Serum Levels of a Human Lung Tumor-associated Antigen Using an Improved Radioimmunoassay

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ABSTRACT

A human lung tumor-associated antigen, previously purified to apparent homogeneity from an extract of a small cell tumor, was radiiodinated with Bolton-Hunter reagent for use in a competitive protein-binding radioimmunoassay. A panel of 215 sera was assembled from normal individuals and pretreatment patients with lung cancer, benign lung disease, and nonlung cancers, and lung tumor antigen in each was quantitated using the radioimmunoassay. The mean of normals was 0.92 ± 0.43 (S.D.) µg/ml (n = 88), and values greater than 2 standard deviations above the mean (1.78 µg/ml) were considered positive. Positive rates in lung cancers of the following histological types were found: adenocarcinoma, 60% (9 of 15); squamous cell, 42% (13 of 31); large cell, 17% (3 of 18); and small cell, 19% (3 of 16). In addition, 13% (3 of 23) of other cancers, 0% (0 of 24) of benign lung disease, and 2% (2 of 88) of normals were positive. Approximately one-third of Stage I patients in the squamous cell and adenocarcinoma groups were positive while two-thirds of patients with more advanced Stage III disease in these categories showed elevations.

INTRODUCTION

A number of reports have appeared concerning human lung tumor-associated antigens (1, 2, 4-10, 12, 13, 15) since the first report by Yachi et al. (14) in 1968. In most of these, the antigens were found to be shared by various histological types of lung tumors although Bell and Seetharam (1) detected an antigen present only on the plasma membranes of oat cell tumors. Our laboratory has been concerned with the isolation and biochemical characterization of human lung tumor-associated antigens, and we have recently reported on the purification and partial biochemical characterization of a protein isolated from a human lung tumor which appears to be human lung tumor associated (11). This conclusion was based on the observation that precipitin lines by Ouchterlony analysis could be demonstrated in 86% (84 out of 98) of soluble aqueous extracts of lung tumors of all histological types but not in extracts of 12 normal adult lungs, 12 normal tissues other than lung, and fetal lung tissue. Twelve of 13 extracts of non-lung tumors were also negative, as were normal sera.

In order to better define the distribution and occurrence of this protein, referred to here as LTA, we developed a RIA capable of detecting less than 10 ng of LTA. An initial screen of a small panel of lung cancer and normal sera using this RIA suggested generally higher levels of LTA in lung cancer sera relative to normal individuals (3). Using a modification of this RIA, which is reported here, we have now examined a larger and more extended panel of pathological and normal sera. These results form the basis of this report.

MATERIALS AND METHODS

All lung cancer serum specimens were kindly provided by Dr. J. Concannon, Allegheny General Hospital, Pittsburgh, Pa. These were pretreatment specimens from newly diagnosed patients with lung cancers of various histological types. Normal sera were obtained from the NIH Clinical Center Blood Bank. Serum samples from patients with benign lung disease and non-lung cancers were obtained from Dr. Vay L. W. Go, Mayo Clinic-NCI Serum Diagnostic Bank, Mayo Clinic, Rochester, Minn. The benign lung disease group included patients with pneumonitis, chronic lung abscess, empyema, bronchiectasis, broncholithiasis, inflammatory fibrosis, hamartoma, and necrotizing, caseating and cavitating granulomas. Non-lung cancers consisted of one ovarian carcinoma, 3 prostatic cancers, one carcinoma of the prostate and bladder, 3 cervical carcinomas, 12 sarcomas, and one uterine, one endometrial, and one breast carcinoma.

RIA. LTA was purified and radiolabeled as described previously (3). 125I-LTA was preadsorbed with Pansorbin (Calbiochem-Behring Corp., La Jolla, Calif.) as follows. A 10% Pansorbin suspension (0.5 ml) was mixed with 5 ml of PBS containing BSA (1 mg/ml) and then centrifuged 10 min at 3000 x g. The pellet (approximately 0.05 ml packed volume) was suspended with 3 ml of 125I-LTA (1.5 x 105 cpm) in PBS containing 30% glycerol. After 2 hr at 4°, the suspension was centrifuged 20 min at 12,000 x g, and the supernatant was filtered through glass wool. Typically, 8% of the radioactivity was removed by this procedure.

The RIA was performed in 1.5 ml polypropylene microcentrifuge tubes by the addition of the following reagents in sequence: (a) PBS containing BSA (1 mg/ml), sufficient amount such that the final volume was 0.5 ml; (b) either unlabeled LTA as standard displacer for construction of standard curve, 1 to 100 µl (3.2 to 320 ng LTA) of a highly purified preparation of LTA (Ref. 11, Pool 2), or 15 µl of serum (150 µl of a 1:10 dilution with PBS-BSA); (c) 50 µl of a 1:200 dilution (PBS-BSA) of R-201; and (d) 50 µl (15,000 to 30,000 cpm) of 125I-LTA. The mixture was incubated for 20 hr at 4°, after which 50 µl of Pansorbin (10% suspension) were added, and the incubation was continued another 2 hr at 4°. The suspension was centrifuged 2 min at 12,000 x g in a microcentrifuge, the supernatant was removed, and the 125I in the pellet was determined using a γ counter. The data were calculated using a log-log transformation of the binding data as described previously (3). A program was written in MLAB on the NIH DEC-System 10 computer for this purpose.

RESULTS

RIA Modification. The RIA previously in use consistently gave high nonspecific binding in the range of 7 to 10%, with a maximum binding of about 20% at an antibody final dilution of 1:10,000 (3). By increasing the antibody concentration to a final dilution of 1:2,000 and by preadsorbing the Bolton-Hunter-radiiodinated LTA with Pansorbin, the average nonspecific binding was reduced to 2.1 ± 0.4% (S.D.) while the
addition of varying amounts of purified, unlabeled LTA. Free and antibody-bound antigen were separated with Pansorbin. 

consistent as evidenced by the reproducibility of the slope and average maximum binding was increased to 43.9 ± 2.4% (Table 1). This form of the RIA proved to be remarkably average maximum binding was increased to 43.9 ± 2.4% (Table 1). This form of the RIA proved to be remarkably consistent as evidenced by the reproducibility of the slope and Y-intercept values obtained in 6 separate experiments. Preadsorption of the radiolabeled antigen with Pansorbin removed about 8% of the radioactivity which is thought to be immunoglobulin contamination acquired during the affinity immunoadsorption step of the purification procedure. In fact, sodium dodecyl sulfate:gel electrophoresis of the purified, radiolabeled LTA preparation indicated 2 minor peaks of radioactivity at M, 50,000 and M, 27,700, which probably represent immunoglobulin heavy and light chains (see Chart 9 and Ref. 3). Adsorption with Pansorbin preferentially reduced these 2 components while not significantly affecting the major antigen peak (data not shown).

The precision of the RIA was demonstrated by assaying 15 individual normal serum specimens on 3 separate occasions. The range of values obtained was 0.28 to 1.44 µg/ml. A fair degree of consistency was evident when low, medium, or high values obtained in different assays were compared. The average coefficient of variation for these 15 samples in 3 separate assays was 10%.

LTA Levels in Normal versus Pathological Sera. Using this RIA, we then examined a panel of 215 sera for LTA content. All patients' sera were preoperative, pretherapy specimens. Untreated sera from pretreatment patients and normal donors were assayed for LTA content using the RIA modified as described in the text. The mean ± S.D. of 88 normal sera was 0.92 ± 0.43 µg/ml. Two standard deviations above the mean, 1.78 µg/ml, was established as the cutoff, and all values greater than this were considered to be positive.

Two standard deviations above the mean, 1.78 µg/ml, was established as the cutoff, and all values greater than this were considered to be positive. Using this criterion, we determined the number of samples within each group which possessed elevated LTA values. Within the lung cancer group, the following positive rates were observed with: squamous cell carcinoma, 13 of 31 (42%); adenocarcinoma, 9 of 15 (60%); large-cell carcinoma, 3 of 18 (17%); and small-cell carcinoma, 3 of 16 (19%). It is clear that certain histological types (squamous cell and adenocarcinoma) show more frequent elevations than do others (large- and small-cell carcinomas). A more accurate assessment of these frequencies, however, must await analysis of a much larger panel of specimens within each group.

These data can also be analyzed in terms of the stage of the disease to see if LTA elevations are restricted to advanced disease or detectable at earlier times. Since serum LTA elevations occurred more frequently in patients with squamous cell and adenocarcinoma tumors, these patients were categorized in terms of the stage of their disease. Table 3 shows that, with squamous cell carcinoma, LTA-positive rates increased...
from 29% in Stage I to 58% in Stage III. Likewise, in the adenocarcinoma group, positive rates increase from 50% in Stage I to 80% in Stage III. Overall, the rates increased from 37% in Stage I to 65% in Stage III. Thus, although it is evident that more frequent LTA elevations are associated with more advanced disease, the fact that more than one-third of the early-disease, Stage I patients scored as positive is encouraging.

**DISCUSSION**

The modified version of the RIA for LTA reported here represents a significant improvement over the earlier form. The decrease in background to approximately 2% along with the concomitant increase in maximum binding to 44% provided a useful operating binding range which was highly reliable and reproducible. This was clearly evident from the results obtained between assays. In 6 separate assays, the slope and y-intercept of the standard curve were virtually superimposable. In addition, when assayed repeatedly, a small panel of sera demonstrated minimal interassay variation. Thus, the assay could be used with confidence to quantitate serum LTA levels for comparative purposes.

The reproducibility and reliability of the present assay can be contrasted with the former version, which had a much more narrow operating range of binding. Although the current assay and the first version gave qualitatively similar results, the quantitative aspects differed markedly. Thus, the previous normal serum LTA level of 17 ng/ml is considerably lower than the value of 0.92 µg/ml obtained here. Although not entirely clear, the principal reason for this is thought to be the uncertainty in the earlier quantitation due to a very narrow binding range. (The difference between background and maximum binding was approximately 1000 cpm.) In this version of the assay, 7000 to 9000 cpm separate background from maximum binding, providing a more reliable assay. The fact that many of the parameters of the RIA have been changed to produce the current version such as incubation volume, antibody dilution, LTA preparation used as displacer, Pansorbin-adsorbed label, etc., probably also contributes to a difference in the absolute levels. Nevertheless, the conclusions are the same, and the values reported here should be considered more accurate.

The most encouraging aspect of this study is the ability of the RIA to discriminate lung cancer patients from normal individuals and patients with benign lung or non-lung malignant disease. Although in this study patients with lung tumors of squamous cell and adenocarcinoma histologies demonstrated more frequent LTA elevations than did those with large or small cell carcinomas, the distribution of elevated LTA levels according to histological type must await verification with a much larger panel. In fact, this finding was somewhat of a surprise as LTA can be demonstrated uniformly in crude extracts of tumors of all 4 histological types. Why LTA from large or small cell tumors is not found as frequently in the serum is a matter of conjecture. It may be due to a more tightly membrane-associ- ated form in these tumors or, alternatively, there may be posttranslational modifications occurring which result in more rapid clearance from the circulation leading to lower serum levels. It would be useful from a clinical viewpoint if this issue could be clarified and the application of LTA as a marker extended to these 2 histological types as well.

The finding that 3 of 23 patients with non-lung cancers had elevated LTA levels is at least partly attributable to the arbitrarily selected cutoff value. Hopefully, the specificity of the assay can be improved by adjustments in this, as well as other, parameters. It is worth noting that the 3 elevations were found in patients with a cervical carcinoma, a prostatic carcinoma, and a chondrosarcoma and that these cancers were also represented in the group which was negative for LTA. Thus, the LTA elevations in the non-lung cancers did not show a clear association with a particular tumor type.

The lack of elevated LTA levels observed in preoperative patients with benign lung disease was particularly encouraging and suggests the possible utility of this assay in differential diagnosis.

The average value of LTA found in the sera of normal individuals was slightly less than 1 µg/ml. These data were not analyzed in terms of the age or sex of the donors, nor were smokers compared to nonsmokers in this study; this is a question which obviously requires attention. The inhibitory capacity of normal sera suggests a nonspecific protein effect in the binding assay or specific inhibition by some serum component. Studies are underway to determine if a relationship exists between LTA and a serum protein which would lead to interference in the RIA. Preliminary experiments suggest a weakly immunogenic relationship between LTA and a serum glycoprotein, which we have identified as AGP. We found that nearly 1000 times as much AP as LTA is required to give equivalent inhibition in the RIA. Since AGP is normally present at about 0.5 to 1.5 mg/ml in serum, this would appear as approximately 1 µg/ml of LTA in this assay. Furthermore, we found LTA and AGP to be physically distinct proteins, and compensation for this cross-reactivity may allow for the development of a more functional and clinically useful test for LTA. Studies along these lines are in progress.

**ACKNOWLEDGMENTS**

The authors are grateful to Dr. R. B. Herberman for helpful discussion and criticism.

**REFERENCES**

4. Frost, M. J., Rogers, G. T., and Bagshawe, K. D. Extraction and preliminary

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**Table 3**

LTA elevations in early versus advanced lung cancer

<table>
<thead>
<tr>
<th>Diagnosis histology</th>
<th>Stage</th>
<th>No. Positive/No. Tested</th>
<th>% positive</th>
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</thead>
<tbody>
<tr>
<td>Squamous</td>
<td>I</td>
<td>5/17</td>
<td>29</td>
</tr>
<tr>
<td>Squamous</td>
<td>II</td>
<td>7/12</td>
<td>58</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>I</td>
<td>5/10</td>
<td>50</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>II</td>
<td>4/5</td>
<td>80</td>
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Manuscript in preparation.


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