Airway Secretory IgA Concentrations in Patients with Lung Cancer*

Evaluation of the Uninvolved Lung

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To determine whether concentrations of the primary airway immunoglobulins (SIgA, IgG) are altered in the uninvolved lung of patients with lung cancer, we determined concentrations of SIgA and IgG in bronchial washings recovered from a proximal airway of the uninvolved lung in 24 patients with lung cancer and in ten patients with benign lung disease. When standardized for the amount of total protein recovered (SIgA/TP, IgG/TP), bronchial washings recovered from the uninvolved lung of lung cancer patients demonstrated a significantly decreased SIgA/TP ratio compared to control subjects (.14±.02 vs .31±.05, SEM, p<.005). There were no differences in the IgG/TP ratios. Lung cancer patients with a decreased serum albumin (<3.2 g/dl) had a significantly decreased SIgA/TP ratio in bronchial washings compared to patients with a higher serum albumin (.08±.03 vs .18±.04, SEM, p<.05). The decreased relative concentration of airway SIgA in lung cancer patients may adversely affect airway defenses against bacterial colonization. (Chest 1989; 95:1265-68)

Pneumonia is a frequent complication in patients with lung cancer and often develops as a result of mechanical obstruction of an airway by tumor.1 Alterations of various respiratory defense mechanisms may also contribute to an increased frequency of respiratory infections in these patients, including altered cellular immune function and defects in alveolar macrophage function.2-6 Secretory IgA (SIgA) is the predominant immunoglobulin in upper airway secretions and contributes to respiratory defense by preventing bacterial adherence to airway epithelial cells.7,8 Previous studies in lung cancer patients have demonstrated that secretory IgA concentrations are often increased in bronchial washings recovered from the cancer-involved lung when compared to the uninvolved lung.9,10 In this study, we sought to determine whether airway concentrations of SIgA and IgG in a proximal airway of the uninvolved lung of patients with lung cancer were altered when compared to airway concentrations in a control patient population.

METHODS

Patient Population

All patients were men hospitalized at the Kansas City Veterans Administration Medical Center, and informed consent was obtained from all patients. In all cancer patients, biopsy specimens obtained at bronchoscopy confirmed the diagnosis of bronchogenic carcinoma. The histologic diagnoses of the 24 lung cancer patients include the following: squamous cell, 12; small cell, 5; adenocarcinoma, 3; large cell, 1; and undifferentiated, 3. The control patient population included ten patients being evaluated for the following: a history of hemoptysis, determined to be secondary to bronchitis (4), a peripheral lung nodule subsequently determined to be benign by resection (4), or pneumonitis (2). In these patients, washings were obtained from a mainstem airway which appeared normal. Characterization of the patient population is provided in Table 1. A serum albumin concentration was obtained on admission in all patients. At the time of bronchoscopy, several of the patients and control subjects were receiving antibiotic treatment for bronchitis. No patient was known to be colonized by Gram-negative bacteria, although sputum cultures were not performed on all patients.

Collection of Bronchial Washings

Patients were premedicated with atropine and meperidine and also received topical anesthesia with tetracaine spray. Airways were evaluated for evidence of endobronchial disease using flexible fiberoptic bronchoscopy. In patients with suspected lung cancer, bronchial washings were initially obtained from the uninvolved lung. In control patients, bronchial washings were obtained from a roentgenographically normal lung without evidence of endobronchial abnormalities. Bronchial washings were obtained from the left mainstem bronchus or the bronchus intermedius by directly instilling 10 ml saline solution followed by immediate aspiration. This was repeated once for a total instilled volume of 20 ml. Appropriate biopsies, brushings, and washings were subsequently obtained from the abnormal lung.

Assays on Bronchial Washings

Bronchial washing samples were filtered through fine gauze to remove mucus and then centrifuged for ten minutes at 1000 g. Supernatants were aspirated and transferred to polystyrene tubes and stored at -70°C. Immunoglobulin concentrations in bronchial washings were determined by a double antibody sandwich enzyme-linked immunosorbent assay (ELISA). The ELISA was performed by coating 96 well EIA plates with 50 μl/well of rabbit antihuman secretory piece of IgA (1:500 dilution) or 5 μg/ml affinity purified goat antihuman IgG. Coating was in carbonate buffer (pH 9.6) for

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concentrations were calculated by linear regression analysis.

Statistics

The differences between groups were assessed by unimpaired
Student's t-test with correction for unequal variances when appro-
perate or Wilcoxon rank sum.

RESULTS

The recovery of bronchial washing fluid was similar
in the control population and patients with lung cancer
(7.3 ml ± 2.0 vs 8.0 ml ± 2.6) (Table 1).

Absolute Concentrations of Total Protein, SIgA and
IgG

The absolute concentration (µg/ml) of protein in bronchial washing fluid was higher in washings recovered
from the uninvolved lung of lung cancer patients
compared to control patients (566 ± 103 vs 279 ± 98
µg/ml, SEM, p = 0.4). The absolute concentration of
IgG was also increased in bronchial washings from the
uninvolved lung in lung cancer patients but was not
significantly greater than control patients (50 ± 9 vs
40 ± 18, µg/ml, SEM). The mean concentration of
SIgA was decreased in bronchial washings from the
uninvolved lung compared to washings from control
patients, although differences were not statistically
significant (56 ± 10 vs 69 ± 21, µg/ml, SEM) (Table 2).

Immunoglobulin/Total Protein Ratio

Secretory IgA and IgG concentrations were also
evaluated after standardization of samples by determin-
ing the ratio of immunoglobulin to total protein
(SIgA/TP, IgG/TP). Total protein was utilized for stan-
dardization as it is reported to provide a more reliable
standardized value than albumin.11,12 The mean SIgA/TP ratio in bronchial washings recovered from the
uninvolved lung in patients with lung cancer was

Table 1—Patient Populations*

<table>
<thead>
<tr>
<th></th>
<th>Smoking History (Pack Years)</th>
<th>Bronchial Washing Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control patients</td>
<td>10</td>
<td>60 ± 11</td>
</tr>
<tr>
<td>Lung Cancer</td>
<td>24</td>
<td>64 ± 5</td>
</tr>
</tbody>
</table>

*Values expressed as mean ± SD.

Table 2—Absolute Concentrations (µg/ml) of Protein and Immunoglobulins Recovered in Bronchial Washings*

<table>
<thead>
<tr>
<th>Protein</th>
<th>SIgA</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control patients</td>
<td>N = 10</td>
<td>279 ± 96</td>
</tr>
<tr>
<td>Lung Cancer</td>
<td>N = 24</td>
<td>566 ± 103†</td>
</tr>
</tbody>
</table>

*Values expressed as mean ± SEM.
†p<0.05 compared to control patients.

16 hours at 4° to 8°C. Plates were brought to room temperature and
washed with PBS containing 0.05 percent Tween 20. Blocking was
then performed for one to two hours at room temperature with 100 µl/well of a 2 percent w/v solution of bovine albumin in PBS. Plates
were washed and bronchial washing samples and standards diluted
in Tris-HCl buffer containing 0.02 percent Tween 20 (pH 8.0) were
added at 50 µl/well and incubated two hours at room temperature.
Standards included purified human secretory IgA and purified
human IgG. Quadruplicate wells were prepared for each bronchial
washing sample and standard dilutions, which were included in
each assay. Plates were washed and conjugates diluted in PBS
containing 0.02 percent Tween 30 were added at 50 µl/well
and incubated for two hours at room temperature. Conjugates included phosphatase labeled goat antihuman IgA (alpha-chain) or IgG.
Plates were washed and the substrate p-nitrophenyl phosphate 1
mg/ml in diethanolamine buffer was added at 100 µl/well. The
enzymatic reaction was stopped after 15 minutes (IgG system) and
30 minutes (IgA system) with 50 µl/well 0.5 N NaOH. Optical density
of each well was determined with a microscan microplate reader
equipped with a 405 nm wavelength filter. Immunoglobulin concen-
trations were subsequently determined by linear regression analysis.
Nonspecific binding of bronchial washing samples, standards, and
conjugates was minimal (OD<0.03) and was subtracted from sample
and standard OD values. There was no cross-reactivity of rabbit
antihuman secretory piece of IgA with human IgG or goat antihu-
man IgG with human secretory IgA. Control studies showed a very
low level (1 percent) of cross-reactivity between rabbit anti-human
secretory piece of IgA and human serum IgA. Calibration curves
for secretory IgA and IgG were performed and demonstrated correlation coefficients greater than 0.99. (Fig 1).

Total protein concentrations in bronchial washings were deter-
moved using a protein assay kit (Bio-Rad Laboratories, Richmond,
CT). Bovine plasma albumin was used as a standard, and protein

FIGURE 1. Calibration curves using purified SIgA and purified IgG.
A (upper) and B (lower).

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Airway Secretory IgA Concentrations in Lung Cancer (Wessellus et al)
Table 3—Immunoglobulin/Total Protein Concentrations in Bronchial Washings from Uninvolved Lung*

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Control Patients</th>
<th>Lung Cancer All Pts</th>
<th>Lung Cancer alb &lt;3.2 g/dl</th>
<th>Lung Cancer alb &gt;3.2 g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>(N)</td>
<td>(10)</td>
<td>(24)</td>
<td>(10)</td>
<td>(14)</td>
</tr>
<tr>
<td>SIgA/TP</td>
<td>.31±.05</td>
<td>.14±.02†</td>
<td>.06±.03‡</td>
<td>.18±.04†</td>
</tr>
<tr>
<td>IgG/TP</td>
<td>.11±.03</td>
<td>.10±.02</td>
<td>.12±.03</td>
<td>.09±.02</td>
</tr>
</tbody>
</table>

* alb is serum albumin; values expressed as mean ± SEM.
†p<0.05 compared to control patients.
‡p<0.05 compared to control patients and lung cancer, alb >3.2 g/dl.

significantly decreased compared to washings recovered in control patients (.14±.11 vs .31±.17, SEM, p<.05) (Table 3). Lung cancer patients with a serum albumin value of less than 3.2 g/dl demonstrated a greater reduction in SIgA/TP ratio than patients with a higher serum albumin (.08±.03 vs .18±.04 SEM, p<.05).

The mean IgG/TP ratio in bronchial washings was not different in washings recovered from the uninvolved lung in lung cancer patients compared to control patients (.11±.08 vs .10±.07 SEM) (Table 3). There was also no difference in the mean IgG/TP ratio in the lung cancer patients with serum albumin <3.2 g/dl compared to other lung cancer patients (.12±.03 vs .09±.02 SEM.)

**Discussion**

Infection of the respiratory system is a presenting symptom in approximately 10 percent of patients with lung cancer and is a frequent complication and cause of death in these patients.1 Multiple factors may contribute to an increased incidence of pneumonia in these patients including impairment of mucociliary clearance mechanisms caused by endobronchial tumor, as well as impairment of other respiratory defense mechanisms. Previous studies have noted impaired cellular immune function and diminished numbers of circulating T-helper lymphocytes in patients with lung cancer.24 Studies of alveolar macrophage function utilizing macrophages recovered from lung cancer patients have demonstrated decreased macrophage chemotaxis and phagocytic function.5,6

Concentrations of airway immunoglobulins, in particular SIgA, may be important in host defense against bacterial respiratory infections. Secretory IgA is an effective agglutinating antibody inhibiting bacterial growth and adherence to epithelial surfaces.7,8 Previous studies have demonstrated an inverse relationship between sputum levels of IgA and bacterial adherence to tracheal cells.13 Bacterial adherence is an important initial step in bacterial colonization of airway epithelial surfaces. In this study, we noted that the standardized concentrations of SIgA (SIgA/TP) in bronchial washings recovered from the uninvolved lung of patients with lung cancer were decreased compared to washings obtained in a group of patients with nonmalignant pulmonary disease including six patients with an inflammatory process (bronchitis/pneumonitis) and four patients with benign lung nodules. Although the pathologic findings in these patients were different from the lung cancer patients, they were similar in age and smoking history. In contrast to the difference in SIgA concentrations, there were no differences between these groups in standardized airway concentrations of IgG (IgG/TP). The approximate 50 percent decrease in relative SIgA concentration noted in the uninvolved lung of lung cancer patients may contribute to increased bacterial adherence to airway epithelial cells, increasing the likelihood of airway bacterial colonization.

In our study, SIgA was present in higher concentrations in bronchial washings than IgG. This finding is in agreement with other studies that have specifically evaluated immunoglobulin content of airway lining fluid.14 In contrast, studies that evaluated more peripheral bronchoalveolar lavage fluid recovered predominantly IgG.11 The relative concentrations of SIgA and IgG vary depending on the location in the respiratory tract that is sampled.15 Sample dilution is a major variable affecting concentration of various components of airway secretions recovered during fiberoptic bronchoscopy. Both albumin and total protein have been used to normalize the concentrations of other proteins; however, for standardization of SIgA concentrations, the use of total protein is associated with less variability.15 All bronchial washing samples in our study were, therefore, normalized using total protein content of the bronchial washing fluid.

Malnutrition is known to decrease secretory IgA concentrations in the respiratory tract and may be an important factor affecting airway concentrations in lung cancer patients.16 Studies in malnourished children demonstrate decreased upper respiratory concentrations of SIgA at the same time that serum levels of IgA are increased.16 In our study, lung cancer patients with a decreased serum albumin (<3.2 g/dL) had SIgA/TP concentrations that were significantly decreased compared to patients with higher serum albumin concentrations. The group of patients with a higher serum albumin, however, also had decreased SIgA/TP ratio compared to control patients. This may be a result of a mild degree of malnutrition present in
these patients compared to control patients or may indicate that in lung cancer patients, factors other than nutrition alter airway SIgA concentrations. Since the concentration of total protein was increased in bronchial washings from lung cancer patients, it is also possible that the differences found were caused by increased local permeability in the airways of patients with lung cancer. Increased local permeability would increase local IgG levels but would not increase SIgA which is synthesized in the bronchial mucosa. Although this mechanism could account for the differences between groups, it seems unlikely to account for the difference noted in lung cancer patients with different nutritional status.

Previous studies have reported increased concentrations of SIgA recovered in bronchial washings from the cancer-involved lungs. In these studies, SIgA recovery was not standardized using protein content in the bronchial washings; however, absolute airway concentrations in the cancer-involved lung were noted to be significantly increased compared to concentrations in the uninvolved lung. The mechanism of this increased concentration of SIgA in the cancer-involved lung is not clear but may be due to increased local synthesis of SIgA by plasma cells in cancer-involved bronchial mucosa. In our study, we also noted an increase in absolute concentrations of SIgA recovered from the cancer-involved lung (data not shown); however, standardization of recovery was difficult due to frequent blood contamination and marked variability in total protein content in these bronchial washings.

In summary, comparison of immunoglobulin/total protein ratios in bronchial washings from the uninvolved lung of lung cancer patients and bronchial washings from a control group of patients demonstrates a decreased SIgA/TP ratio in the uninvolved lung in patients with lung cancer. The mechanism of the decrease in SIgA/TP ratio is not clear although malnutrition or local changes in permeability may be contributing factors. None of the lung cancer patients in this study was known to have Gram-negative bacterial colonization, so the clinical significance of the decreased concentration of SIgA in these patients is unclear. A decrease in airway SIgA concentration may promote increased bacterial adherence to airway epithelial cells which would increase the likelihood of bacterial colonization of airways in these patients.

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