Thorax, 1980, **35**, 1–8

Editorial

Bronchoalveolar lavage

The interesting paper from the Brompton group[1] represents one of the first British contributions to address the question, "can bronchoalveolar lavage (BAL) be used to determine the diagnosis and management of cryptogenic fibrosing alveolitis (CFA)?" We will set their observations as a more general question—namely, what can the examination of products of BAL teach us about lung cell function in health and disease?

Some historical comments may serve as an introduction. In 1847, Green reported his method of catheterising the larynx and bronchi to the Medical and Surgical Society of New York.[2] The technique was condemned as an anatomical impossibility and an unwarranted innovation in practical medicine, and he was asked to resign from membership.

Bronchoalveolar lavage was first used at Yale in 1922 in the management of phosgene poisoning. This approach has now been extended to cystic fibrosis, asthma, and alveolar proteinosis. In the latter it represents the sole available therapy. In 1961, Myrvik[3] showed how this simple lavage procedure could be used in rabbits to obtain lung macrophages in a "high state of purity." This seminal observation spawned a new discipline, pulmonary cell biology, which focused initially on the distinctive features of lung as opposed to other tissue macrophages. The more recent development is the application of BAL to man in health and disease. This application was begun by a study[4] performed on gentlemen "enjoying" the hospitality of the Governor of the great State of Florida. This study provided the first evidence that in vivo cigarette smoking induced a highly active macrophage which consumed more oxygen and glucose than the quiescent unsmoked macrophage.

The observations have now been extended to disease by several American groups, notably at the National Institutes of Health,[5,6] the Brompton group,[1] and a considerable group of continental European workers.[7] The major development has been the demonstration that lavaged cells reflect the cell population derived from lung biopsies in at least two diseases, namely sarcoidosis and CFA. This development is certainly of theoretical interest since it permits the examination of pathogenetic mechanisms in man. Additionally, the clinical importance is stressed in Haslam's paper in which information affecting the diagnosis and management of CFA is presented.

We will highlight some selected aspects of BAL. A recent detailed review is in press.[8]

Address for reprint requests: Dr JBL Gee, Department of Medicine, Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510, USA.

Normal lungs

SOLUBLE COMPONENTS OF LAVAGE FLUID

A number of materials present in normal lavage fluid is listed in table 1 and presented elsewhere.[9,10] The humoral defence mechanisms are represented by IgG and secretory IgA. There are two main sources for the immunoglobulins found in respiratory secretions: local synthesis in the submucosa and transport from the intravascular immunoglobulin pool. Both are important, but the relative contribution of each to the normal immunoglobulin content of lung lavage fluids has not been measured. Opsonins of the IgG class are clinically important in the lung—for example, in *Pseudomonas* infection.[11] IgG and IgA are also both important as antitoxins and in neutralisation of viruses. The IgA inhibits bacterial and virus mucosal adherence. Other phlogistic factors in lung lavage fluid include a sizeable list of cellular and bacterial-derived chemotactic factors, the centrally important clotting—Kallikrein systems, and complement components. Small amounts of classical pathway components have been detected in lavage fluids and C_3b has been shown to attach bacteria to macrophages, a prerequisite for phagocytosis.

Antiproteinases are a necessary component in the delicate regulation of the activity of such tissue destructive proteinases as elastase. The main antiproteinase in lavage fluid is α_1-antitrypsin but other less well-characterised antiproteinases have been detected in bronchial secretions. Additionally, a number of lysosomal enzymes—for example, β-glucuronidase—have been found in BAL fluids.

Table 1 *Lung lavage: adult non-smoker normal values*

Cells	
Macrophages	90%±7·0
Lymphocytes	15·2%±2·6
T lymphocytes	47%
B lymphocytes	15%
"Null" cells	38%
Polymorphonuclear leucocytes	1·0%
Eosinophils and basophils	Normally not present

Lipids‡	
Neutral liquids	
Cholesterol	31·3±16·3
Free fatty acids	18·8±11·8
Triglyceride	18·7± 8·7
Cholesterol-esters	38·1±12·7
Phospholipids	
PC	57·4
PE	15·2
PG	11·0
Sphingomyelin and PI and PS	12·4
Unknown	4·1

*Proteins**	
Humoral immune substances	
SIgA	0·72±0·12
dimeric (11S)	60–80%
monomeric (7S)	9–13%
higher polymers	11%
free secretory component	Present but not quantified
free "J" chain	?
IgG	0·3±0·02
IgM	Present in small quantities in 5% of control
IgD	?
IgE	77·2±8·2 mg/ml
Complement	
C_3	Present
C_4	0·28†
C_6	0·011†
C_9	Present
Factor B	Present
Antiprotease	
α_1–antitrypsin	2·63±0·17
Other	
Albumen	1·42±0·22 mg/ml
Transferrin	Present but not quantified
α_2–macroglobulin	Present but not quantified

*Proteins are expressed as mean ± SE of ratio to albumen as denomenator.
†Bronchial fluid/serum ratio of means.
‡Expressed as percentage of total neutral or phospholipid phosphorous.

The significance of the small amounts of enzymatic activity in health is not yet clear. The lipid materials obtained by BAL from nonsmokers have also been characterised.[12]

LAVAGE CELL POPULATION

The alveolar macrophage (AM) constitutes the largest single cell population in BAL fluid and is the subject of two recent reviews.[13 14] Apart from exhibiting the general characteristics of macrophages, including receptors for C_3b and F_c portion of IgG molecule, bactericidal activity, phagocytosis, and response to opsonising antibodies and to macrophage inhibition factors, two particular distinctive features have been described in AM which have great potential relevance to pulmonary disease.

While the macrophages themselves form the first line of defence by virtue of their inherent and immunologically enhanceable secretory, phagocytic, and bactericidal activity, they can also release a chemotactic factor which attracts and mobilises the more vigorous scavenging and microbicidal polymorphonuclear leucocyte (PMN).[51] This AM-derived chemotactic factor can also elicit lysosomal enzyme release from the PMN as has been demonstrated by measurements of PMN elastase.[16] Thus, macrophage-modulated PMN activity can not only serve defensive functions, but can also evoke the release of tissue destructive enzymes.

A second feature of lung macrophages is their capacity to adapt to chemical changes in the external environment. This activity depends on the AM's biosynthetic capacity which derives from a rich endoplasmic reticulum. Such responses include adaptations to changes in oxygen tension and to the presence in the environment of such diverse materials as haemoglobin and cigarette smoke. As regards oxygen tension, the AM are naturally exposed to oxygen tension of approximately 100 mmHg. The AM contain high antioxidant enzyme activities (superoxide dismutase, catalase, and glutathione peroxidase). The activity of one of these enzymes, superoxide dismutase, rises in the maturing AM as fetal maturity also develops so that with the transfer of the fetus to air breathing at birth adequate antioxidant enzymes are available.[17] Culture of isolated macrophages from two tissues, the aerobic lung and anaerobic peritoneum, have also shown adaptation in both the antioxidant enzymes and the relative contributions of anaerobic and aerobic energy sources employed by the macrophage. This effect is also apparent as the oxygen tension is varied in the culture medium system.[18] Additional adaptations include the development of arylhydrocarbon hydroxylase activity in the macrophages obtained from

cigarette smokers[19] and also the development of haem oxygenase when AM are cultured in the presence of haemoglobin.

The second major cell population in BAL are lymphocytes (for recent reviews, see[10 20]). In normal man, these comprise 5–30% of the lavage cell population. As in peripheral blood, T cells predominate, and there are suggestions that the blastogenic response to mitogenic stimuli is normal in normal human lung lymphocytes and definitely reduced in those of smokers.[21] Most of the lymphokines associated with peripheral blood lymphocytes have been described in human lung lymphocytes. This technique of washing respiratory lymphocytes out of the lower respiratory tract has the obvious limitation of only sampling luminal cells which may not reflect accurately the composition of lymphocytes in submucosal and interstitial areas in all disease states. It should be noted that as many as one-third of lavaged lung lymphocytes cannot be classified accurately at present (null cells). These may represent immature cells without fully developed surface markers or a subpopulation of lymphocytes whose function is currently unknown. Studies of lymphocytes from the human lung place the "pulmonologist" in an unusual position of being able to study the local cellular immune mechanism of his organ of interest in a highly specific way.

Other cells found in the lavage fluid include occasional eosinophils, basophils, and mast cells. Mast cells sit along the mucosal surfaces allowing, in allergic individuals, an explosive IgE-mediated release of phlogistic factors.

Another cell found in the lavage fluid is the PMN, which is normally sequestered in the pulmonary circulation.[14] However, BAL performed on healthy young subjects yielded up to 3% in one study,[22] and even less in other studies.[8] Apart from their phagocytic and scavenging action, the PMN can cause major local tissue damage since they contain such proteinases as elastase and collagenase. These enzymes can degrade extracellular connective tissue protein substrates at the neutral pH of normal extracellular fluid. Polymorphonuclear leucocytes also release powerful oxidising materials, superoxide anion, H_2O_2, and the hydroxyl-free radical.[14] These materials are highly efficient bactericidal agents but also injure the membranes both of the PMN themselves and of other adjacent cells, for example endothelial cells. In this respect, the monocyte phagocyte series, which also generate these oxidants, have better developed intracellular antioxidant systems and thus release less oxidants into the tissues.[23] This and other differences between the suave macrophage and the waspish PMN are important for lung defences since the unstimulated AM can maintain normal lung sterility without tissue injury while an aggressive lung defence by PMN can be potentially highly injurious to the lung. Thus it is appropriate to minimise the role of the PMN in the normal lung, a point discussed next in relation to smoking.

Abnormal lungs

SMOKING

The effects of chronic cigarette smoking on lung cell function are in some ways most appropriately studied in man, an approach in which the technique of BAL is important. Bronchoalveolar lavage yields four to five times more cells from the lungs of cigarette smokers and these cells are 95% macrophages.[4 8] This increased lavage cell yield reflects the large numbers of macrophages present in biopsy specimens of terminal air spaces of otherwise healthy subjects.[24] These macrophages are richly granular and contain non-biodegradable kaolinite particles derived from contamination of the tobacco leaf with soil minerals.[13 25] Since most British cigarette tobacco is "Virginian," some colonial soil has come home to roost!

Human AM so exposed in vivo are activated cells. They contrast with the impaired function observed when lavaged AM from healthy experimental animals are exposed in vitro to cigarette smoke. The latter show impairments of phagocytosis, protein synthesis, and energy metabolism, particularly at the mitochrondrial level.[24] In man, the AM from cigarette smokers are much more active, showing increases in the intracellular levels of lysosomal hydrolases, angiotensin convertase,[26] and lactate dehydrogenase.[22] Cell adherence and lysozyme secretion rates are also enhanced in cigarette smokers' macrophages.[22] Whether human AM contain or secrete more tissue injuring proteinases, such as elastase, is not yet clear.[22 27]

This activation of the individual AM is enhanced further by the sharp increase in the number of air-space macrophages mentioned previously. These macrophage responses can be interpreted as clearance and detoxification mechanisms but also raise the spectre of tissue injury since lysosomal hydrolases and secreted proteinases from AM can readily evoke tissue injury. This potential tissue injury by either AM or PMN derived enzymes can be augmented in the smoker since oxidants, either in cigarette smoke itself or alternatively released

from the phagocytes themselves, are now known to impair the antiprotease function of α_1-antitrypsin.[27] We mentioned above, the relative paucity of PMN in the lavage fluid from normal subjects. As judged by both differential cell counts and measurements of a PMN-marker enzyme (myeloperoxidase), there is little or no increase in the percentage of PMN present in the lavage fluid from "normal" *young* smoking subjects.[22] However, since there is an absolute increase in the total cells yielded by lavage, the total number of PMN rises by a similar amount. It is probable that as subjects grow older and continue to smoke, the number of PMN removable by lavage may rise.[8] Indeed, to the extent that the smoking is associated with chronic bronchitis and attacks of purulent sputum, it would hardly be surprising to find increased numbers of PMN within the small airways and hence also in the lavage fluid. In older smoking subjects, therefore, PMN enzymes such as elastase become important and could readily cause tissue injury. The sputum of cystic fibrosis patients with attacks of bacterial infection contain very large quantities of PMN elastase.

Under these circumstances, the AM assume another role, namely that of scavenging elastase released from PMN.[22 28] It is apparent, therefore, that there is much to be learned about the dual role of the AM, which can both stimulate PMN elastase release and also take up and inactivate this same elastase.

LUNG DISEASES

Table 2 contains some current information on the lavage characteristics in some selected lung diseases, two of which deserve particular comment.

In CFA there is, as first described by the Vermont group,[29] substantial evidence of an increased PMN yield in BAL fluid.[5 6] Similar results have been obtained in asbestosis as opposed to simple asbestos exposure without lung fibrosis; in the latter, a normal lavage cell population has been reported.[30] While there is some suggestion of increased lymphocyte yield, this is certainly less pronounced and less well defined. Additionally, immune complexes have also been found both in the circulating serum but, at least so far, not in BAL fluid. In the latter, increases in IgG and IgA also occur. The association of such complexes with PMN implies that these cells would release lysosomal enzymes and tissue injuring oxygen reduction products. Additionally, such complexes identified in patients with idiopathic pulmonary fibrosis are suspected to cause spontaneous release of alveolar macrophage-derived PMN chemotactic factors.[31] This mobilisation would, therefore, compound the inflammatory sequence. While the sequence of events appears a likely pathogenetic mechanism in CFA, we do not currently know the source of such complexes. Whether immune complexes also occur in asbestosis is not yet known.

Both the NIH group[8] and Haslam and colleagues[1] have shown a reasonable correlation between the cellular features of open lung biopsies and the cell population derived by BAL in cryptogenic fibrosing alveolitis. This point, therefore, justifies the use of the less invasive BAL to assess intrapulmonary pathology so avoiding the morbidity of an open lung biopsy.

In sarcoidosis, a different picture emerges.[8] Lymphocytes comprise from 10–70% of the lavaged cells, mean values of 35% and 23% having been reported.[6 26] Even higher "lavage lymphocytosis" occurs (mean 66%) in hypersensitivity pneumonitis which also increases the lavage fluid immunoglobulin levels (IgG and IgM).[6] As with CFA, in sarcoidosis the BAL cell yield also relates closely to the biopsy cell population and is

Table 2 *Bronchoalveolar lavage fluid characteristics in selected diseases*

Disease state	*Cell*	*Fluid*
Smoking	↑ Yield AM and ? PMN	—
Cryptogenic fibrosing alveolitis (asbestosis)	↑ PMN, ? ↑ lymphocytes	↑ IgG
Sarcoidosis	Lymph–AM rosettes, ? active T lymphocytes	↑ IgG, ↑ IgM
Silicosis	↑ Type II pneumocytes	—
Idiopathic haemosiderosis	RBC containing AM	—
Proteinosis	Degraded AM	↑ Igs, unique soluble proteins
Lung carcinoma	↓ T lymphocytes	↑ IgG and IgA
Cystic fibrosis	↑ PMN	Elastase, mucoid pseudomonas, abnormal glycoproteins
Hypersensitivity pneumonitis	↑ Lymphocytes	↑ IgG, IgM
Eosinophilic granuloma	↑ PMN and Eos	—
Histocytosis X	Cells with cytoplasmic X bodies	—
Lipoid pneumonia	Fat laden AM	—

reproducible from lobe to lobe in the same patient. The lymphocytes obtained by BAL in sarcoidosis are largely T cells which are in an activated state. In sarcoidosis, the BAL T lymphocytosis is in contrast with the peripheral blood T cell lymphopenia, a feature indicating the usefulness of BAL to examine the lymphocytes specifically associated with the granuloma itself.[32] One may expect further work elucidating the subsets of T cells and the relative prevalence of suppressor T cells will be of considerable interest since material from these cells may well account for the skin anergy so frequent in sarcoidosis.[33] One additional characteristic of sarcoidosis is the presence in the lavage of lymphocyte-macrophage rosettes. These are in a sense "mini-granulomata."

Studies of the AM in sarcoidosis have also been undertaken.[26 33] These demonstrate an increase in the intracellular lysozyme and angiotensin-convertase level so suggesting that the AM are a source of both of these enzymes. These enzymes are raised in the sera of many sarcoidosis patients. Parenthetically, the term angiotensin-convertase is too narrow a term for this enzyme activity since the activity is also capable of attacking bradykinin analogues. The biological significance of this kininase is not yet clear but the increased activity of this enzyme in macrophages occurs in other granulomatous disorders such as schistosomiasis.

Space forbids more than a mention of other disorders which might yield up their secrets by the use of BAL. Some of these are indicated in table 2 and are more fully reviewed elsewhere.[8]

Clinical value of bronchoalveolar lavage

The clinical usefulness of BAL is partly determined by its complication rate. For the most part, the common complications are those inherent in the fibreoptic bronchoscopy itself: reactions to the topical anaesthetic, trauma caused by faulty insertion, hypoxaemia as a result of occlusion of the bronchi by the wedged scope or instillation of lavage fluids and bronchospasm. A common complication of lavage itself, occurring in up to 17% of lavages, is a moderate transient fever (up to 101°F). This usually requires only symptomatic treatment. In skilled hands, BAL has no mortality and only minimal morbidity.

The diagnostic usefulness of any even modestly invasive procedure, such as BAL, depends on the relation between the precision with which the clinical data establish the diagnosis and the specificity of the information yielded by the procedure. Examples of this kind of information available from bronchoalveolar lavage in various diseases are indicated in table 2. While more experience is required to define the precision with which the cellular and chemical features of a given disease are defined by BAL, some features may be virtually pathognomic. Two examples may be given—the macrophages containing red cells present in idiopathic pulmonary haemosiderosis,[34] and the proteinaceous materials present in alveolar proteinosis.[35 36] In industrial lung disease, the diagnosis is usually established by the clinical picture and the exposure history. Bronchoalveolar lavage does not at present yield sufficient material for chemical analysis. However, studies of lavaged AM may prove helpful in multiple occupational exposures—for instance, in detecting lipid laden macrophages in the lavage from patients exposed to two potentially toxic materials, oils and tungsten carbide, in steel rope manufacture. The refinement of physio-chemical analytical techniques for the microdetection of metals may change this. Other diagnostic features of BAL—notably those that depend on differential cell counts—may be less precise since there is considerable variation in the cell population in both health and disease. Further, the effects of smoking superimposed on those of the disease need to be considered.

There are some problems in the standardisation of the lavage procedure itself and also in knowing how best to adjust for the variable volume of fluid recovered.[8] These concerns apply to both the cellular and soluble components. An additional concern about the latter is the distinction between the haematogenous and lung tissue origins for soluble materials. This may be minimised by expressing such materials as IgG in terms of the albumin content of the lavaged fluid. In spite of these concerns, reproducibility appears to be reasonably good.

The other side of the coin is the precision of the clinical data. In some instances, for example, *Pneumocystis carinii* pneumonitis, the bedside diagnosis is usually far from clear and requires specific diagnostic tests which can be performed on BAL cells.[37 38] In others, such as sarcoidosis and CFA, the diagnosis is frequently clear on conventional clinical grounds and may be supported by less invasive tests such as measurements of the serum lysozyme and angiotensin-converting enzyme levels. Thus, while CFA and sarcoid can clearly be distinguished from one another by BAL cell characteristics,[8 39] this may only rarely be really necessary. We suspect that BAL will not be a routine diagnostic procedure under these circum-

stances, but may prove helpful where the diagnosis is clinically less apparent.

Management of lung diseases

We support the suggestions of the Brompton group that BAL could well provide help in therapeutic decisions. Such help is certainly needed in both CFA and sarcoidosis. In both these disorders the use and dosage of corticosteroids is based on empirical trial and error and often undertaken in the face of irreversible fibrosis. Haslam *et al*[1] suggest that the percentage of lymphocytes in the BAL from CFA patients is related to steroid responsiveness. Likewise, there are suggestions that lymphocyte predominance in the BAL is associated with an active phase of sarcoidosis, a phase that usually shows a rapid immediate response to corticosteroids.[8] In hypersensitivity pneumonitis lymphocytes also predominate.[8] In the acute state, this group of disorders is highly steroid sensitive. Thus, BAL lymphocytosis is broadly suggestive of therapeutic responsiveness. However, do we need this information for deciding the treatment plan? For clinically apparent hypersensitivity pneumonitis the clinical response is clear enough on its own. For sarcoid there is, at least in our minds,[33] a need for a medium-term controlled trial, at least where overt pulmonary infiltration is present, since the place and dosage of corticosteroids are not clearly defined and established lung dysfunction is frequently not reversible. We have discussed elsewhere the use of serum levels of angiotensin-converting enzyme and lysozyme, the humorocellular assessment of sarcoidosis by bronchoalveolar lavage, and the extent of tissue involvement (gallium scan) as several relevant factors in defining the "activity" of this disease.[33] In CFA, which demonstrates only modest steroid responsiveness, we badly need some guidelines which might be particularly applicable in the early stages of the disease.

Conclusion

Bronchoalveolar lavage has been and will continue to be a powerful investigative tool. It has taken pulmonary disease over 50 years since Virchow to discover the function of the isolated cell and that required the aid of BAL!

We can now define cellular mechanisms in lung disease, particularly in man. Apart from permitting us to study disorders unique to man, such as cigarette smoking and sarcoidosis, BAL overcomes the real species variations inherent in experimental models. We believe that such knowledge will now enable lung physicians to take two leaves from the rheumatologist's book and develop rational approaches to the therapy of lung tissue injury. One example of this approach adopts the use of lysosomotropic drugs. Specific inhibitors of critical enzymes are introduced into the right place to treat certain lung diseases. Inhibitors of tissue injury enzymes (for example, elastase) can be coupled to particles which, if phagocytosed by PMN or AM, might become localised within the lysosomes or secretory granules of these cells or both. This localisation is important since this is usually the site at which such tissue injuring enzymes are either stored or transported from the cytoplasm to the cell exterior. A start in this direction has been made with the chloromethyl ketone inhibitors of elastase which have been coupled to insoluble microspheres comprising aggregates of albumin.[40] Such aggregates are likely to be readily phagocytosed and, after the intralysosomal degradation of the albumin, the inhibitor might be free to act at the appropriate location. To date, work with these microspheres has only employed intravenous injection resulting in 50% of the material localising in the lungs, where it persists with a half-life of 17 days. Whether aerosol delivery would result in lysosomal sequestration in lung phagocytes is not yet known. Approaches of this type will require considerable ingenuity but this technique is in its infancy. Perhaps, one day, such aerosols may do more good than the "mysterious mystical mists" all too often used in some respiratory therapy departments!

Another approach to treating inflammatory disease favoured by the rheumatologists is the non-steroidal anti-inflammatory drugs which have, so far, had little place in the chest physician's black bag. Not all these agents act on PMN or macrophage function when studied in vitro. Their in vivo effects will be much more complex and may be double-edged. However, BAL offers an opportunity to examine them mechanistically in the setting of natural lung disease in man.

Bronchoalveolar lavage has taught us much of disease mechanisms. Now it may not only suggest which patients to treat but also teach us something about treatment strategies.

We appreciate the opportunity to see preprints of certain articles.[1 8 16 27 28] Our work is supported by grant HL 19237 from the National Institutes of

Health. Robert B Fick is a Fellow of the Parker B Francis Foundation.

J BERNARD L GEE AND ROBERT B FICK JR
Department of Medicine (Pulmonary Section), Yale University School of Medicine, New Haven, Connecticut 06510, USA

References

1 Haslam PL, Turton CWG, Heard B *et al.* Bronchoalveolar lavage in pulmonary fibrosis: comparison of cells obtained with lung biopsy and clinical features. *Thorax* 1980; **35**:9–18.

2 Patterson EJ. History of bronchoscopy and esophagoscopy for foreign body. *Laryngoscope* 1926; **36**:157–75.

3 Myrvik QN, Leake ES, Fariss B. Studies on pulmonary alveolar macrophages from the normal rabbit: a technique to procure them in a high state of purity. *J Immunol* 1961; **86**:128–32.

4 Harris JO, Swenson EW, Johnson JE. Human alveolar macrophages: comparison of phagocytic ability, glucose utilization, and ultrastructure in smokers and nonsmokers. *J Clin Invest* 1970; **49**:2086–96.

5 Crystal RG, Fulmer JD, Roberts WC, Moss ML, Line BR, Reynolds HY. Idiopathic pulmonary fibrosis: clinical, histologic, radiographic, physiologic, scintigraphic, cytologic, and biochemical aspects. *Ann Intern Med* 1976; **85**:769–88.

6 Weinberger SE, Kelman JA, Norton AE *et al.* Bronchoalveolar lavage in interstitial lung disease. *Ann Intern Med* 1978; **89**:459–66.

7 Voisin C. *Colloque International Sur le Lavage Broncho-Alveolaire Chez L'homme.* Institut Pasteur de Lille, 7–9 Février 1980; in press.

8 Hunninghake GW, Gadek JE, Kawanami O, Ferrans VJ, Crystal RG. Inflammatory and immune processes in the human lung in health and disease: evaluation by bronchoalveolar lavage. *Am J Pathol* 1979; **97**:149–206.

9 Reynolds HY, Newball HH. Analysis of proteins and respiratory cells obtained from human lungs by bronchial lavage. *J Lab Clin Med* 1974; **84**: 559–73.

10 Reynolds HY, Newball HH. Fluid and cellular milieu of the human respiratory tract. In: Kirkpatrick CH, Reynolds HY, eds. *Immunologic and Infectious Reactions in the Lung.* New York: Marcel Dekker, 1976:3–27.

11 Reynolds HY, Thompson RE. Pulmonary host defenses. II. Interaction of respiratory antibodies with *Pseudomonas aeruginosa* and alveolar macrophages. *J Immunol* 1973; **111**:369–80.

12 Finley TN, Ladman AJ. Low yield of pulmonary surfactant in cigarette smokers. *N Engl J Med* 1972; **286**:233–7.

13 Mason RJ. Metabolism of alveolar macrophages. In: Brain JD, Proctor DF, Reid LM, eds. *Respiratory Defense Mechanisms, part 2.* New York: Marcel Dekker, 1977:893–926.

14 Gee JBL, Khandwala AS. Motility, transport, and endocytosis in lung defense cells. In Brain JD, Proctor DF, Reid LM, eds. *Respiratory Defense Mechanisms, part 2.* New York: Marcel Dekker, 1977:927–82.

15 Kazmierowski JA, Gallin JI, Reynolds HY. Mechanisms for the inflammatory response in primate lungs. *J Clin Invest* 1977; **59**:273–81.

16 Hunninghake GW, Gadek JE, Crystal RG. Mechanisms by which cigarette smoke attracts polymorphonuclear leukocytes to lung. *Chest* 1980; in press.

17 Autor AP, Frank L, Roberts RJ. Developmental characteristics of pulmonary superoxide dismutase: relationship to idiopathic respiratory distress syndrome. *Pediat Res* 1976; **10**:154–8.

18 Liu J, Simon LM, Phillips JR, Robin ED. Superoxide dismutase (SOD) activity in hypoxic mammalian systems. *J Appl Physiol* 1977; **42**:107–10.

19 McLemore TL, Corson MA, Martin RR *et al.* Cytotoxicity and AHH enzyme induction in human pulmonary macrophages and lymphocytes cultured with asbestos (abstr.). *Am Rev Respir Dis* 1979; **119**:229A.

20 Bienenstock J, Clancy RL, Percy DYE. Bronchus associated lymphoid tissue (BALT): Its relationship to mucosal immunity. In: Kirkpatrick CH, Reynolds HY, eds. *Immunologic and Infectious Reactions in the Lung.* New York: Marcel Dekker, 1976:29–58.

21 Daniele RP, Dauber JH, Rossman MD. Lymphocyte populations in the bronchoalveolar air spaces: recent observations in normals and asymptomatic smokers. In: Voisin C, ed. *Colloque International Sur le Lavage Broncho-Alveolaire chez L'homme.* Institut Pasteur de Lille, 7–9 Février, 1980; in press.

22 Hinman LM, Stevens CA, Matthay RA, Gee JBL. Elastase and lysozyme activities in human alveolar macrophages: effects of cigarette smoking. *Am Rev Respir Dis* 1980; in press.

23 Adler LM, Gee JBL, Root RK. H_2O_2 formation and utilization by human monocytes (abstr). *Clin Res* 1978. **26**:522A.

24 Gee JBL, Khandwala AS, Boynton BR. Effects of cigarette smoke on alveolar macrophage function. In: Lee SD, Mudd JB, eds. *Assessment of Toxic Effects of Environmental Pollutants.* Ann Arbor, Michigan: Ann Arbor Science Publishers, 1979: 77–85.

25 Green GM, Jakab GJ, Low RB, Davis GS. Defense mechanisms of the respiratory membrane. *Am Rev Respir Dis* 1977; **115**:479–514.

26 Hinman LM, Stevens CA, Matthay RA, Gee JBL. Angiotensin-convertase activities in human alveolar macrophages: effects of cigarette smoking and sarcoidosis. *Science* 1979; **205**:202–3.

27 Janoff A, White RK, Carp H, Harel S, Deering

R, Lee D. Lung injury induced by leukocytic protease. *Am J Pathol* 1979; **97:**111–36.

28 Campbell EJ, White RR, Senior RM, Rodriguez RJ, Kuhn C. Receptor-mediated binding and internalization of leukocyte elastase by alveolar macrophages in vitro. *J Clin Invest* 1979; **64:**824–33.

29 Davis GS, Brody AR, Craighead TE. Analysis of airspace in interstitial lung disease. *Am Rev Respir Dis* 1978; **118:**7–15.

30 Bignon J, Atassi K, Jaurand MC, Geslin P, Solle R. Cellular and protein content analysis of bronchoalveolar lavage fluid from patients with idiopathic pulmonary fibrosis and asbestosis (abstr). *Am Rev Respir Dis* 1978; **117:**56.

31 Gadek J, Hunninghake G, Lawley T, Kelman J, Fulmer J, Crystal R. Role of immune complexes in amplifying the alveolitis of idiopathic pulmonary fibrosis (abstr). *Clin Res* 1978; **26:**446A.

32 Hunninghake GW, Fulmer JD, Young RC Jr, Gadek JE, Crystal RG. Localization of the immune response in sarcoidosis. *Am Rev Respir Dis* 1979; **120:**49–57.

33 Gee JBL, Bodel PT, Zorn SK, Hinman LM, Stevens CA, Matthay RA. Sarcoidosis and mononuclear phagocytes. *Lung* 1978; **155:**243–53.

34 Drew WL, Finley TN, Golde DW. Diagnostic lavage and occult pulmonary hemorrhage in thrombocytopenic immunocompromised patients. *Am Rev Respir Dis* 1977; **116:**215–21.

35 Bell DY, Hook GER. Pulmonary alveolar proteinosis: analysis of airway and alveolar proteins. *Am Rev Respir Dis* 1979; **119:**979–90.

36 Bhattacharyya SN, Lynn WS. Structural studies on the oligosaccharides of a glycoprotein isolated from alveoli of patients with alveolar proteinosis. *J Biol Chem* 1977; **252:**1172–80.

37 Drew WL, Finley TN, Mintz L, Klein HZ. Diagnosis of *pneumocystic* carinii pneumonia by bronchopulmonary lavage. *JAMA* 1974; **230:**713–5.

38 Kelley J, Landis JN, Davis GS, Trainer TD, Jakab GJ, Green GM. Diagnosis of pneumonia due to pneumocystis by subsegmental pulmonary lavage via the fiberoptic bronchoscope. *Chest* 1978; **74:**24–28.

39 Reynolds HY, Fulmer JD, Kazmierowski JA, Roberts WC, Frank MM, Crystal RG. Analysis of cellular and protein content of bronchoalveolar lavage fluid from patients with idiopathic pulmonary fibrosis and hypersensitivity pneumonitis. *J Clin Invest* 1977; **59:**165–75.

40 Martodam RR, Twumasi DY, Liener IE, Powers JC, Nishino N, Krejcarek G. Albumin microspheres as carrier of an inhibitor of leukocyte elastase: potential therapeutic agent for emphysema. *Proc Natl Acad Sci USA* 1979, **76:**2128–32.