review of the substantial research literature about normal and abnormal disease-related changes in BALF is beyond the scope of this article but is addressed in many of the reviews about BAL. A recent update about BAL has appeared from the European BAL Task Force [summarized, 39]. Several other perspectives bridge the last 10 years well, specifically related to interstitial pulmonary fibrosis [12, 76, 128], which has remained important as a clinical problem requiring innovative and better forms of therapy [34, 65]. Still a curious dichotomy exists between the delineation of immunopathogenic changes that suggest new kinds of anti-inflammatory or antifibrotic therapy and lack of

a good correlation for BALF cellular profiles, especially for PMN, with patient responsiveness to therapy or prognosis of IPF (CFA) [125, 130]. Patients with IPF, however, have been found to have the pro-inflammatory cytokine IL-8 present in BALF and IL-8 mRNA was expressed in alveolar macrophages; the level of mRNA for IL-8 correlated with the percent or number of PMN in BALF [8, 59, 75, 116]. Mechanisms of neutrophilic alveolitis should be resolved in the future with more sophisticated scrutiny of cells and other BALF components retrieved from affected airways and alveoli.

As illustrated in Figure 2, the use or interest in BAL was reflected by the striking number of publications that began to appear. A few years after the initial human BAL studies with FOB appeared in the mid 1970s, a 40-fold increase occurred in published human lavage studies, which plateaued about 15 years later, but paper output remains constant. BAL animal model research reflected a similar pace. However, just the number of publications does not reveal the evolution and change in the use and application of BAL. Whereas early on human BAL reports emphasized findings from or application pertaining to diffuse alveolar/interstitial diseases from prominent lung investigating groups, publications in the late 1980s indicated some important changes, perhaps healthy ones. Because BAL analysis had not become a routine test in pulmonary clinical practice for lack of a good correlation with prognosis or treatment outcome in patients with DILD, BAL use widened to other more useful applications, such as microbial recovery in lung infections, especially in HIV immunocompromised patients with *Pneumocystis carinii,* and to other groups of diseases. Illnesses increasingly studied were asthma [114]; occupational inhalation diseases (asbestos, organic antigen exposure, and airborne metals); lung cells in HIV-infected patients [135]; acute lung injury resulting in adult respiratory distress syndrome [118]; or complications of organ transplantation, including lung, bone marrow, and even liver transplantation. However, many studies each year continued to be published about sarcoidosis, perhaps because BALF changes are usually distinctive in this and other granulomatous lung diseases. Although BAL had been performed in children usually with lung illness, the first reports of BALF analysis in healthy children without parenchymal lung involvement appeared in the mid 1990s [70, 86, 102], extending the spectrum of the procedure. Inevitably, some of the early and most enthusiastic proponents of BAL research phased out or redirected interests to other lung problems or avenues of therapy, such as gene delivery to the lungs. Yet another generation of colleagues continues, and the biannual conferences on BAL flourish—the 7th Conference on BAL was held June 28–July 1, 2000, in Krakow, Poland.

As noted, the overall number of human BAL-related publications persists at about 400 articles/y, although the peak occurred in the 1989–90 span. Articles also reflect increasingly a higher proportion of lower impact scientific journals for about 25–30% of the articles began to be published in secondary journals, beginning in 1988 continuing throughout the 1990s. This illustrates that BAL findings or its application have pushed out into a more local environment, as occurs with medical technology once it moves beyond the academic centers. However, many of the prominent investigators, particularly in Europe, have authored BAL reviews in native language publications.

Considerable discussion already has reviewed evidence that a BALF cellular analysis made during the initial evaluation of patients with DILD, particularly with IPF or CFA, has not always predicted response to corticosteroid treatment nor prognosis

well, especially when the PMN percentage is the primary indicator. When other inflammatory cells in BALF are considered, the profile is more indicative, especially if lymphocytes are increased in IPF (CFA); elevated eosinophils portend a poor response to corticosteroids usually. Moreover, the alveolitis as sampled by BAL may not reflect the histologic findings in lung biopsy specimens or cellular changes in alveolar septa/interstitial tissue for IPF [38A]; however, correlations between BAL cells and tissue cell extractions are better for lymphocytic alveolitis and granulomatous lung disease.

This lack of diagnostic specificity of BALF differential cells allowed another imaging modality to become popular, which has greatly altered the evaluation and monitoring of patients with DILD, namely HRCT lung scans [27]. From HRCT scans, patterns of lung involvement suggest specific diagnoses, findings of ground-glass appearance are equated with parenchymal cellularity, and serial scans can monitor patient progress [74, 132]. Dependence on HRCT scans has become integral to patient management. However, as found in several reports [73, 81], combining expert clinical acumen and HRCT scan interpretation still does not provide a confident clinical diagnosis for a significant percentage of patients, and tissue verification with open lung biopsy [81] is still needed. Would incorporation of BALF analysis be helpful then in changing the clinician's confidence in a diagnosis [119] or altering subsequent evaluation?

Future Role for BAL Sampling

The technical aspects of BAL have been assembled and reviewed thoroughly [121] and updated recently [39]; consensus appears good about its indications, optimal ways to perform lavage, and how to analyze the cellular and soluble components. However, unless a clinical laboratory is prepared to process BALF samples frequently, the analysis remains time consuming, and verification can be problematic. The use of computer-assisted software for comparisons with other laboratories' databases [25, 26], or atlas-type reference material of cytopathic appearances of BALF cells prepared by cytopathologists may be useful [10], if the application includes a broad variety of lung diseases. Improving the quality of cytopathic analysis and reproducibility of results [3] is still needed. With more confidence in reported laboratory results, BAL differential cell counts may be more helpful and acceptable for clinical diagnostic reasoning. As already mentioned, it is likely that BALF analysis will remain of added value for improving diagnostic accuracy in the evaluation of patients with DILD and prove to be complementary to clinical acumen and HRCT lung scans [73, 81].

Increasingly, more elderly patients are being encountered with perhaps milder or an insidious form of IPF [93] in whom more-limited or less-invasive evaluation can be performed or is clinically appropriate. In healthy, elderly volunteers (>60 y of age), preliminary BAL results compared cellular changes that might provide clues to altered immune processes (CD_4 accumulation of lymphocytes) that possibly contribute to illness (UIP) in older patients [2]. Other analytic modalities such as specimens of condensed exhaled air from the lungs [111], local nasal mucosal washings, or induced fresh sputum specimens will be used also to quantitate inflammation or relevant cytokine expression.

Something of a renaissance for BALF analysis is predicted, but within the confines of clinical research protocols. New or different applications of BAL will continue and

become more innovative, especially as the technique is used to examine different lung diseases. As mentioned for Figure 2, published research that initially reflected DILD has shifted during the past 15 years to study many other important diseases, such as asthma, lung infection in immuncompromised patients, bronchitis/COPD, organ transplantation, acute lung injury, etc. [92]. Potentially useful insights for all these diseases, especially allergic, occupational inhalation exposure(s), and bronchitis, will derive from study of mucosal–alveolar space fluids obtained discreetly from the nasopharynx, conducting airways, and alveolar microenvironment and all contrasted. Sensitive new techniques are needed to sample discrete areas [57] as with minute absorptive pledgets applied to the airway lining surface. Bronchitis/COPD associated with cigarette smoking is an obvious choice. Because the entire respiratory tract is exposed and at risk for illness, selective sampling of portions of the airways, including BAL for alveolar-emphysema mechanisms, and interrelating results will provide new insights. Integrating surfactant and its proteins into the host defense scheme is an active research area [77, 78] for which BAL recovery is essential and has been a longstanding product of BAL research already [28, 80, 99]. Moreover, surfactant dysfunction is involved with ARDS [39], an injurious process that is receiving more research attention, including with BAL.

As manipulation of mucosal surfaces to resist infectious agents becomes more feasible by regulation of cytokines [94], study of antigen-presenting cells, such as dendritic cells and airway macrophages, is focusing on the lungs. BAL is a method to retrieve these dendritic cells [126]. Moreover, BAL cells are obtained from the distal airways and alveolar surface affected by a disease process. By subjecting these cells to an array of DNA sequences representing portions of human genes, a microscan for activated genes or products can be made that will give information directly about what genes are involved or are contributing to understanding the cause of many lung diseases that remain without a defined origin.

In conclusion, the procedure of BAL retrieves cells and soluble substances from the lining fluid of the distal airways and alveolar units, containing immunologic components of the lung's epithelial surface. In many instances it was assumed that BALF also reflected the milieu of the interstitium or parenchyma as well, and this is not always the case. Alveolar surface changes in disease (alveolitis) are not necessarily a continuum of an interstitial process, although the compartments are contiguous; they may remain separate or unique. Nonetheless, lavage sampling of the airway and alveolar surfaces provides a specimen that is involved with a disease process or in close approximation to it. This approach will continue to be helpful as more details of the normal lung are found and pathogenesis of diseases are explored.

Acknowledgements. The author appreciates Mrs. Susan Crawford's preparation of the manuscript. The helpful review of the manuscript by Dr. Patricia Haslam was greatly appreciated; as well as helpful comments received from Dr. Stephen I. Rennard, Dr. Robert L. Baughman, Dr. Gianpetro Semenzato, and Dr. Ronald G. Crystal.

This article was the basis for a presentation at the 7th International Conference on Bronchoalveolar Lavage on June 28, 2000, at the Medical College of Jagellonian University, Kraków, Poland.

References

1. Abbas AK, Murphy KM, Sher A (1996) Functional diversity of helper T lymphocytes. Nature 383: 787–793

2. Albera C, Gagliardi L, Caggese D, Palmulli P, et al (2000) Age-related modification of BAL cell profile in UIP and sarcoidosis (abstract). Curr Pneum 4(1):19
3. Baughman RP, Kleykamp BO, Hunninghake GW (2000) Increasing the quality of hematopathology laboratories in interpreting bronchoalveolar lavage samples (abstract). Curr Pneum 4(1):15
4. Bergmann KC, Kramer H, Wiesner B (1981) Bronchoalveolar lavage fluid—a new material in pulmonology. Erkrankungen Atmungsorgane 157:34–40
5. Bronchoalveolar lavage (1992) In: Baughman RP (ed) Mosby Year Book, Inc., St. Louis, pp 1–297
6. Cantrell ET, Warr GA, Busbee DL, Martin RR (1973) Induction of aryl hydrocarbon hydroxylase in human pulmonary alveolar macrophages by cigarette smoking. J Clin Invest 52:1881–1884
7. Carlens E (1949) A new flexible double-lumen catheter for bronchospirometry. J Thorac Surg 18:742–746
8. Carré PC, Mortenson RL, King TE Jr, Noble PW (1991) Increased expression of the interleukin-8 gene by alveolar macrophages in idiopathic pulmonary fibrosis. J Clin Invest 88:1802–1810
9. Carrington CB, Gaensler EA, Coutu RE, Fitzgerald MX, Gupta RG (1978) Natural history and treated course of usual and desquamative interstitial pneumonia. N Engl J Med 298:801–809
10. Chlap Z (2000) Multimedial atlas of BAL cytopathology. 1. Outline of the contents. 2. Possibility to use the atlas as CD-ROM (abstract). Curr Pneum 4(1):27
11. Cohen AB, Cline MJ (1971) The human alveolar macrophage: isolation, cultivation in vitro and studies of morphologic functional characteristics. J Clin Invest 50:1390–1398
12. Cooper JAD Jr (2000) Pulmonary fibrosis—pathways are slowly coming to light (perspective). Am J Respir Cell Mol Biol 22:520–523
13. Costabel U (1991) Bronchoalveolar lavage: a standardized procedure or a technical dilemma? Eur Respir J 4:776–777
14. Costabel U, Teschler H, Guzman J, Kroegel C (1990) Bronchoalveolar lavage: interim evaluation following 10 years clinical use. Med Klin 85:376–387
15. Crystal RG, Bitterman PR, Rennard SI, Hance AJ, et al (1984) Interstitial lung diseases of unknown cause: disorders characterized by chronic inflammation of the lower respiratory tract. N Engl J Med 310:154–166, 235–244
16. Crystal RG, Fulmer JD, Roberts WC, Moss ML, et al (1976) Idiopathic pulmonary fibrosis—clinical, histologic, radiologic, physiologic, scintigraphic, cytologic and biochemical aspects. Ann Intern Med 85:769–788
17. Crystal RG, Reynolds HY, Kalica AR (1986) Bronchoalveolar lavage. The report of an international conference. Chest 90:122–131 (second BAL Conference 5/16–18/1984, Columbia, Maryland)
18. Daniele RP, Altose MD, Rowland DR Jr (1975) Immunocompetent cells from the lower respiratory tract of normal human lungs. J Clin Invest 59:986–996
19. Daniele RP, Elias JA, Epstein PE, Rossman MD (1985) Bronchoalveolar lavage: role in the pathogenesis, diagnosis, and management of interstitial lung disease. Ann Intern Med 102:93–108
20. Dauber J, Rossman M, Daniele RP (1979) Bronchoalveolar cell populations in acute sarcoidosis: observations in smoking and nonsmoking patients. J Lab Clin Med 94:862–871
21. Davis GS (1986) Bronchoalveolar lavage and the technological dilemma. Am Rev Respir Dis 133: 181–183
22. Davis GS, Brody AR, Craighead JE (1978) Analysis of airspace and interstitial mononuclear cell populations in human diffuse interstitial lung disease. Am Rev Respir Dis 118:7–15
23. Davis GS, Giancola MS, Costanza MC, Low RB (1982) Analyses of sequential bronchoalveolar lavage samples from healthy human volunteers. Am Rev Respir Dis 126:611–616
24. Davis GS, Landis JN, Brody AR, Graham WGB, et al (1975) Characteristics of diffuse lung disease reflected by pulmonary lavage. Am Rev Respir Dis 111:933–934 (Abstract, Internation Conference on Lung Diseases, Montreal, Canada, May 1975)
25. Drent M, Jacobs JA, Cobben NAM, et al (2000) Predicting computer program using cellular BAL fluid analysis results: an update. Curr Pneum 4(1):15
26. Drent M, vanNierop MAMF, Gerritsen FA, et al (1996) A computer program using BALF-analysis results as a diagnostic tool in interstitial lung diseases. Am J Respir Crit Care Med 153:736–741
27. du Bois RM (1994) Diffuse lung disease: an approach to management. BMJ 309:175–179
28. Finley TN, Ladman AJ (1972) Low yield of pulmonary surfactant in cigarette smokers. N Engl J Med 286:223–227
29. Finley TN, Swenson EW, Curran WS, Huber GL, Ladman AJ (1967) Bronchopulmonary lavage in normal subjects and patients with obstructive lung disease. Ann Intern Med 66:651–658

30. Fulmer JD (1982) Bronchoalveolar lavage. Am Rev Respir Dis 126:961–963
31. Garcia JG, Keogh BA (1984) Bronchoalveolar lavage: a decade later. Irish Med J 77:399–401
32. Gee JBL, Fick RB Jr (1980) Bronchoalveolar lavage. Thorax 35:1–8
33. Gibson PG, Robinson BW, McLennan G, Bryant DH, Breit SN (1989) The role of bronchoalveolar lavage in the assessment of diffuse lung diseases. Aust N Z J Med 19:281–291
34. Goldstein RH, Fine A (1995) Potential therapeutic initiatives for fibrogenic lung diseases—impact of basic research on tomorrow's medicine. Chest 108:848–855
35. Goldstein RA, Rohatgi PK, Bergofsky EH, Block ER, et al (1990) Official American Thoracic Society statement—Clinical role of bronchoalveolar lavage in adults with pulmonary disease. Am Rev Respir Dis 142:481–486
36. Green GM (1970) The J. Burns Amberson lecture–in defense of the lung. Am Rev Respir Dis 102: 691–703
37. Harris JA, Swenson EW, Johnson JE (1970) Human alveolar macrophages: comparison of phagocytic, glucose utilization, and ultrastructure in smokers and nonsmokers. J Clin Invest 49:2086–2096
38. Haslam PL (1984) Bronchoalveolar lavage. Semin Respir Med 6:55–70
38A. Haslam PL (1990) Evaluation of alveolitis by studies of lung biopsies. Lung (suppl) pp 984–992
39. Haslam PL, Baughman RP (1999) Report of ERS Task Force: guidelines for measurement of acellular components and standardization of BAL. Eur Respir J 14:245–248
39A. Haslam PL, Dewar A, Butchers P, Primett ZS, et al (1987) Mast cells, atypical lymphocytes, and neutrophils in bronchoalveolar lavage in extrinsic allergic alveolitis—comparison with other interstitial lung diseases. Am Rev Respir Dis 135:35–47
40. Haslam PL, Turton CWG, Heard B, Lukoszek A, et al (1980) Bronchoalveolar lavage in pulmonary fibrosis: comparison of cells obtained with lung biopsy and clinical features. Thorax 35:9–18
41. Haslam PL, Turton CWG, Lukoszek A, Salsbury AJ, et al (1980) Bronchoalveolar lavage fluid cell counts in cryptogenic fibrosing alveolitis and their relation to therapy. Thorax 35:328–339
42. Howard P (1990) Clinical usefulness of bronchoalveolar lavage. Eur Respir J 3:377–378
43. Hunninghake GW, Fulmer JD, Young RC Jr, Gadek JF, Crystal RG (1979) Localization of the immune response in sarcoidosis. Am Rev Respir Dis 120:49–57
44. Hunninghake GW, Gadek JE, Kawanami O, Ferrans VJ, Crystal RG (1979) Inflammatory and immune processes in the human lung in health and disease: evaluation by bronchoalveolar lavage. Am J Pathol 97:149–206
45. Hunninghake GW, Kawanami O, Ferrans VJ, Young RC, et al (1981) Characterization of inflammatory and immune effector cells in the lung parenchyma of patients with interstitial lung disease. Am Rev Respir Dis 123:407–412
46. Ikeda S (1970) Flexible bronchofiberscope. Ann Otol Rhinol Laryngol 79:916–919
47. Ikeda S, Yanai N, Ishikawa S (1968) Flexible bronchofiberscope. Keio J Med 17:1–16
48. Jones KP, Edwards JH, Reynolds SP, et al (1990) A comparison of albumin and urea as reference markers in bronchoalveolar lavage fluid from patients with interstitial lung disease. Eur Respir J 3:152–156
49. Keimowitz RI (1964) Immunoglobulins in normal human tracheobronchial washings: a qualitative and quantitative study. J Lab Clin Med 63:54–59
50. Keogh BA, Bernardo J, Hunninghake GW, Line BR, et al (1983) Effect of intermittent high dose parenteral corticosteroids on the alveolitis of idiopathic pulmonary fibrosis. Am Rev Respir Dis 127:18–22
51. Klech H, Hutter C (1990) Clinical guidelines and indications for bronchoalveolar lavage (BAL): report of the European Society of Pneumology Task Force on BAL. Eur Respir J 3:937–974
52. Krieger B, Blinder L, Inchausti BC (1989) Clinical utility of bronchoalveolar lavage in a general hospital. Arch Intern Med 149:1605–1607
53. Lawrence EC, Blaese RM, Martin RR, Stevens PM (1978) Immunoglobulin secreting cells in normal human bronchial lavage. J Clin Invest 62:832–835
53A. Leatherman JW, Michael AF, Schwartz BA, Hoidal JR (1984) Lung T cells in hypersensitivity pneumonitis. Ann Intern Med 100:390–392
54. Le Lavage broncho-alveolaire chez l'homme-colloque (1979) Proceedings edited by Biserte G, Chrétien J, Voisin C, Institut National de La Santé et de la Recherche Médicale, rue de Tolbiac, Paris, France, pp 1–544

55. Levin DC, Wicks AB, Ellis JH Jr (1974) Transbronchial lung biopsy via the fiberoptic bronchoscope. Am Rev Respir Dis 110:4–12
56. Liebow AA, Steer A, Billingsley JG (1965) Desquamative interstitial pneumonia. Am J Med 39:369–404
57. Lindahl M, Ståhlborn B, Tagesson C (1999) Newly identified proteins in human nasal and bronchoalveolar lavage fluids: potential biomedical and clinical applications. Electrophoresis 20:3670–3676
58. Low RB, Davis G, Giancola MS (1978) Biochemical analyses of bronchoalveolar lavage fluids of normal healthy volunteers. Am Rev Respir Dis 118:863–875
59. Lynch JP III, Standiford TJ, Rolfe MW, et al (1992) Neutrophilic alveolits in idiopathic pulmonary fibrosis: the role of interleukin-8. Am Rev Respir Dis 145:1433–1439
60. Mann PEG, Cohen AB, Finley TN, Ladman AJ (1971) Alveolar macrophages: structural and functional differences between nonsmokers and smokers of marijuana and tobacco. Lab Invest 25:111–120
61. Marcy TW, Merrill WW, Rankin JA, Reynolds HY (1987) Limitations of using urea to quantify epithelial lining fluid recovered by bronchoalveolar lavage. Am Rev Respir Dis 135:1276–1280
62. Martin WJ, Williams DE, Dines DE, Sanderson DR (1983) Interstitial lung disease. Assessments by bronchoalveolar lavage. Mayo Clin Proc 58:751–757
63. Martin WR, Padrid PA, Cross CE (1990) Bronchoalveolar lavage. Clin Rev Allergy 8:305–332
64. Masson PL, Heremans JF, Prignot J (1965) Studies on the proteins of human bronchial secretions. Biochim Biophys Acta 111:466–478
65. Mason RJ, Schwarz MI, Hunninghake GW, Musson RA (1999) Pharmacological therapy for idiopathic pulmonary fibrosis—past, present and future (NHLBI workshop summary 9/9–10/1998). Am J Respir Crit Care Med 160:1771–1777
66. Matthys H, Costabel U (1982) Diagnosis of interstitial lung diseases using bronchoalveolar lavage. Deutsche Med Wochenschr 107:740–742
67. Merrill W, O'Hearn E, Rankin J, Naegel G, et al (1982) Kinetic analysis of respiratory tract proteins recovered during a sequential lavage protocol. Am Rev Respir Dis 126:617–620
68. Merrill WW, Reynolds HY (1983) Bronchial lavage in inflammatory lung disease. Clin Chest Med 4:71–84
69. Métras H (1953) Le cathetériseme bronchique. In: Métras H, Charpin J, eds. Le Cathetérisme Bronchique. Paris, Vigot Frères, pp 55–65
70. Midulla F, Villani A, Merolla R, Bjermer L, et al (1995) Bronchoalveolar lavage studies in children without parenchymal lung disease: cellular constituents and protein levels. Pediatr Pulmonol 20:112–118
71. Morrison HM, Stockley RA (1988) The many uses of bronchoalveolar lavage. BMJ Clin Res Ed 296:1758
72. Myrvik QN, Leake ES, Fariss B (1961) Lysozyme content of alveolar and peritoneal macrophages from the rabbit. J Immunol 86:133–136
73. Nishimura K, Izumi T, Kitaichi M, et al (1993) The diagnostic accuracy of high-resolution computed tomography in diffuse infiltrative lung diseases. Chest 104:1149–1155
74. Orens JB, Kazerooni EA, Martinez FJ, et al (1995) The sensitivity of high-resolution CT in detecting idiopathic pulmonary fibrosis proved by open lung biopsy. Chest 108:109–115
75. Ozaki T, Hayashi H, Tani K, Ogushi F, et al (1992) Neutrophil chemotactic factors in the respiratory tract of patients with chronic airway diseases or idiopathic pulmonary fibrosis. Am Rev Respir Dis 145:85–91
76. Paine R, Ward PA (1999) Cell adhesional molecules and pulmonary fibrosis. Am J Med 107:268–279
77. Phelps DS (1995) Pulmonary surfactant modulation of host-defense function. Appl Cardiopulm Pathophys 5:221–229
78. Phelps DS (2000) Surfactant regulation of host-defense function in the lung: a question of balance. Pediatr Pathol Mole Med. In press
79. Pingleton SK, Harrison GR, Stechschulte DJ, Wesselius LJ, et al (1983) Effect of location, pH and temperature of instillate in bronchoalveolar lavage in normal subjects. Am Rev Respir Dis 128:1035–1037
80. Pratt SA, Finley TN, Smith MH, Ladman AJ (1969) A comparison of alveolar macrophages and pulmonary surfactant (?) obtained from the lungs of human smokers and nonsmokers by endobronchial lavage. Anat Rec 163:497–504
81. Raghu G, Mageto YN, Lockhart D, Schmidt RA, et al (1999) The accuracy of the clinical diagnosis of new-onset idiopathic pulmonary fibrosis and other interstitial lung disease: a prospective study. Chest 116:1168–1174

82. Ramirez-RJ, Kieffer RF, Ball WC (1965) Bronchopulmonary lavage in man. Ann Intern Med 63:819–828
83. Rankin JA (1988) What is today's role for bronchoalveolar lavage? J Respir Dis 9:113–128
84. Rankin JA, Naegel GP, Reynolds HY (1986) Use of a central laboratory for analysis of bronchoalveolar lavage fluid. Am Rev Respir Dis 133:186–190
85. Rankin JA, Snyder PE, Schachter EN, Matthay RA (1984) Bronchoalveolar lavage—its safety in mild asthma. Chest 85:723–728
86. Ratjen F, Bredendiek M, Zheng L, et al (1995) Lymphocyte subsets in bronchoalveolar lavage fluid of children without bronchopulmonary disease. Am J Respir Crit Care Med 152:174–178
87. Rennard SI (1990) Future directions for bronchoalveolar lavage. Lung 168:1050–1060
88. Rennard SI, Basset G, Lecossier D, O'Donnell K, et al (1986) Estimations of the absolute volume of epithelial lining fluid recovered by bronchoalveolar lavage using urea as an endogenous marker of dilution. J Appl Physiol 60:532–538
89. Rennard SI, Ghafouri M, Thompson AB, Linder J, et al (1990) Fractional processing of sequential bronchoalveolar lavage to separate bronchial and alveolar samples. Am Rev Respir Dis 141:208–217
90. Reynolds HY (1982) Immunologic lung disease. Chest 81:626–631
91. Reynolds HY (1986) Idiopathic interstitial pulmonary fibrosis: Contribution of bronchoalveolar lavage analysis. Chest 89:139S–144S
92. Reynolds HY (1987) Bronchoalveolar lavage—state of art. Am Rev Respir Dis 135:250–263
93. Reynolds HY (1998) Diagnostic and management strategies for diffuse interstitial lung disease. Chest 113:192–202
94. Reynolds HY (2000) Advances in understanding pulmonary host defense mechanisms: dendritic cell function and immunomodulation. Curr Opin Pulm Med 6:209–216
95. Reynolds HY, Atkinson JP, Newball HH, Frank MM (1975) Receptors for immunoglobulin and complement on human alveolar macrophages. J Immunol 114:1813–1819
96. Reynolds HY, Chrétien J (1984) Respiratory tract fluids: analysis of content and contemporary use in understanding lung disease. DM 30:1–103
97. Reynolds HY, Fulmer JD, Kazmierowski JA, Roberts WC, Frank MM, Crystal RG (1977) Analysis of cellular and protein components of bronchoalveolar lavage fluid from patients with idiopathic pulmonary fibrosis and hypersensitivity pneumonitis. J Clin Invest 59:165–175
98. Reynolds HY, Kazmierowski JA, Newball HH (1975) Specificity of opsonic antibodies to enhance phagocytosis of *Pseudomonas aeruginosa* by human alveolar macrophages. J Clin Invest 56:376–385
99. Reynolds HY, Newball HH (1974) Analysis of proteins and respiratory cells obtained from human lungs by bronchial lavage. J Lab Clin Med 84:559–573
100. Reynolds HY, Thompson RE (1973) Pulmonary host defenses I. Analysis of protein and lipids in bronchial secretions and antibody responses after vaccination with *Pseudomonas aeruginosa.* J Immunol 111:358–368
101. Reynolds HY, Thompson RE (1973) Pulmonary host defenses II. Interaction of respiratory antibodies with *Pseudomonas aeruginosa* and alveolar macrophages. J Immunol 111:369–380
102. Riedler J, Grigg J, Stone C, et al (1995) Bronchoalveolar lavage cellularity in healthy children. Am J Respir Crit Care Med 152:163–168
103. Robinson BW, James A, Rose AH, Sterrett GF, Musk AW (1988) Bronchoalveolar lavage sampling of airway and alveolar cells. Br J Dis Chest 82:45–55
104. Rogers RM, Braunstein MS, Shuman JF (1972) Role of bronchopulmonary lavage in the treatment of respiratory failure—a review. Chest 62(suppl 2):95S–106S
105. Rossi GA (1986) Bronchoalveolar lavage in the investigation of disorders of the lower respiratory tract. Eur J Respir Dis 69:293–315
106. Rossi GA, Sacco O, Vasallo F, Degli Innocenti L, Ravazzoni C (1988) Bronchoalveolar lavage during fiberoptic bronchoscopy: what has it brought to pulmonary medicine? Respiration 54:49–58
107. Rudd RM, Haslam PL, Turner-Warwick M (1981) Cryptogenic fibrosing alveolitis-relationships of pulmonary physiology and bronchoalveolar lavage to response to treatment and prognosis. Am Rev Respir Dis 124:1–8
108. Sackner MA, Wanner A, Landa J (1972) Applications of bronchofiberoscopy. Chest 62(suppl):70–78
109. Saltini C, Hance AJ, Ferrans VJ, Basset F, et al (1984) Accurate quantification of cells recovered by bronchoalveolar lavage. Am Rev Respir Dis 130:650–658
110. Scadding JG, Hinson KFW (1967) Diffuse fibrosing alveolitis (diffuse interstitial fibrosis of the lungs) correlation of histology at biopsy with prognosis. Thorax 22:291–304

111. Scheideler L, Manke H-G, Schwulera U, et al (1993) Detection of nonvolatile macromolecules in breath—a possible diagnostic tool. Am Rev Respir Dis 148:778–784
112. Semenzato G, Chilosi M, Ossi E, Trentin L, et al (1985) Bronchoalveolar lavage and lung histology—comparative analysis of inflammatory and immunocompetent cells in patients with sarcoidosis and hypersensitivity pneumonitis. Am Rev Respir Dis 132:400–404
113. Smiddy JF, Ruth WE, Kerby GR, Renz LE, Raucher C (1971) Flexible fiberoptic bronchoscope (letter). Ann Intern Med 75:971
114. Smith DL, Deshazo RD (1993) Bronchoalveolar lavage in asthma. An update and perspective. Am Rev Respir Dis 148:523–532
115. Smith LJ (1981) Bronchoalveolar lavage today. Chest 80:251–252
116. Southcott AM, Jones KP, Li D, Majumdar S, et al (1995) Interleukin-8: differential expression in lone fibrosing alveolitis and systemic sclerosis. Am J Respir Crit Care Med 151:1604–1612
117. Springmeyer SC (1987) The clinical use of bronchoalveolar lavage. Chest 92:771–772
118. Steinberg KP, Mitchell DR, Maunder RJ, Milberg JA, et al (1993) Safety of bronchoalveolar lavage in patients with adult respiratory distress syndrome. Am Rev Respir Dis 148:556–561
119. Stoller JK, Rankin JA, Reynolds HY (1987) The impact of bronchoalveolar lavage cell analysis on clinicians' diagnostic reasoning about interstitial lung disease. Chest 92:839–843
120. Strumpf IJ, Feld MK, Cornelius M, et al (1981) Safety of fiberoptic bronchoalveolar lavage in evaluation of interstitial lung disease. Chest 80:268–271
121. Technical recommendations and guidelines for bronchoalveolar lavage (BAL)—report of the European Society of Pneumology Task Group on BAL (1989) Klech H, Pohl W (eds) Eur Respir J 2:561–585
122. Travis WD, Matsui K, Moss J, Ferrans VJ (2000) Idiopathic nonspecific interstitial pneumonia: prognostic significance of cellular and fibrosing patterns—survival comparison with usual interstitial pneumonia and desquamative interstitial pneumonia. Am J Surg Pathol 24:19–33
123. Turner-Warwick ME, Haslam PL (1986) Clinical applications of bronchoalveolar lavage: an interim view. Br J Dis Chest 80:105–122
124. Turner-Warwick ME, Haslam PL (1987) Clinical applications of bronchoalveolar lavage. Clin Chest Med 8:15–26
125. Turner-Warwick ME, Haslam PL (1987) The value of serial bronchoalveolar lavages in assessing the clinical progress of patients with crytogenic fibrosing alveolitis. Am Rev Respir Dis 135:26–34
126. VanHaarst JMW, deWit HJ, Drexhage HA, Hoogsteden HC (1994) Distribution and immunophenotype of mononuclear phagocytes and dendritic cells in the human lung. Am J Resp Cell Mol Biol 10:487–492
127. Waldmann RH, Jurgensen PF, Olsen GN, Ganguly R, Johnson JE (1973) Immune response of the human respiratory tract: immunoglobulin levels and influenza virus vaccine antibody response. J Immunol 111:38–41
128. Ward PA, Hunninghake GW (1999) Lung inflammation and fibrosis. Am J Respir Crit Care Med 157:S123–S129
129. Warr GA, Martin RR, Sharp PM, Rossen RD (1977) Normal human bronchial immunoglobulin and proteins: effects of cigarette smoking. Am Rev Respir Dis 116:25–30
130. Watters LC, Schwarz MI, Cherniak RM, Waldron JA, et al (1987) Idiopathic pulmonary fibrosis. Pretreatment bronchoalveolar lavage cellular constituents and their relationships with lung histopathology and clinical response to therapy. Am Rev Resp Dis 135:696–704
131. Weinberger SE, Kelman JA, Elson NA, Young RC, et al (1978) Bronchoalveolar lavage in interstitial lung disease. Ann Inter Med 89:459–466
132. Wells AU, Hansell DM, Rubens MB, et al (1993) The predictive value of appearance of thin section computed tomography in fibrosing alveolitis. Am Rev Respir Dis 148:1076–1082
133. Yeager HM, William MC, Beckman JF, Bayly TC, et al (1976) Sarcoidosis: analysis of cells obtained by bronchial lavage. Am Rev Respir Dis 113:96–100
134. Yeager H, Zimmet SM, Schwartz SL (1974) Pinocytosis by human alveolar macrophages: comparison of smokers and nonsmokers. J Clin Invest 54:247–251
135. Young KR, Rankin JA, Naegel GP, et al (1985) Bronchoalveolar lavage cells and proteins in patients with the acquired immunodeficiency syndrome—an immunologic analysis. Ann Intern Med 103:522–533

Accepted for publication: 8 August 2000