

# Detection of Tumor-associated Antigens in the Sera of Lung Cancer Patients by Three Monoclonal Antibodies<sup>1</sup>

Masaki Hirota, Kiyoyasu Fukushima, Paul I. Terasaki,<sup>2</sup> Glenn Y. Terashita, Jane Galton, and Masaaki Kawahara

Department of Surgery, School of Medicine, University of California at Los Angeles, Los Angeles, California 90024

## ABSTRACT

One hundred sixty-one sera from lung cancer patients, including 46 samples from patients who had not yet received treatment were screened for tumor-associated antigens with 3 monoclonal antibodies, CSLEX1, CSLEA1, and CLEX5, by a new cell binding inhibition assay. We had previously determined that the antigens recognized by CSLEX1 and CSLEA1 are sialosylated Lewis<sup>x</sup> and sialosylated Lewis<sup>a</sup>, respectively. Either of these two antibodies alone reacted with about 65% of the 46 untreated patients' sera. Eighty-seven % of the 46 showed positive results with at least one of the two antibodies.

The CLEX5 monoclonal antibody is presented here as recognizing a potential tumor-associated antigen. CLEX5 reacted with 54% of the 46 sera from nontreated lung cancer patients. When the results for all three antibodies were combined, the percentage of positive sera was 89% (of 46).

Some interesting patterns in the serum levels of the antigens detected by these antibodies were observed. Levels of sialosylated Lewis<sup>x</sup> were significantly higher in sera from nontreated advanced stage (III and IV) patients ( $P < 0.0003$ ). In addition, levels of the antigens detected by CSLEX1 and CSLEA1 were dependent on whether or not the patient had been receiving treatment. These observations suggest potential applications of monoclonal antibodies to diagnosis and monitoring of therapies.

## INTRODUCTION

The advent of monoclonal antibodies has given new hope for assays to determine tumor-associated antigens in the sera of cancer patients. Monoclonal antibodies which identify antigens in the sera of patients have been described previously (1-4).

Recently we have developed 3 monoclonal antibodies which detect antigens existing in cancer tissues as well as in the sera of cancer patients. One of the antibodies reacts with the sialosylated Le<sup>x</sup><sup>3</sup> determinant, a potential tumor-associated marker (5, 6) and was designated CSLEX1 (cytotoxic sialosylated Le<sup>x</sup>). The second antibody, CSLEA1 (7), is similar but not identical to NS-19-9 (8, 9) and reacts with sialosylated Le<sup>a</sup>, which is considered to be tumor associated (10). The third antibody, CLEX5, reacts with a nonsialosylated sugar residue. With the use of these 3 antibodies we show here that among patients with lung

cancer as many as 89% of sera drawn from patients before therapy are reactive.

## MATERIALS AND METHODS

**Monoclonal Antibodies.** The monoclonal antibodies used in this study were produced according to procedures described by Köhler and Milstein (11). The CSLEX1 antibody was IgM, the CSLEA1 was IgG, and the CLEX5 was IgM. The same extract of stomach cancer tissue was used as the immunogen in the production of both CSLEX1 and CLEX5. A mixture of 3 colon cancer cell lines was used as the immunizing agent for CSLEA1.

The epitopes of CSLEX1 and CSLEA1 are sialosylated Le<sup>x</sup> and sialosylated Le<sup>a</sup>, respectively, as previously reported (6, 7). The reaction pattern of CLEX5 is similar to that of anti-Le<sup>x</sup> antibody. Although its precise epitope has yet to be determined, CLEX5 reacts with a nonsialosylated sugar residue.

**Human Sera.** The serum panel used in this study consisted of sera from 161 unselected patients with lung cancers (74 adenocarcinoma, 48 squamous cell, 32 small cell, and 7 large cell carcinoma), 115 unselected patients with benign lung diseases (48 pulmonary tuberculosis, 16 chronic bronchitis, 13 sarcoidosis, 10 bronchial asthma, 9 acute pneumonia, 6 pulmonary emphysema, 65 bronchiectasis, 4 silicosis, and 4 fungus infections of the lung) and 120 unselected healthy blood donors. These sera were kindly provided by Nagasaki University Hospital (Nagasaki, Japan) and National Kinki Chuo Hospital (Osaka, Japan). Sera were stored at -80°C until used. The International Union Against Cancer classification system was used to categorize the patients into stages I-IV according to tumor size and metastasis to regional lymph nodes or other organs. Stage I corresponds to the earliest diagnosable disease state while stage IV indicates the most advanced state. One hundred forty-one of the 161 lung cancer sera were from advanced stage (III and IV) cases. Forty-six of the 161 lung cancer sera were from patients who had not had clinical therapy in the 6 mo prior to bleeding.

**Target Cell Lines.** HL60, an acute promyelocytic leukemia cell line, was the target cell for CSLEX1 in the cell-binding inhibition assay. This cell line was obtained from the American Type Culture Collection. M7609, a colon cancer cell line, was used as the target for both CSLEA1 and CLEX5. M7609 was kindly provided by Dr. M. Fukushima (Hiroaki University, Hiroaki, Japan). Both cell lines were maintained in RPMI 1640 medium supplemented with 15% fetal calf serum.

For the cell-binding inhibition assay, target cells were fixed overnight in 0.5% glutaraldehyde [in phosphate-buffered saline (0.01 M sodium phosphate: 0.15 M sodium chloride, pH 7.4)] at 4°C, washed, and resuspended in the buffer at a concentration of  $6-8 \times 10^6$ /ml.

**Cell-binding Inhibition Assay.** The cell-binding inhibition assay was performed as described in an earlier report (12). In brief, Terasaki microtest wells were coated with appropriately diluted antibody by incubating overnight at 4°C. After the trays were washed, 2-fold serial dilutions of serum in phosphate-buffered saline were added, followed by a 2-h incubation at room temperature. The trays were washed again, then target cells were added and incubated for 10 min at room temperature. Trays were then tilted at an angle of 60 degrees for 30 min. During this time, unbound cells fell to one side of the well. The trays were then laid flat for reading with an inverted phase contrast light microscope. The

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<sup>2</sup> To whom requests for reprints should be addressed at UCLA Tissue Typing Laboratory, 15-30 Rehabilitation Center, 1000 Veteran Avenue, Los Angeles, CA 90024.

<sup>3</sup> The abbreviations used are: Le<sup>x</sup>, Lewis<sup>x</sup>; Le<sup>a</sup>, Lewis<sup>a</sup>.

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Table 1  
Screening of a serum panel with 3 monoclonal antibodies by cell-binding inhibition assay

Type of sera	No. of sera tested	No. of positive samples			Combined results <sup>a</sup>
		CSLEX1	CSLEA1	CLEX5	
Adenocarcinoma	74	34 (45.9) <sup>b</sup>	27 (36.5)	33 (44.6)	54 (77.0)
Squamous cell carcinoma	48	16 (33.3)	19 (40.0)	27 (56.3)	37 (77.1)
Small cell carcinoma	32	9 (28.1)	16 (50.0)	17 (53.1)	24 (75.0)
Large cell carcinoma	7	4 (57.1)	3 (42.9)	4 (57.1)	6 (85.7)
Lung cancer total <sup>c</sup>	161	63 (39.1)	65 (40.4)	81 (50.3)	121 (75.2)
Benign lung diseases	115	0 (0.0)	0 (0.0)	19 (16.5)	19 (16.5)
Healthy control	120	2 (1.7)	0 (0.0)	1 (0.6)	3 (2.5)

<sup>a</sup> Samples positive with at least one antibody.

<sup>b</sup> Numbers in parentheses, percentage.

<sup>c</sup> Lung cancer groups include sera from both treated and nontreated patients.

results were scored according to the percentage of the well surface covered by bound cells as follows: 0–10% coverage, score 1; 10–30% 2; 30–60%, 4; 60–80%, 6; and 80–100%, 8. The reported titers of inhibition show the titer of serum that inhibited the cell binding as indicated by a score of 1 or 2.

RESULTS

For CSLEX1 and CLEX5, sera with cell-binding inhibition titers of 1:16 and higher were considered positive. Two of 120 normal sera were positive with CSLEX1 (Table 1), with titers of 1:16 and 1:32. One of the 120 normal sera was positive with CLEX5, showing a titer of 1:32. For CSLEA1, sera with inhibition titers of 1:8 and higher were considered positive. None of the normal sera was positive with CSLEA1. Neither CSLEX1 nor CSLEA1 were inhibited by any of the 115 sera from patients with benign lung diseases. However, CLEX5 reacted with 19 of 115 sera (16.5%) including 8 of 48 pulmonary tuberculosis, 4 of 16 chronic bronchitis, 3 of 9 acute pneumonia, 2 of 6 pulmonary emphysema, and 2 of 5 bronchiectasis sera.

All normal and benign disease sera not mentioned above either showed no inhibition or had titers of inhibition below the indicated cutoff levels. Depending on the antibody, between 75 and 97% of the normal and benign disease sera showed no inhibition.

Each of the 3 monoclonal antibodies described here reacted with approximately 40–50% of the 161 sera from lung cancer patients, with 75.2% of these sera reacting with at least one of the 3 antibodies. Gross differences between the reactivities of the 4 subgroups of lung cancer sera were not observed with any of the antibodies (Table 1).

Lung cancer patients were classified as either treated or nontreated. Treatments included chemotherapy, radiation therapy, and/or operations. The nontreated group included patients showing evidence of tumor loading who had not been treated in the 6 mo prior to bleeding. There were a total of 46 samples in the nontreated group. As shown in Chart 1, CSLEX1 alone reacted with 67.4% of 46 sera, including 83.3% of 24 sera in the lung adenocarcinoma subgroup and 46.7% of squamous cell carcinoma sera. CSLEA1 alone reacted with 63.0% of 46 sera, including 66.7% of adenocarcinoma sera and 53.3% of squamous cell carcinoma sera. Eighty-seven % of 46 sera reacted with at least one of these 2 antibodies, including 96% of adenocarcinoma sera, and 73% of squamous cell carcinoma sera. Eighty-nine % of 46 sera reacted with at least one of the 3 antibodies.

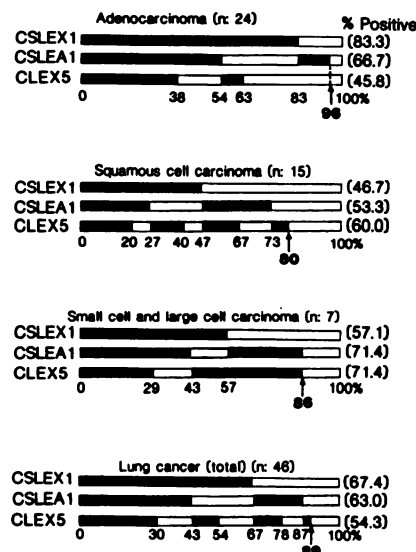


Chart 1. Reaction patterns of 46 sera from nontreated lung cancer patients tested by cell-binding inhibition with 3 monoclonal antibodies. ■, positive reactions. Results are summarized as percentages of positive samples, with **boldface numbers** indicating the total percentages of sera in each group reacting with at least one of the antibodies.

The lung cancer patients in the nontreated group were divided into 2 subgroups according to the clinical stage of disease. As shown in Chart 2, the percentages of positive sera in stages III and IV were higher than those for the nonadvanced stages. However, only the results for CSLEX1 showed a statistically significant difference ( $P < 0.0003$ , Wilcoxon paired rank sum test).

The results for all of the 161 lung cancer sera were summarized according to the treatment status (treated or nontreated) of the patients and shown in Chart 3. Statistically significant ( $P < 0.002$ ) differences between treated and nontreated groups were observed for CSLEX1 and CSLEA1 but not for CLEX5.

DISCUSSION

With the use of 2 primary monoclonal antibodies, CSLEX1 and CSLEA1 and a supplementary antibody, CLEX5, potential tumor-associated antigens were detected in as many as 89% of sera from lung cancer patients before treatment. From this and related findings, we wish to draw 3 conclusions on the serological characteristics of tumor-associated antigens.

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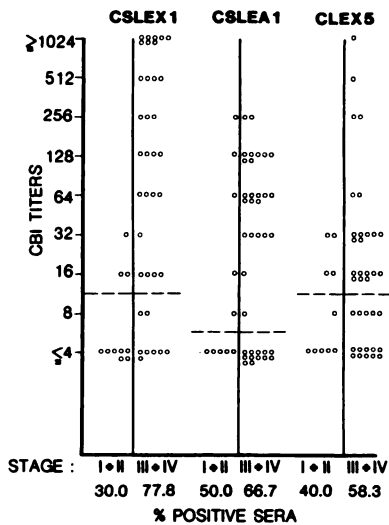


Chart 2. Relationship between the stage of lung cancer and the cell-binding inhibition (CBI) titers of 46 sera from nontreated patients tested with 3 monoclonal antibodies. ---, cutoff values for each antibody. For CSLEX1 and CLEX5, sera with inhibition titers of 1:16 and higher were considered positive. For CSLEA1, sera with inhibition titers of 1:8 or higher were considered positive.

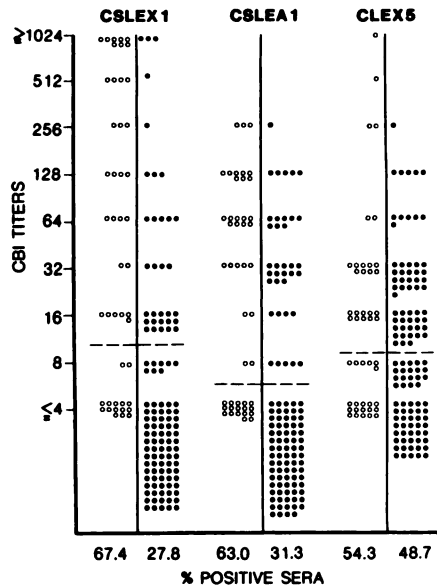


Chart 3. Effect of clinical treatment on cell-binding inhibition (CBI) titers for sera from 46 patients who had not had treatment (O) and 115 patients who had been receiving treatment (●). ---, cutoff values for each antibody. For CSLEX1 and CLEX5, sera with inhibition titers of 1:16 and higher were considered positive. For CSLEA1, sera with inhibition titers of 1:8 and higher were considered positive.

With a combination of monoclonal antibodies, the ability to detect tumor-associated antigens is increased. For example, in the cases of the sialosylated forms of Le<sup>a</sup> and Le<sup>x</sup>, some cancer sera contain both antigens whereas others contain only one of the 2 antigens. When the 3 antibodies are used in combination, as many as 89% of the lung cancer sera from nontreated patients show a positive reaction. Also, the levels of sialosylated Le<sup>x</sup> in serum are associated with the type of lung cancer, with significantly higher titers observed in the lung adenocarcinomas.

In addition with regard to the relationship between the stage of disease and antigen levels, nontreated patients with more advanced cancers have significantly higher inhibition titers with

CSLEX1. It appears that the serum level of sialosylated Le<sup>x</sup> is related to tumor burden. Though somewhat higher positive percentages of advanced stage sera were also observed with both CSLEA1 and CLEX5, the increases were not as striking as with CSLEX1.

Furthermore serum levels of the tumor-associated antigens recognized by CSLEX1 and CSLEA1 seem to be sensitive to whether or not the patient had received treatment. Though we have yet to complete our testing of serum samples from individual patients at timed intervals, it appears from these early studies that therapy decreases the serum levels of these tumor-associated antigens. It would thus be likely that the effectiveness of therapy could be monitored by determining the serum levels of these antigens.

Information of the secretor status of the patients was not available for this study. We are currently studying the relationship between the secretor status of test subjects and their serum antigen levels, since these antigens are blood group associated.

Although the cell-binding inhibition assay was utilized here, the radioimmuno-sandwich assay has been shown to yield similar results (13). More so than the method, specificity of the monoclonal antibody is critical. For example, although each of the 3 antibodies used here individually reacted with between 54 and 67% of lung cancer sera overall, the antibodies often did not react with sera of the same patients. The CLEX5 antibody had the highest false-positive rate, reacting with 17% of the sera from patients with benign lung diseases, whereas CSLEX1 and CSLEA1 produced false-positive reactions in none of the sera from the benign group. Unlike carcinoembryonic antigen which lacks the specificity needed for cancer diagnosis (14), the sialosylated forms of Le<sup>a</sup> and Le<sup>x</sup> as detected by CSLEA1 and CSLEX1 may become valuable in distinguishing between benign and malignant lung diseases.

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