Jpn. J. Cancer Res. 85, 211–219, February 1994

A New Serum Tumor Marker, CAM 123-6, Highly Specific to Pulmonary Adenocarcinoma

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KL-6, a circulating mucin-like glycoprotein, is a pulmonary adenocarcinoma-associated antigen and is also regarded as an indicator of disease activity of interstitial pneumonitis. KL-6 has extensive heterogeneous antigenic determinants and consists of multiple heterogeneous antigen molecules. We have searched for circulating KL-6-associated glycoproteins with superior diagnostic value to KL-6 as a tumor marker for pulmonary adenocarcinoma. A new murine monoclonal antibody EH-123 reacting with an asialosugar chain on KL-6 was established. A new KL-6-associated molecule detected by a bimonoclonal bideterminant sandwich assay using the EH-123 antibody as a catcher and horseradish peroxidase-labeled KL-6 as a tracer was designated as CAM 123-6. In 59% (22 of 37) of patients with pulmonary adenocarcinoma, serum levels of CAM 123-6 were abnormally elevated and the positive rate increased with the progression of clinical stage. Elevated levels were not detected in normal individuals or in patients with benign lung diseases, other histologic types of lung cancer, gastric cancer, colon cancer or breast cancer. CAM 123-6 was more specific to pulmonary adenocarcinoma than carcinoembryonic antigen (CEA), but the sensitivity of CAM 123-6 for pulmonary adenocarcinoma was similar to that of CEA. CAM 123-6 is a promising candidate as a serum tumor marker for pulmonary adenocarcinoma.

Key words: Tumor marker — Cancer-associated mucin — CAM 123-6 — EH-123 antibody — KL-6 antibody

Many tumor-associated mucin-like glycoproteins, such as CA 19-9,[1] CA 72-4[2] and MUSE11,[3] have been reported to be useful for serological diagnosis of malignancies and to contribute to the adherent properties of cancer cells.[4, 5] We also reported circulating antigens, such as KL-3,[6] KL-6,[6] and CAM-14.[7] KL-6 was discovered as a circulating and pulmonary adenocarcinoma-associated mucin-like glycoprotein and classified as Cluster 9 (MUC1) of lung tumor and differentiation antigens on the basis of immunohistochemical and flow cytometric findings (The 3rd International IASLC Workshop on Lung Tumor and Differentiation Antigens, September 9-11, Zurich, Switzerland). However, serum KL-6 is abnormally elevated in sera from patients with active diffuse lung diseases.[8–11] The antigenicity of mucins is heterogeneous. It may be due to differences of either mucin gene expression or core protein glycosylation, or both.[12, 13] We supposed that KL-6 produced by cancer cells might have epitopes different from that derived from nonmalignant epithelial cells in diffuse lung diseases. We therefore searched for circulating KL-6-associated mucins which are more specific to pulmonary adenocarcinoma than the original KL-6. LISA 1-6, which is one of the KL-6-associated mucins, has higher sensitivity and specificity to pulmonary adenocarcinoma than KL-6.[14] However, LISA 1-6 is increased in sera from patients with pulmonary squamous cell carcinoma, pulmonary small cell carcinoma or benign lung diseases. Its sensitivity and specificity to pulmonary adenocarcinoma are not satisfactory.

We have now found a new KL-6-associated mucin which is more specific to pulmonary adenocarcinoma than either KL-6 or LISA 1-6. In this paper, we describe the new cancer-associated antigen, CAM 123-6, which is highly specific to pulmonary adenocarcinoma, and we discuss the possible clinical usefulness of CAM 123-6.

MATERIALS AND METHODS

Hybridoma selection A total of 1.5×10^7 cells of an adenocarcinoma cell line YMB-S, which expresses high levels of KL-6 and is a subclone of YMB-1 cells [Japanese Cancer Research Resources Bank (JCRB) #0823],[15] was immunized into a BALB/c mouse. Hybridomas were produced by fusing splenocytes from the mouse and P3-NS-1-Ag4/1 (NS-1) murine myeloma cells by the method of Köhler and Milstein.[16] Culture supernatants from uncloned hybridomas were examined for their reactivities to epitopes on the pulmonary adenocarcinoma-associated antigen KL-6[6] by means of reversed indirect enzyme-linked immunosorbent assay (RI-ELISA).[14, 17] In RI-ELISA, two sets of specimens were used as antigens: pleural effusions which were pooled from 7 patients with pulmonary adenocarcinoma (cancer effusion) or 6 patients with tuberculous pleurisy (tuberculous effusion).

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RI-ELISA RI-ELISA was performed according to the procedures described previously.[14, 17] In brief, each well of a 96-well Microtest plate for enzyme immunoassay (EIA) was sensitized with 100 μl of 10 μg/ml of goat anti-mouse immunoglobulin antibodies (Cappel Lab., Cochranville, PA, USA) at room temperature for 1 h. The plate was washed with Dulbecco's phosphate-buffered saline (DPBS) (0.01 mol/liter phosphate-0.14 mol/liter NaCl, pH 7.4) containing 0.05% Tween 20 and 0.1% bovine serum albumin (BSA), then 100 μl per well of hybridoma culture supernatant was added and the plate was incubated at room temperature for 1 h. The wells were washed again and 100 μl of antigen specimen diluted 5-fold with mouse serum buffer [1% normal mouse serum (Cedarlane Lab., Ontario, Canada)-10% fetal calf serum (FCS)-DPBS] was added and incubated overnight at 37°C. The plate was washed, and 100 μl of horseradish peroxidase (HRP)-labeled KL-6 (IgG1) (HRP-KL-6) antibody diluted 5,000-fold with mouse serum buffer was added to each well and incubated at 37°C for 3 h. The wells were washed, then 100 μl of OPDA solution (0.3% *o*-phenylenediamine dihydrochloride-0.02% H_2O_2-0.15 mol/liter citrate-phosphate buffer, pH 4.9) was added and allowed to react for 30 min. The reaction was stopped by adding 100 μl of 2 mol/liter HCl, and the intensity of color (A_{492}) was determined. Hybridoma clones whose culture supernatant reacted with the cancer effusion more strongly than the tuberculous effusion were selected for cloning. Finally, EH-123 monoclonal antibody (IgM) was chosen for further evaluation. Antibody was purified from ascites produced in BALB/c mice by means of gel filtration with TSK Gel HW 55S (Toyo Soda Ltd., Ube, Yamaguchi) and ion-exchange chromatography with DEAE Sepharose CL6B (Pharmacia, Uppsala, Sweden) as described elsewhere.[18]

Conjugation of monoclonal antibody with HRP KL-6 antibody was conjugated with HRP using the method of Nakane and Kawaoi,[19] as previously reported.[6]

Quantification of CAM 123-6 A soluble tumor-associated antigen which was detected by a sandwich assay using EH-123 antibody as a catcher and KL-6 antibody as a tracer was designated as CAM 123-6. In brief, a 96-well Microtest plate for EIA was sensitized with 100 μl of 10 μg/ml of purified EH-123 antibody at room temperature for 2 h. The plate was washed with PBS (0.05 mol/liter phosphate-0.1 mol/liter NaCl, pH 6.5) containing 0.05% Tween 20 and 0.1% BSA, then 100 μl of antigen specimen diluted 10-fold with dilution buffer (0.1% normal mouse serum-10% FCS-PBS) was added to each well and incubated overnight at room temperature. The wells were washed and 100 μl of HRP-KL-6 antibody diluted 300-fold with dilution buffer was added and incubated at room temperature for 3 h. The plate was again washed, and 100 μl of OPDA solution was added and allowed to react for 30 min. The reaction was stopped by adding 100 μl of 2 mol/liter HCl and the absorbance (A_{492}) was measured.

For quantification of CAM 123-6, a pleural effusion from a patient with pulmonary adenocarcinoma was used as a standard reference sample. This reference pleural effusion contained 15,600 U/ml of both CAM 123-6 and KL-6.

Quantification of KL-6 Serum levels of KL-6 were measured with a sandwich-type ELISA using immobilized KL-6 antibody and HRP-KL-6 antibody, as previously reported.[6]

Quantification of carcinoembryonic antigen Carcinoembryonic antigen (CEA) was measured using a radioimmunoassay kit available commercially (CEA RIA BEAD, Dainabot Co., Tokyo).

Clinical materials Sera and pleural effusions from patients with several types of malignancies and benign lung diseases, and sera from healthy individuals were provided by several hospitals. The patients were diagnosed on the basis of pathological and clinical examinations. The healthy individuals had no remarkable past clinical history and showed no abnormality in follow-up examinations, including analysis of peripheral blood cells, liver function test, renal function test, urinalysis, fecal examination and upper gastrointestinal tract examination. The diagnosis of malignant effusion was made on the basis of cytopathologic detection of malignant cells in the effusion. The diagnosis of tuberculous effusion was made on the basis of either the detection of *Mycobacterium tuberculosis* in the pleural fluid or the findings from a histologic pleural biopsy specimen.

Biochemical properties of antigens The sensitivity of epitopes recognized by EH-123 antibody (EH-123 epitopes) to sodium metaperiodate (Wako Pure Chemical Ind., Osaka), neuraminidase from *Vibrio cholerae* (Boehringer Mannheim GmbH, Mannheim, Germany), pronase from *Streptomyces grisens* (Calbiochem Corp., La Jolla, CA, USA) and trypsin (Flow Lab. Inc., North Ryde, NSW, Australia) was determined by measuring the change of binding of EH-123 antibody to the target antigen. A malignant pleural effusion dot-blotted on a nitrocellulose membranes (Bio-Rad Lab., Richmond, CA, USA) was treated with sodium metaperiodate or neuraminidase at various concentrations at 37°C for 30 min. The membranes were washed with DPBS containing 0.05% Tween 20 and 0.1% BSA, and incubated with DPBS containing 2% BSA at room temperature for 2 h. Then they were stained with EH-123 and KL-6 antibodies by the indirect immunoperoxidase method (avidin-biotin-peroxidase complex method) using Vectastain mouse IgM and IgG kits, respectively (Vector Lab., Burlingame, NJ, USA), as previously reported.[20] Non-fixed YMB-S adenocarcinoma cells, which strongly

express both EH-123 epitopes and epitopes recognized by KL-6 antibody (KL-6 epitopes), were treated with 0.25% pronase or 0.25% trypsin, and stained by means of the indirect immunofluorescence method as reported previously.[21]

The molecular weight of the soluble antigen was measured by sodium dodecyl sulfate-4% polyacrylamide gel electrophoresis (SDS-PAGE), Western blotting and immunostaining, as described elsewhere.[22] The antigen was obtained from pleural effusion from a patient with pulmonary adenocarcinoma.

The molecular weights of CAM 123-6 and KL-6 were determined by molecular sieving. A malignant pleural effusion from a patient with pulmonary adenocarcinoma was fractionated by gel filtration with TSK Gel HW 55S. Mouse monoclonal IgM (Mr 900,000), thyroglobulin (669,000) and BSA (67,000) were used as standard markers. The CAM 123-6 and KL-6 levels in the eluate fractions were measured by using the sandwich-type ELISA mentioned above.

Reactivity with tissue sections Formalin-fixed and paraffin-embedded tissue sections from various cancers and normal tissues were stained with EH-123 and KL-6 antibodies by the indirect immunoperoxidase method (avidin-biotin-peroxidase complex method) using Vectastain mouse IgM and IgG kits, respectively.[20]

RESULTS

Serum levels of CAM 123-6 and KL-6 Serum levels of CAM 123-6 are shown in Fig. 1. The level was 0.8 ± 0.4 U/ml (mean ± SD) in 40 normal individuals. A cut-off level of 2.1 U/ml was determined, because all of the normal individuals were negative at that level. Fifty-nine percent of patients with pulmonary adenocarcinoma (22 of 37) were positive. However, the patients with pulmonary squamous cell carcinoma or small cell carcinoma were all negative. With respect to the clinical stage of pulmonary adenocarcinoma, the positive rate of CAM 123-6 was 22% (2 of 9) for stages I and II, 64% (9 of 14) for stage III and 79% (11 of 14) for stage IV. However, all the patients with gastric cancer (n=10), colon cancer (n=4), or breast cancer (n=13) were negative. The positive rate was 20% (2 of 10) of patients with hepatocellular cancer and 25% (2 of 8) of patients with pancreatic cancer or cholangiocarcinoma. Though abnormally high levels of KL-6 were observed in 64% (18 of 28) of patients with benign lung diseases including interstitial pneumonitis, sarcoidosis, diffuse panbronchiolitis and pulmonary tuberculosis, none of them showed abnormally elevated levels of CAM 123-6. In pulmonary adenocarcinoma, CAM 123-6 and KL-6 were both positive in 27% (10 of 37) and both negative in 32% (12 of 37).

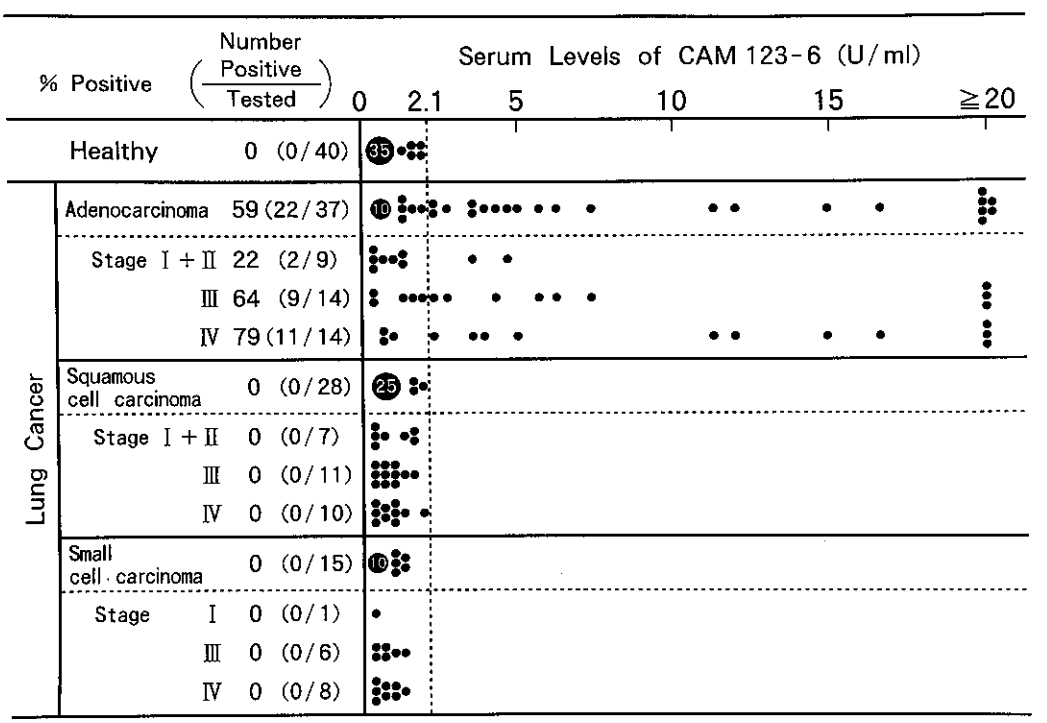

Fig. 1. CAM 123-6 levels in sera from patients with lung cancer and healthy controls.

Twelve patients showed positive CAM 123-6 and negative KL-6, and 3 patients positive KL-6 and negative CAM 123-6 (Fig. 2).

Combination of CAM 123-6 and CEA Serum levels of CAM 123-6 and CEA were compared in the same samples from 27 patients with pulmonary adenocarcinoma. CAM 123-6 and CEA were both positive in 41% (11 of 27) and both negative in 22% (6 of 27). Five patients showed positive CAM 123-6 and negative CEA, and 5 patients positive CEA and negative CAM 123-6.

CAM 123-6 levels in pleural effusions CAM 123-6 levels in pleural effusions were examined. A cut-off level of 11.0 U/ml was determined, where all of the tuberculous effusions were negative. Abnormally high levels of CAM 123-6 were observed in 80% (20 of 25) of patients with pulmonary adenocarcinoma (Fig. 3).

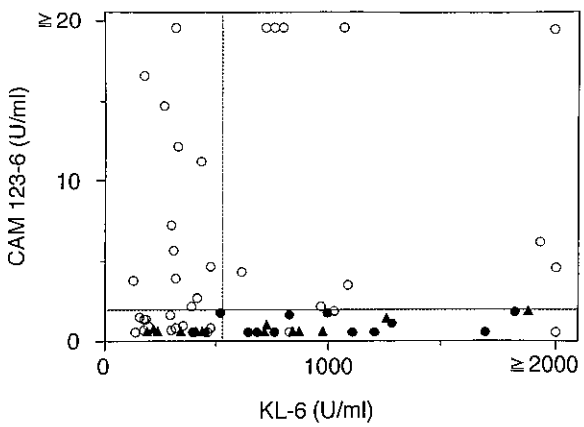

Fig. 2. Correlation between serum levels of CAM 123-6 and KL-6 in patients with pulmonary adenocarcinoma, pulmonary tuberculosis and diffuse lung diseases. Open circles, pulmonary adenocarcinoma (37 cases); closed circles, pulmonary tuberculosis (13 cases); closed triangles, diffuse lung diseases (15 cases).

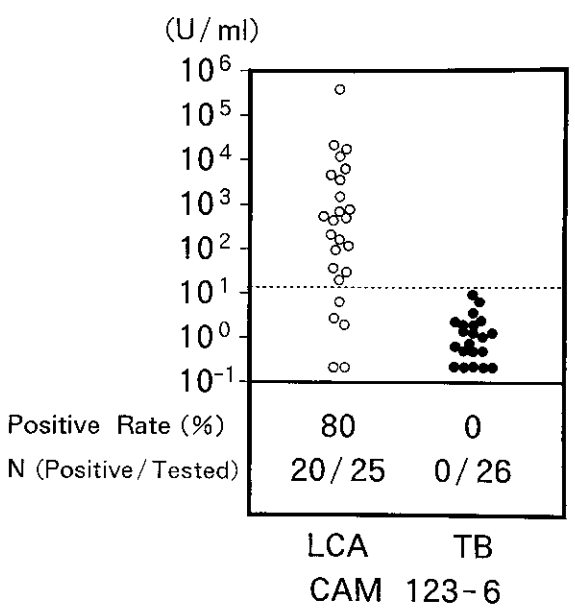

Fig. 3. CAM 123-6 levels in pleural effusions from patients with pulmonary adenocarcinoma and tuberculous pleurisy.

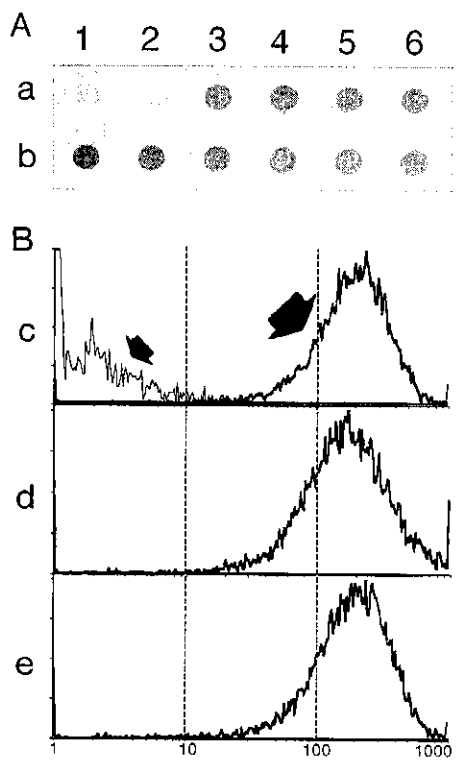

Fig. 4. Biochemical properties of EH-123 epitope. (A) Analysis of the binding of EH-123 antibody to a pleural effusion dot-blotted on a nitrocellulose membrane, which was treated with sodium metaperiodate (line a) and neuraminidase (line b). a-1, 100 mmol/liter; a-2, 10 mmol/liter; a-3, 1 mmol/liter; a-4, 100 μmol/liter; a-5, 10 μmol/liter; a-6, control. b-1, 100 mU/ml; b-2, 10 mU/ml; b-3, 1 mU/ml; b-4, 100 μU/ml; b-5, 10 μU/ml; b-6, control. The binding of EH-123 antibody to the antigen treated with 10 mmol/liter or more of sodium metaperiodate decreased, while the binding of EH-123 antibody to the antigen treated with a high concentration of neuraminidase did not decrease. (B) Flow cytometric analysis of the binding of EH-123 antibody to non-fixed YMB-S cells treated with 0.25% pronase (d) or 0.25% trypsin (e). The large arrow indicates the histogram of nontreated cells stained by EH-123 antibody and the small arrow that of nontreated cells stained by culture supernatant of NS-1 cells. The fluorescence of the cells treated with 0.25% pronase was slightly weaker than that of nontreated cells, while that of the cells treated with 0.25% trypsin was as strong as that of nontreated cells.

Biochemical properties of the antigens EH-123 epitope was sensitive to sodium metaperiodate and weakly sensitive to pronase, but was resistant to neuraminidase and trypsin (Fig. 4). KL-6 epitope was sensitive to sodium metaperiodate and neuraminidase and weakly sensitive to pronase, but was resistant to trypsin, in accordance with the results described previously.[6] By SDS-PAGE, Western blotting and immunostaining, the soluble antigen recognized with EH-123 antibody was shown to be polydisperse, with molecular weights in the range between 200 kDa and 900 kDa. On molecular sieving, CAM 123-6 in a malignant pleural effusion showed two peaks at fraction numbers 63 and 66, while KL-6 showed one peak at fraction number 68 (Fig. 5). Mouse IgM (Mr 900,000), thyroglobulin (669,000) and BSA (67,000) were eluted at fraction numbers 77, 79 and 96, respectively.

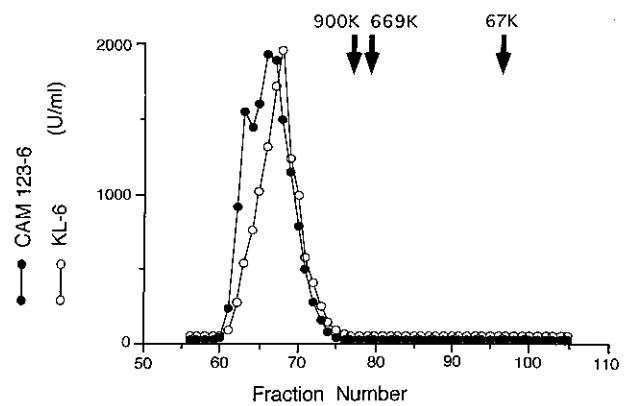

Fig. 5. A pleural effusion from a pulmonary adenocarcinoma patient was gel-filtered on TSK gel HW 55S. The peaks of molecular weight standards, mouse IgM (Mr 900,000), thyroglobulin (669,000) and BSA (67,000) were observed at fraction numbers 77, 79 and 96, respectively. CAM 123-6 exhibited two peaks at fraction numbers 63 and 66, while KL-6 showed one peak at fraction number 68.

Table I. Reactivity of Monoclonal Antibodies with Malignant Tissues

Tissues	Monoclonal antibodies	
	EH-123	KL-6
Lung		
Adenocarcinoma	+～++ [a](5/5)[b]	++～+++ (5/5)
Squamous cell carcinoma	− (0/5)	+～+++ (5/5)
Small cell carcinoma	− (0/2)	− (0/2)
Pancreatic cancer	+ (2/3)	+++ (3/3)
Breast cancer	++ (3/3)	+++ (3/3)
Gastric cancer	+～+++ (3/3)	++ (2/3)
Colorectal cancer	+～++ (3/3)	+ (2/3)
Hepatocellular cancer	++ (1/2)	++ (1/2)

a) Positive reactivity was scored based on the rate of positively stained cells as follows: +, <10%; ++, 10–75%; +++, >75%.
b) Positive number/tested number.

Reactivity with tissue sections The reactivities of monoclonal antibodies EH-123 and KL-6 with tissue sections are shown in Tables I and II. EH-123 antibody reacted with all tissue sections from 5 patients with pulmonary adenocarcinoma (Fig. 6A), but did not react with tissue sections from 5 patients with squamous cell carcinoma (Fig. 6C). KL-6 antibody reacted strongly with all tissue sections from 5 patients with pulmonary adenocarcinoma (Fig. 6B) and 5 patients with squamous cell carcinoma (Fig. 6D). EH-123 epitope was negative on pneumocytes (Fig. 6E), but was positive on mucous cells of the bronchial gland (Fig. 6G). KL-6 epitope was positive on pneumocytes (Fig. 6F) and serous cells of the bronchial gland (Fig. 6H). A difference of the distribution between the two epitopes was also observed in other normal

Table II. Reactivity of Monoclonal Antibodies with Normal Tissues and Cells

Tissues	Monoclonal antibodies	
	EH-123	KL-6
Lung		
Pneumocyte	−[a] (0/4)[b]	+++ (4/4)
Bronchiole	− (0/3)	++～+++ (3/3)
Bronchus	− (0/2)	− (0/2)
Bronchial gland		
Serous cell	− (0/2)	+++ (2/2)
Mucous cell	+～++ (2/2)	− (0/2)
Thyroid		
Follicular cell	− (0/1)	++ (1/1)
Pancreas		
Acinus cell	+++ (1/1)	− (0/1)
Duct	− (0/1)	+++ (1/1)
Islet cell	− (0/1)	− (0/1)
Breast		
Ductal epithelium	+ (1/1)	+++ (1/1)
Lobular epithelium	+ (1/1)	+++ (1/1)
Stomach		
Surface mucous cell	− (0/1)	− (0/1)
Gland	+++ (1/1)	− (0/1)
Colon		
Epithelium	− (0/2)	− (0/2)
Liver		
Hepatocyte	− (0/1)	− (0/1)
Bile duct	− (0/1)	+++ (1/1)

a) Positive reactivity was scored based on the rate of positively stained cells as follows: +, <10%; ++, 10–75%; +++, >75%.
b) Positive number/tested number.

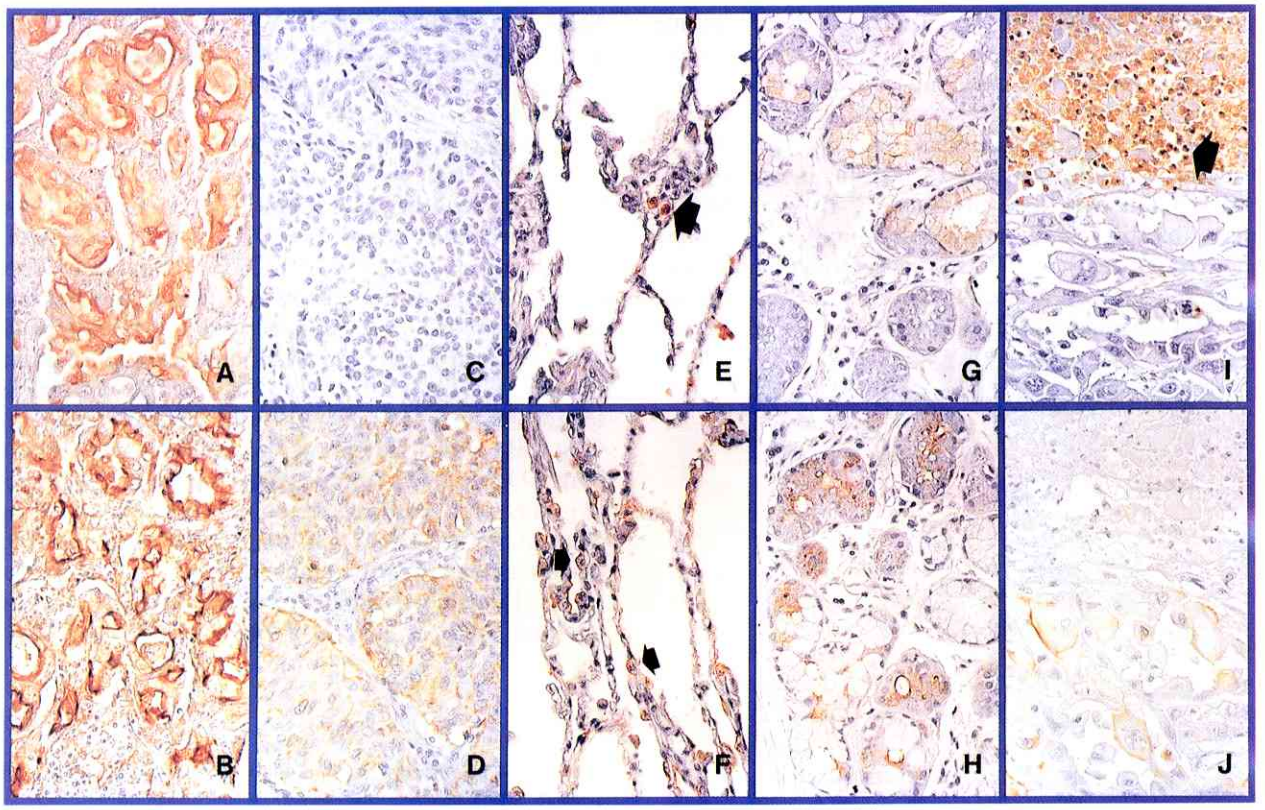

Fig. 6. Formalin-fixed and paraffin-embedded tissue sections were stained with EH-123 antibody (A, C, E, G, I) and KL-6 antibody (B, D, F, H, J) by an avidin-biotin-peroxidase complex method. A and B, well-differentiated pulmonary adenocarcinoma; C and D, well-differentiated squamous cell carcinoma; E and F, normal peripheral lung; G and H, bronchial glands; I and J, poorly differentiated squamous cell carcinoma with infiltrated inflammatory cells. E and I, EH-123 antibody reacted with granulocytes (large arrow). F, KL-6 antibody reacted with type II pneumocytes (small arrow).

tissues such as thyroid follicular cells, pancreatic ductal and acinus cells, hepatic bile-ductal cells and blood cells. It is interesting that EH-123 epitope was expressed in leukocytes such as granulocytes and monocytes, as confirmed by using immunofluorescence staining and flow cytometry (Fig. 6I). EH-123 antibody stained 96% of granulocytes, 77% of monocytes and less than 5% of lymphocytes, while KL-6 antibody did not react with such leukocytes.

DISCUSSION

The soluble cancer-associated antigen CAM 123-6 showed a high diagnostic value for pulmonary adenocarcinoma. The sensitivity was 59% (22 of 37), the specificity was 97% (152 of 156) and the diagnostic accuracy was 90% (174 of 193) for pulmonary adenocarcinoma. The positive rate of CAM 123-6 in pulmonary adenocarcinoma increased with progression of clinical stage. None of the patients with other histologic types (squamous cell carcinoma and small cell carcinoma) of lung cancer showed an elevated serum level of CAM 123-6. CEA,[23] SLX,[24] NCC-ST-439[25] have been reported to be useful for serological diagnosis of pulmonary adenocarcinoma. However, abnormally high levels of these markers have also been observed in patients with pulmonary squamous cell carcinoma and small cell carcinoma.[26–28] Elevated serum levels of KL-6 and LISA 1-6 have been observed in 18% (4 of 22) and 37% (11 of 30) of patients with pulmonary squamous cell carcinoma and 8% (1 of 13) and 50% (10 of 20) of patients with small cell carcinoma,[6, 14] respectively. Thus, CAM 123-6 may be useful for discriminating pulmonary adenocarcinoma from other histologic types of lung cancers and evaluating the advanced clinical stage in patients with pulmonary adenocarcinoma.

High levels of false-positive rate in benign lung diseases such as diffuse lung diseases and pulmonary tuberculosis have been reported with some tumor markers for pulmonary adenocarcinoma such as CEA and SLX.[29, 30]

Fifty percent (9 of 18) of patients with interstitial pneumonitis and 14% (18 of 130) of patients with pulmonary tuberculosis showed abnormally high levels of CEA.[29] Twenty-two percent (4 of 18) and 49% (20 of 41) of patients with interstitial pneumonitis and 4% (5 of 130) of patients with pulmonary tuberculosis showed abnormally elevated levels of SLX.[29, 30] Moreover, abnormally high levels of KL-6 and LISA 1-6 were observed in 21% (12 of 57) and 6% (2 of 31) of patients with benign lung diseases,[6, 8, 14] respectively. In this study, though elevated serum levels of KL-6 were observed in 64% (18 of 28) of patients with benign lung diseases including diffuse lung diseases and pulmonary tuberculosis, none of them showed abnormally elevated levels of CAM 123-6. In patients with pulmonary adenocarcinoma, however, the positive rate of CAM 123-6 was higher than that of KL-6. CAM 123-6 is better able to discriminate pulmonary adenocarcinoma from benign lung diseases.

In other malignancies, elevated serum levels of CAM 123-6 were observed only in 20% (2 of 10) of patients with hepatocellular cancer and 25% (2 of 8) of patients with pancreatic cancer or cholangiocarcinoma. We reported that serum levels of KL-6 were elevated in 52% (17 of 33) of patients with pulmonary adenocarcinoma, 44% (4 of 9) of patients with pancreatic cancer, and 40% (8 of 20) of patients with breast cancer.[6] The positive rate of LISA 1-6 was observed in 92% (11 of 12) of patients with pancreatic cancer and 63% (25 of 40) of patients with pulmonary adenocarcinoma.[14] These results suggest that CAM 123-6 is more specific to pulmonary adenocarcinoma than either KL-6 or LISA 1-6. Abnormally high levels of tumor markers such as CEA,[23] SLX,[24] NCC-ST-439[25] have been frequently observed in patients with adenocarcinoma of other many origins.[27, 31, 32] CAM 123-6 may be helpful for discriminating pulmonary adenocarcinoma from adenocarcinomas derived from various other organs. Our results suggest that CAM 123-6 is more specific to pulmonary adenocarcinoma than CEA, but the sensitivity of CAM 123-6 for pulmonary adenocarcinoma was similar to that of CEA. CAM 123-6 was able to show increased sensitivity in combination with CEA.

The soluble CAM 123-6 in a pleural effusion from a patient with pulmonary adenocarcinoma showed a molecular weight of >900 kDa and was larger than KL-6 on molecular sieving. SDS-PAGE, Western blotting and immuno-staining indicated that the soluble antigen recognized by EH-123 antibody was polydisperse, with molecular weights in the range between 200 kDa and 900 kDa. This characteristic is typical of mucins.[33] EH-123 antibody recognized nonsialylated carbohydrate chains, while KL-6 antibody recognized sialylated carbohydrate chains. In cancer-associated mucins such as CA19-9,[1] SLX[24] and NCC-ST-439,[25] the epitopes recognized by the corresponding monoclonal antibodies are all sialylsugar residues. Though the structure of EH-123 epitope has not been fully characterized, this is the first time that an asialosugar has been shown to be a cancer-associated epitope highly specific to pulmonary adenocarcinoma.

Immunohistochemical studies revealed wide differences of distribution between EH-123 and KL-6 epitopes. EH-123 antibody recognized pulmonary adenocarcinoma, but not squamous cell carcinoma. However, KL-6 antibody reacted with both tissue sections. The similar staining pattern produced by EH-123 and KL-6 antibodies indicates that the carbohydrates recognized by both antibodies may exist on the same mucin molecule derived from pulmonary adenocarcinoma. On the other hand, the difference in the staining patterns of the bronchial glands and other normal tissue sections suggests that the two carbohydrate antigens are produced by different cells in normal organs. Furthermore, the difference of distribution between EH-123 and KL-6 epitopes was greater than that between LISA 101 and KL-6 epitopes. These histologic studies indicate that CAM 123-6 is highly specific to pulmonary adenocarcinoma because the two carbohydrate epitopes are present on the same mucin molecule when it is produced by pulmonary adenocarcinoma but hardly exist on the same molecule produced by normal cells. The soluble cancer-associated antigen CAM123-6 seems to be potentially useful as a tumor marker for diagnosing pulmonary adenocarcinoma.

ACKNOWLEDGMENTS

The authors thank the following collaborators for providing sera: Dr. Masayuki Miyake, Kitano Hospital; Dr. Masao Kuwabara and Dr. Masaru Tanabe, Hiroshima Prefectural Hospital; Dr. Nobuo Ueda, Ehime Prefectural Central Hospital; Dr. Taketoshi Ito, Ehime Prefectural Imabari Hospital; Dr. Kazutaka Nishimura, Ehime National Hospital; Dr. Taiji Takada, Saijo Central Hospital; Dr. Akihide Hashimoto and Teiichiro Taguchi, Uchiyama Hospital; Dr. Hiraku Ichiki, Ehime Prefectural Niihama Hospital; Dr. Seiichiro Watanabe, Kinki Central Hospital; and Dr. Kazuki Nishihara, Hiroshima Kyoritsu Hospital. This work was supported in part by a Grant-in Aid for Scientific Research (No. 03807040) from the Ministry of Education, Science and Culture, Japan.

(Received October 13, 1993/Accepted November 1, 1993)

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