Bronchoalveolar Lavage and the Immunology of Primary Lung Cancer

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Bronchoalveolar lavage (BAL) is a powerful tool with which the immunology of the lung in health and disease can be studied. This technique has been successfully used to characterize localized humoral and cell-mediated responses in sarcoidosis and a number of other interstitial pneumonitides. In contrast, BAL in patients with lung cancer has resulted in some confusion regarding the extent and type of local and systemic immunity in these patients. This review summarizes some of the data obtained from these patients via BAL, but does not attempt to explain the reported discrepancies. The objective of this review is rather to identify gaps which exist in our knowledge of the environmental factors influencing pulmonary immunity in primary lung cancer.

Bronchogenic carcinoma is now the most common fatal malignancy in the United States. It is estimated that 85,000 men and 36,000 women will die of this disease in 1984. This compares to less than 50,000 deaths from chronic obstructive pulmonary disease; moreover, overall five-year survival rates in lung cancer average a dismal 9 percent. A number of diagnostic procedures have been examined in hopes that they may aid in the early detection of this disease. While many of these are still in a developmental stage, little progress has been made.

Bronchoalveolar lavage (BAL) is a useful procedure which can be used to sample the cellular, chemical, and humoral constituents of the alveolar milieu. Adapting this technique to the fiberoptic bronchoscope has resulted in a number of clinical studies, most of which have concentrated on either normal subjects or patients with diverse but predominantly interstitial lung disease. Today, BAL is becoming a standardized procedure and many sources of variability have been identified. Likewise, studies on the physiologic consequences of BAL have documented its safety in healthy subjects and patients with interstitial disease. Unfortunately, patients with chronic obstructive pulmonary disease (and lung cancer) may present technical difficulties which can limit the return of lavage fluid. The application of BAL to the study of lung cancer is illustrated in Figure 1.

Lymphocytes and macrophages have been the subject of intense interest in both animal models and some human cancers. Information obtained from animals suggest that the pulmonary alveolar macrophage (PAM) is a powerful arm of tumor immune surveillance and can be stimulated to cytotoxicity for tumors metastatic to the lung. While much data on this cell have accumulated from animal models, the effects of the human bronchoalveolar microenvironment in which bronchogenic carcinoma has developed is less well studied. This article examines the data generated by BAL which relates to the condition of the bronchoalveolar milieu in primary lung cancer. Little attention will be given to systemic immunity since it has been covered in previous reviews. Rather than review the myriad of immunologic interrelationships which have been studied in animals, our intent will be to review what is known about pulmonary immunity and the role which the microenvironment plays in human lung cancer. Our approach is outlined in Table 1.

The Search for Specific Antigens

Antigens to which an immunotherapeutic attack on a tumor can be stimulated have been extensively studied without much success. Nonetheless, even if antigen-guided antitumor immunotherapy were not possible, a simple blood test to diagnose preclinical carcinoma might prove extremely valuable. Carcinoembryonic antigen (CEA) was found in elevated concentrations in the serum, pleural effusion, and bronchial washings of patients with lung cancer. Lemarie et al studied four groups of patients undergoing physiologic saline lavage. The CEA immunoenzymatic assay was applied...
to lavage fluid from normal subjects, noncancer pulmonary disease patients, bronchogenic carcinoma patients, and patients with pulmonary metastases from diverse primary tumors. Compared to the noncancer subjects whose plasma and lavage CEA values averaged <14 ng/ml, the primary lung cancer patients exhibited CEA concentrations >10 ng/ml in their lavage fluid. The nonprimary (metastatic) group had plasma and lavage findings similar to the primary cancer group.

Merrill et al.29 studied BAL and plasma values of CEA using modified competitive radioimmunoassay in nonsmoking and smoking volunteers. The two groups were similar in age, sex, cell count, lavage fluid volume, and total protein recovery. These investigators found the mean BAL concentration of CEA per milligram of total protein in smokers was twice that of nonsmokers. These levels were much higher than plasma levels (42 to 76 ng/mg for concentrated lavage fluid vs .04-.08 ng/ml for plasma). There was a weak correlation between smoking in pack-years and the CEA level; however, most of the elevation in mean CEA in the lavage fluid in smokers was attributable to only seven of the 28 smokers. It would be interesting to know if this elevation is predictive of future malignancy of the airway of this subgroup. The CEA is an attractive early step in an ongoing search for a sensitive and specific tumor-associated antigen which could prove to be diagnostic and/or prognostic.

**THE PRESENCE OF ANTIBODIES**

Matching the intensity of the search for tumor-specific antigens is the investigation into nonspecific and tumor-specific antibodies. Zeromski et al.30 analyzed tissue blocks of 22 patients with resected primary lung cancer and 12 patients with either nonprimary cancer or nonmalignant diseases. These resected tissue specimens were labeled immunofluorescently for the five classes of immunoglobulins. The sera of these patients and 42 patients with other lung diseases were also examined. The IgA staining cells were frequently observed in the area closest to the tumor while IgG, IgM, and IgD staining cells were less frequently observed. No IgE stained cells were observed. There was no difference referable to tumor cell type, and IgA stained cells predominated in the lymph nodes closest to the malignancy. Elevations of IgA were also observed in the serum of 62 percent of the cancer patients with inoperable patients having higher levels than those in the operable group. Elevations of serum IgG were also higher in the cancer group; however, levels varied greatly among individuals. These findings were also supported by the findings of Gerstl et al.31

Mandel et al.32 extended their observations of immunoglobulins in secretions in a variety of nonpulmonary

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**Table 1—Bronchoalveolar Immunology of Lung Cancer**

<table>
<thead>
<tr>
<th>Specific antigens</th>
<th>Tumor associated antibodies</th>
<th>Cell mediated immunity</th>
<th>Blood lymphocytes</th>
<th>T-lymphocyte subsets</th>
<th>Macrophage/monocyte</th>
<th>Cytotoxicity</th>
<th>Activation</th>
<th>Chemotaxis</th>
<th>Effect on natural killer cells</th>
<th>Effect of immune stimulants</th>
<th>Pinocytosis</th>
<th>Production of oxygen intermediates</th>
<th>Immune phagocytosis</th>
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Figure 1. Schema of the use of bronchoalveolar lavage (BAL) in the study of the humoral and cellular constituents of the environment of lung cancer.
malignancies to lung cancer. "Bronchial washings" of ten normal general anesthesia patients without lung disease were studied. The remaining control subjects exhibited bronchiectasis, tuberculosis, and pneumonitis. Of the cancer patients, most had squamous cell tumors, but adenocarcinoma and undifferentiated malignancies were also studied. Specimens were obtained by lavage using a rigid bronchoscope, lyophilized, and later analyzed by qualitative immunoelectrophoresis and quantitative radial immunodiffusion. Attempts at standardization of these lavage concentrates by potassium and calcium determinations proved more reproducible than relating immunoglobulin levels to lavage total protein. Significantly elevated lavage IgG/K and IgA/K ratios were found in the involved vs noninvolved lungs of the cancer patients.

Paluch and Ioachim also reported elevated IgG/K, IgA/K levels in bronchial washings of patients with squamous cell carcinoma, adenocarcinoma, and patients with inflammatory disease in comparison to autopsy controls without lung disease. These immunoglobulins were obtained by dissociation of immune complexes and were then analyzed by indirect immunofluorescence against human lung cancer models (HLU T and PC). Positive fluorescence was seen against the adenocarcinoma and squamous carcinomas but not against normal cells.

Secretory IgA-S was also found in in vitro analysis of supernatants of mucosal tissue cultures from normal, tuberculosis, and carcinoma patients. These investigators also found increased levels of IgA-S associated with carcinoma compared to normal tissue.

Inglehart et al. studied patients with benign disease and 47 patients with primary bronchogenic carcinoma. These patients had bronchial washings through a fiberoptic bronchoscope and a subgroup had bilateral samples obtained via rigid bronchoscopy. Significant elevations of secretory IgA as determined by competition radioimmunoassay were observed in the nonsmall cell carcinoma patients. Eighty percent of the carcinoma patients had values >300 μg/ml, and secretions from the tumor-bearing lung showed a 50 percent higher elevation of IgA compared to the contralateral lung. These findings have led the investigators to propose this as a method for early diagnosis. The mechanism remains obscure, but a local secretory functional derrangement rather than an active immunologic reaction has been proposed.

CELL-MEDIATED IMMUNITY: THE LYMPHOCYTE

Cell-mediated immunity as mediated via T-lymphocytes either as direct tumor cell cytotoxicity or via lymphokine-stimulation of PAM remains as the centerpiece of a burgeoning research activity. At the time of this writing, it appears that studies of the lymphocyte as a mediator of immune surveillance against lung cancer have been limited to peripheral blood lymphocytes (PBL) or surgically removed lymph nodes. The absence of studies of lymphocytes from BAL of lung

![Diagram](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21466/ on 06/27/2017)
cancer *vis-a-vis* sarcoïdosis probably relates to the yield of lymphocytes with BAL being much greater in the latter entity. It has been known for some time that cell-mediated immunity decreases as the stage of lung cancer progresses as evidenced by skin test results.\textsuperscript{23,24} Yet, the mechanism by which this decrement occurs is unknown.

In 1976, Yasumoto et al\textsuperscript{28} studied 33 patients with squamous, adenoc, anaplastic, and alveolar cell carcinoma, and seven control subjects, only two of whom were healthy. Peripheral blood lymphocytes (PBL) were separated by Conray-Ficoll gradients and mixed with cultured human squamous cell carcinoma at a ratio of lymphocytes to tumor cells (effector:target ET ratio) of 400:1. Cytotoxicity was assessed as the number of surviving targets stained by May-Grunwald-Giemsa solution after 24 hour incubation. In stage 1 cancer, PBL mean cytotoxicity was 61 percent, however, there was a progressive decrease with increasing tumor progression to stage 4 which was characterized by hilar, mediastinal, intrathoracic, and/or distant metastasis. The regional node lymphocytes from the patients with bronchogenic carcinoma averaged 20 percent to 25 percent cytotoxicity, whereas control subjects varied widely. The results suggest an intrapulmonary regional surveillance, as well as systemic “awareness” of tumor by PBL which appears to wane as the tumor progresses. The same group of investigators extended their observations on PBL cytotoxicity to the effects of radiotherapy of the primary lesion\textsuperscript{27} and found no overall change in cytotoxicity following radiotherapy in patients with early lesions (stages 1 to 3). However, augmentation of cytotoxicity was observed in those with later stages of disease despite a drop in the number of circulating lymphocytes following radiotherapy. There was also a correlation between posttherapy cytotoxicity and roentgenographic reduction in tumor size.

Peripheral blood T-lymphocyte subsets were studied and reported in 1981 by Pucci et al.\textsuperscript{29} The investigators identified subsets via their receptors for IgA (Tα), Fc-IgG (Tγ) and Fc-IgM (Tμ) by rosetting of sheep red blood cells. The study population was comprised of eight patients with bronchogenic carcinoma and 23 patients with colonic carcinoma. Patients with ulcerative colitis, Crohn’s disease, diverticulitis, chronic bronchitis, bronchiectasis, and emphysema made up the age-matched disease control group. Thirty normal age-matched controls were also studied. This study found an increase in circulating Tα cells from those with bronchogenic (16 percent) and colon carcinomas (13 percent) compared to the nonmalignant disease (6.2 percent) and normal control subjects (7 percent). It was suggested that these findings indicate a mucosally-oriented cell-mediated response to these two mucosal malignancies.

Further observations on blood T-lymphocyte subsets were reported by Ginns et al\textsuperscript{30} using monoclonal antibodies and flow cytometry. The OKT4 (inducer/helper), OKT8 (cytotoxic/suppressor), and OKT3 (total T-cell number) were the cell types studied. The sample population consisted of 76 subjects (26 lung cancer, 29 nonsmoking volunteers, and 19 smokers). These investigators found no significant difference in nonsmoking and smoking control subjects in percentages of OKT3 (68 percent), OKT4 (43 percent), and OKT8 (24 percent) lymphocytes. There was, however, an increase in absolute numbers of lymphocytes in the smokers due to an elevation of total cell count. The results in the patients with squamous cell cancer were similar to that of the normal smokers. The adenocarcinoma patients, however, demonstrated a decrease in OKT8 cells. The ratio of OKT4 to OKT8 (helper/suppressor) was elevated in the adenocarcinoma group, but it was reduced in those with metastatic malignancy to the lung. The investigators suggested that the results provided additional evidence of immune recognition, but that lavage studies were needed.

In a study of 69 lung carcinoma patients (squamous, adenocarcinoma, and large cell), Vanký et al\textsuperscript{31} reported lymphocyte proliferation in response to mitogens and cytotoxicity for autologous tumor cells. Blood lymphocytes demonstrating increased DNA synthesis following mitogen stimulation seemed to predict a good prognosis for increased tumor-free period and survival. In contrast, lymphocytes of 53 percent of these patients demonstrated cytotoxicity by lysis of \textsuperscript{51}Cr-labeled tumor targets, but cytotoxicity did not correlate with survival nor relapse.

**MACROPHAGE/MONOCYTE**

Early observations of macrophages within surgically resected tumor tissue stimulated a quest for identification and potential stimulation of this cytotoxic effector cell.\textsuperscript{4} Vose\textsuperscript{44} reported that adherent cells from resected specimens of a variety (lung, breast, gastrointestinal) of malignancies were cytotoxic for autologous tumor targets. These adherent cells phagocyted latex particles, incorporated neutral red, formed EA rosettes with ox red blood cells, and stained with nonspecific esterase and chloroacetate esterase. Alveolar macrophages isolated from patients with lung cancer were cytotoxic for autologous tumor cells at effector:target (E:T) ratios as low as 6:1. Putatively normal cells from tumor-free areas appeared resistant to lysis when used as targets. However, adherent cells from tumor-free lung regions were cytotoxic for the tumor cell targets but not for the normal lung cells.

In 1980, Lemaire et al\textsuperscript{45} reported cytotoxicity of human PAMs obtained via BAL for three human tumor cell models as compared with blood monocytes (PBM) from the same subjects. The study population was
comprised of five patients with bronchogenic carcinoma (squamous, adenocarcinoma, small cell), eight nonsmoking and four smoking normal subjects, and six patients with various infections. The targets used were cell lines from a melanoma, renal cell carcinoma, epidermoid lung carcinoma, and skin fibroblast. Cytotoxicity was defined as the inhibition of uptake of tritiated thymidine, and cytoidal activity was defined as release of thymidine from prelabeled targets. The PAMs at E:T ratios of 4:1 and 8:1 were cytotoxic for these tumor targets and normal skin fibroblasts. Cytoidal activity was less noticeable than cytotoxicity, and the PBMs were significantly less active than the PAMs. Moreover, blood monocytes from tumor patients demonstrated more cytotoxicity than those from normal subjects. There was not, however, a significant increase in the cytotoxicity of the PAMs from the cancer patients vs normal smokers. Cytotoxicity appeared to be related to cell-to-cell contact with transfer of lysosomal enzymes or release of cytotoxic products (e.g., C3a, proteases, oxygen radicals). Since phorbol myristate acetate (PMA), catalase, and superoxide dismutase did not affect tumor target cell kill but did effect fibroblast toxicity, \( H_2O_2 \) generation by the PAMs was discounted.

Contrasting results were reported by Bordignon et al.\(^a\) These workers obtained PBMs from adult volunteers, peritoneal exudate macrophages from postoperative noncancer gynecologic and surgery patients, and PAMs from BAL of patients undergoing bronchoscopy for noninfectious or nonmalignant disease. Target cells were from a murine kidney line. Cytolysis was measured by \(^3\)H-thymidine release from the targets at E:T ratios of 5, 10, 20, and 40:1. Cytostasis was assessed by growth inhibition and assayed spectrophotometrically following methylene blue staining. This group found no PAM cytolytic activity at any E:T ratio. In contrast, the cytolytic activity of nonpulmonary macrophages ranged from 8.5 percent to 37 percent. The PAMs were, however, as cytostatic as macrophages from other sources. In addition, lymphokines from phytohemagglutinin (PHA)-stimulated lymphocyes but not human fibroblast interferon stimulated weak PAM-associated tumor cytolysis.

In 1982, Swinburne et al.\(^a\) reported their observations on the tumoricidal potential of PAMs isolated from patients with a variety of disease entities. This group studied 39 subjects comprised of patients with bronchogenic carcinoma, chronic bronchitis, fibrosing alveolitis, sarcoidosis, “mild bronchial inflammation,” and metastatic melanoma. Target cells were from a human lung adenocarcinoma and tumor cytotoxicity was assessed by the Selenomethionine-75 postlabeling assay. There was no significant difference between the malignant and nonmalignant groups at any macrophage E:T ratio, and mean cytotoxicity ranged from 20 percent at E:T of 0.5:1 to 85 percent at 20:1. The difference in these results and those of Bordignon et al.\(^a\) was attributed to the purification of the cells and target cell chosen. In still another study, Swinburne et al.\(^a\) compared the cytotoxicity of the PAM to that of peripheral blood monocytes (PBM). Monocytes were obtained from an unspecified number of adult volunteers, and human PAMs were obtained by BAL of unspecified patients with various disease entities. Although PAM cytotoxicity increased proportionate to the E:T ratio (91 percent at 20:1), PBM cytotoxicity plateaued at 47 percent at an E:T ratio of 3:1. The PBM were, however, more cytotoxic at low (<3:1) E:T ratios and lipopolysaccharide (LPS) had no stimulatory effect.

Rhodes et al.\(^a\) studied the functional expression of surface receptors for the F, component of IgG (F, receptor) as a marker for PAM activation from normal subjects and patients with nonmetastatic squamous, oat cell, and adenocarcinomas. The number and characteristics of the patients were not specified. Both PBM and PAM were studied with respect to F, receptor expression as assessed by rosetting of bovine erythrocytes. These investigators found an increase in rosette-forming cells in the PBM of the cancer group compared to the normal group. Conversely, depression of rosetting was noted in the PAM of the carcinoma group. Trypsin pretreatment to remove immunoglobulins resulted in increases in rosetting in both normal and carcinoma subjects' macrophages, but the PAM activity remained substantially below the PBM activity. In addition to the PBM and PAM studies, unspecified carcinoma and normal tissues were cultured from biopsied material. Supernatants of lung carcinoma tissue were shown to depress F, receptor expression. These data suggest that depression of PAM F, receptor expression was due to nonimmunoglobulin soluble mediators released from the tumor cells.

Renoux et al.\(^a\) studied chemotaxis of PAMs in malignancy. The BAL was performed on 12 normal volunteers, 20 patients with infection, 38 patients with predominantly squamous cell bronchogenic carcinoma, and eight patients with diverse malignancies metastatic to the lung. Chemotaxis was assessed by migration using Boyden chambers and an 8 \( \mu m \) pore filter. These workers found no difference in random migration, but the primary lung carcinoma patients demonstrated impaired chemotaxis to zymosan-activated serum compared to normal subjects or patients with pulmonary metastases. Similarly, Lemarie et al.\(^a\) found PAM chemotaxis to be significantly lower in patients with bronchial carcinoma than in healthy volunteers. Chemotaxis was more depressed in samples obtained from the area of the tumor than in those from the contralateral lung. In contrast, the presence of lung metastases did not affect chemotaxis. These
observations again suggested an intrinsic dysfunction of the alveolar macrophages in the environs of primary lung cancer.

The ability of macrophages isolated from various sources (eg, blood, milk, peritoneal exudate, and lung) to depress natural killer (NK) activity of PBL was reported by Bordignon et al26 in 1982. The PAM, unlike the cells from other sources, were associated with a dose-dependent inhibition of NK activity resulting in a reduction in cytotoxicity.

Sone et al31,32 reported the effects of immune stimulants on human PAM. The BAL was performed on nonsmoking volunteers and cytotoxicity assessed by release of 3H-thymidine using E:T ratios of 2.5:1 to 40:1. Although the PAM exhibited low but significant cytolytic activity without stimulation, the addition of lipopolysaccharide (LPS) or muramyl dipeptide (MDP) encapsulated in multilamellar phosphatidylcholine-phosphatidylserine liposomes resulted in significant augmentation of tumoricidal activity.

Plowman33 has reported reduced levels of pinocytic activity in PAMs obtained from tumor-bearing lung regions compared to normal regions. Twenty bronchogenic carcinoma patients and ten control subjects were studied. Pinocytosis was assessed by uptake of 199Au labelled colloidal particles of 5 to 20 μm diameter. There was a wide range of uptake (5 percent to 60 percent), but a suggestion of skewness toward reduced pinocytosis was seen in the cancer group.

Chemiluminescence of PAM from bronchogenic carcinoma patients was demonstrated by Weissler et al.34 Chemiluminescence as a manifestation of the production of toxic oxygen intermediates was greater in PBM than PAM from both normal and bronchogenic carcinoma patients. Recently, these workers have also found the PAM ineffective in killing a murine mastocytoma tumor cell model perhaps due to a defect in the production of toxic oxygen intermediates via myeloperoxidase.35

Our own experience suggests no difference in phagocytosis of opsonized sheep red blood cells by PAM from tumor-bearing vs tumor-free lung regions in patients with bronchogenic carcinoma compared to noncancer patients.36 We have, however, seen wide variation in the levels of phagocytosis making interpretation of the statistical analysis of small groups difficult.

CONCLUSIONS

A vast array of humoral and cellular immune mediators may be present in the alveolar milieu of lung cancer patients (Fig 2). While limitations in yield may hamper studies of some cellular subpopulations (eg, T-lymphocytes), studies on the mediators produced by or effective on these cells are feasible using BAL. For example, studies designed to examine the levels of prostaglandins, leukotrienes, or interleukins produced in tumor-associated and tumor-free lobes of the lung might provide valuable information as to the role these environmental substances play in regulating pulmonary immunity.

Overall, the function of the alveolar macrophage as an arm of immune surveillance against bronchogenic carcinoma appears unsettled. Perhaps larger studies using standardized tumor targets and uniform definitions of "cytotoxicity," "cytolysis," and "cytostasis" would be helpful. Studies of other functions of the PAM as it relates to the lymphocyte and how this interaction may be effected by both lymphokines and monokines produced in response to and/or metastatic carcinoma are also needed. The BAL, nevertheless, remains a powerful tool with which many questions concerning the environment, recognition, and destruction of primary lung neoplasms may yet be answered.

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