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MONOCLONAL ANTIBODIES KL-3 AND KL-6 AGAINST HUMAN PULMONARY ADENOCARCINOMA: 2. DETECTION OF SOLUBLE ANTIGEN IN SERA AND PLEURAL EFFUSION

ヒト肺腺癌に対するモノクローナル抗体 KL-3 及び KL-6.
2. 血清及び胸水中の可溶性抗原の検出

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ヒト肺腺癌に対するモノクローナル抗体 KL-3 及び KL-6．2．血清及び胸水中の可溶性抗原の検出

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SUMMARY

Two monoclonal antibodies, KL-3 and KL-6, were produced by immunization with the human pulmonary adenocarcinoma cell line VMRC-LCR. Both antigen determinants recognized by KL-3 and KL-6 antibodies appear to be carbohydrate in nature. The molecular weights of both soluble antigens in pleural effusions were found to be greater than 1,000 K. While elevated levels of KL-3 antigen in sera were found only in those patients with gastric and pancreatic cancer, KL-6 antigen levels were above normal in sera of patients with lung cancer, especially advanced adenocarcinoma, and pancreatic cancer and breast cancer. In benign diseases of the lung and other organs, only sera from patients with active pulmonary tuberculosis who had extensive lung damage had notably elevated KL-6 antigen levels. Serum levels of KL-6 antigen also bore no relationship to those of CEA, CA19-9, CRP, and to BSR. In pleural effusions, the prevalence of lung adenocarcinoma cases with elevated levels of KL-3 and KL-6 antigens was 76% and 82%, respectively, whereas nonmalignant pleural effusions, including those from tuberculosis and cholesterin pleuritis, were mostly shown to have normal levels of these antigens.

These monoclonal antibodies define soluble antigens that, in combination with other tumor markers, may be clinically useful for tumor diagnosis and monitoring tumor progression.

要　約

ヒト肺腺癌細胞株 VMRC-LCR を免疫原として，KL-3 と KL-6 の二つのモノクローナル抗体を作製した．KL-3 抗体と KL-6 抗体により認識される抗原決定基は両者とも糖鎖であると思われた．胸水中のこれら可溶性抗原の分子量は 1,000K 以上であった．血清中の KL-3 抗原値の上昇は，胃癌患者及び膵臓癌患者にのみ認められたが，KL-6 抗原は，肺癌（特に進行した腺癌），膵臓癌及び乳癌の患者の血清中で異常高値を示した．肺やその他の臓器の良性疾患においては，広範な肺障害を示す活動性肺結核患者の血清のみが，顕著に高い KL-6 抗原値を示した．また，血清中の KL-6 抗原値は，血清中の CEA，CA19-9 及び CRP の値や BSR とも相関を示さなかった．胸水においては，KL-3 抗原及び KL-6 抗原値の上昇を示す肺腺癌例の頻度はそれぞれ 76% と 82% であったが，結核及びコレステリン胸膜炎に由来するような非悪性胸水は，ほとんど正常な抗原値を示した．

これらのモノクローナル抗体の認識する可溶性抗原は，ほかの腫瘍マーカーと併用することにより，腫瘍診断並びに腫瘍の進行度の判定において臨床的に有用であるかもしれない．

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INTRODUCTION

Development of methods for early diagnosis and more effective therapy for lung cancer are needed because there is a rising incidence of this disease and its prognosis is poor. Detection of tumor markers in the blood would be a useful method of diagnosis. Tumor markers such as CEA, TPA, NSE, and ferritin are used clinically at present. However, these markers have inadequate sensitivity and specificity and thus, are not quite satisfactory for use in early diagnosis. Therefore, numerous monoclonal antibodies (MoAbs) to tumors have been produced in an attempt to find new tumor markers.[1-10] Antibodies to tumor markers in the sera of lung cancer patients which have been produced and whose clinical usefulness have been reported, such as the CSLEX-1 antibody produced with stomach cancer as the immunogen[11] and the YH 206 antibody produced with lung cancer as the immunogen,[12] however, are extremely few.

We have produced MoAbs KL-3 and KL-6 against the pulmonary adenocarcinoma cell line VMRC-LCR,[13] and these MoAbs appear useful for detecting soluble cancer antigens in sera and pleural effusions.

MATERIALS AND METHODS

Determination of Molecular Weights of the Soluble Antigens

The antigen content in the fractions of malignant pleural effusions gel-filtrated using Sepharose 4B was measured by Sandwich enzyme-linked immunosorbent assay (ELISA) using a 96-well EIA plate which is mentioned later.

Antibody Purification and Sandwich ELISA

Ascites forming after intraperitoneal injection of 5×10^6 hybridoma cells into BALB/c mouse was collected. After ammonium sulfate precipitation of the ascites, KL-3 antibodies were refined using Sephadex G-200 and KL-6 antibodies, using DE52. The purified antibodies were peroxidase-labeled by the method of Nakane and Kawaoi.[14] Briefly, 5 mg horseradish peroxidase Grade I (Boehringer Mannheim, West Germany) was dissolved in 0.3 M carbonate buffer solution, pH 8.1, and then after adding 1% 1-fluoro 2, 4-dinitrobenzene ethanol solution, the solution was dialyzed in 0.01 M carbonate buffer solution, pH 9.5. One milliliter of 0.06 M

緒　言

肺癌の発生率は増加しており，その予後は不良であるため，早期診断法及び有効な治療法の開発が必要である．血液中の腫瘍マーカーの検出は有用な診断法であろう．現在，CEA，TPA，NSE 及びフェリチンなどの腫瘍マーカーが臨床的に用いられている．しかし，これらのマーカーは感度及び特異性が不十分なため，早期診断のための使用に十分に満足すべきものではない．したがって，新しい腫瘍マーカーを検出するために，腫瘍に対する多くのモノクローナル抗体（MoAbs）が産生されている．[1-10] 胃癌を免疫原として産生された CSLEX-1 抗体[11] 及び肺癌を免疫原として産生された YH 206抗体[12] などのように，臨床的有用性が報告されている肺癌患者の血清中腫瘍マーカーに対する抗体は極めて少ない．

我々は肺腺癌細胞株 VMRC-LCR に対するモノクローナル抗体 KL-3 及び KL-6 を作製したが，[13] これらのモノクローナル抗体は血清及び胸水中の可溶性癌抗原を検出するのに有用と考えられる．

材料及び方法

可溶性抗原の分子量の測定

Sepharose 4B を用いてゲル濾過した悪性胸水分画の抗原成分を Sandwich タイプの enzyme-linked immunosorbent assay（ELISA）により後述の96穴 EIA プレートを用いて測定した．

抗原精製及び Sandwich ELISA

BALB/c マウスの腹腔内に 5×10^6 個のハイブリドーマ細胞を注射した後，形成された腹水を採取した．腹水を硫酸アンモニウム沈降した後，KL-3 抗体は Sephadex G-200を用い，また KL-6 抗体は DE52を用いて精製した．これらの精製抗体は，Nakane 及び Kawaoi[14] の方法によりペルオキシダーゼで標識した．簡単に述べると，5 mg の西洋ワサビペルオキシダーゼ Grade I(Boehringer Mannheim, West Germary) を 0.3 M 炭酸緩衝液 pH 8.1に溶解し，1％1-fluoro 2, 4-dinitrobenzene ethanol 溶液を添加した後，0.01M 炭酸緩衝液 pH 9.5で透析した．透析物に 1 ml の

sodium periodate was added to the dialysate, which was incubated for 30 minutes and then 1.0 ml of 0.16 M ethylene glycol was added to the solution, which was incubated for 60 minutes and then dialyzed in 0.01 M carbonate buffer solution again and mixed with 5 mg of the purified antibodies. Three hours later, 5 mg of $NaBH_4$ was added and after being left to stand overnight at 4°C, the solution was dialyzed in phosphate-buffered saline (PBS), and the first peak obtained on gel filtration in a Sephadex G-200 column was defined as peroxidase-conjugated antibody.

0.06 M 過ヨウ素酸ナトリウムを添加し30分間反応させた後，この溶液に0.16 M エチレングリコール 1.0 ml を添加し，60分間反応させた．0.01 M 炭酸緩衝液で再度透析し精製抗体 5 mg と混合した．3時間後，$NaBH_4$ 5 mg を添加し，4℃で一晩放置した後，phosphate-buffered saline（PBS）で透析し，Sephadex G-200カラムによるゲル濾過で，最初に得られたピークをペルオキシダーゼ標識抗体とした．

In the Sandwich ELISA using a 96-well EIA plate (Costar, Cambridge, Mass), 100 μl of 10 μg/ml purified antibodies was dispensed into each well and left standing for one hour at room temperature, after which they were washed with TB-PBS (0.05% Tween 20, 0.1% bovine serum albumin [BSA], PBS). Next, 100 μl of specimen was added and allowed to react for three hours, after which they were washed, followed by the addition of 100 μl of peroxidase-conjugated MoAbs diluted 10-fold with buffered diluent (0.15 M PBS, pH 6.4, 10% normal rabbit serum, and 0.1% BSA) and incubated for 16 hours. Then, after washing, 100 μl of OPDA solution (0.3% *o*-phenylenediamine dihydrochloride, 0.02% H_2O_2, and 0.15 M citrate buffer, pH 4.9) was added and allowed to react for 30 minutes, after which reaction was stopped by the addition of 100 μl of 1N H_2SO_4 and absorbance (OD_{492}) was determined. For the purpose of quantitative determination of antigens in sera and pleural effusions, polystyrene beads were used instead of the plate. Polystyrene beads (1/4 inch, Wako, Japan) were incubated at 37°C for one hour in 100 μg/ml MoAb solution (0.25 M phosphate buffer, pH 7.5) and then cooled in ice water for 10 minutes. The antibody-sensitized beads were allowed to react with 0.3 ml of the specimen at 37°C for three hours in a glass tube. They were then washed three times with physiological saline, and, after adding 0.3 ml of peroxidase-labeled antibodies, diluted 1000-fold with a buffered diluent, were incubated for 16 hours. After washing, the beads were transferred to a polystyrene tube (Elkay Products, Inc., Boston, Mass) and 0.3 ml of OPDA solution was added and allowed to react for 30 minutes, after which 1 ml of 2N hydrochloric acid was added and absorbance (OD_{492}) was measured.

96穴の EIA プレート（Costar, Cambridge, Mass）を用いた Sandwich ELISA 法では，10 μg/ml の精製抗体100 μl を各穴に分注し，一時間室温で放置した後，TB-PBS（0.05% Tween 20, 0.1%ウシ血清アルブミン〔BSA〕, PBS）で洗浄した．次いで検体100 μl を添加し，3時間反応させた後洗浄し，希釈緩衝液（0.15 M PBS, pH 6.4, 10%正常ウサギ血清，及び0.1% BSA）で10倍に希釈したペルオキシダーゼ標識モノクローナル抗体100 μl を添加し，16時間反応させた．洗浄後，100 μl の OPDA 溶液（0.3% *o*-phenylenediamine dihydrochloride, 0.02% H_2O_2，及び0.15 M クエン酸緩衝液，pH 4.9）を添加し30分間反応させた後，1N H_2SO_4 100 μl を添加し反応を停止させ，吸光度（OD_{492}）を測定した．血清及び胸水中の抗原の定量には，培養プレートの代わりにポリスチレン・ビーズを用いた．ポリスチレン・ビーズ（¼ inch, 和光，日本）をモノクローナル抗体溶液（0.25 M リン酸緩衝液，pH 7.5）中で1時間37℃で反応させ，氷水中で10分間冷却した．抗体感作ビーズをガラス管内で3時間，37℃で検体 0.3 ml と反応させた．これを生理食塩水で3回洗浄し，希釈緩衝液で1000倍に希釈したペルオキシダーゼ標識抗体 0.3 ml を添加した後，16時間反応させた．洗浄後，ビーズをポリスチレン管（Elkay Products 社，Boston, Mass）に移し，OPDA 溶液 0.3 ml を添加し30分間反応させた後，2 Nの塩酸 1 ml を添加し吸光度（OD_{492}）を測定した．

For quantification of KL-3 and KL-6 antigens, serial dilution was made of pleural effusions having a large content of either antigen, and several concentrations showing linearity of absorbance were selected and used as standards. Clinical specimens were diluted with a buffered diluent. KL-3 antigens were diluted twofold and KL-6 antigens 40-fold before use.

KL-3及びKL-6抗原の定量化には，いずれかの抗原成分を大量に含有する胸水を連続希釈し，吸光度が直線を示した濃度を選択し，標準検体として用いた．臨床標本は希釈緩衝液で希釈した．使用前に，KL-3抗原は2倍に，KL-6抗原は40倍に希釈した．

RESULTS

Molecular Weights of Soluble Antigens

As shown in Figure 1, the molecular weight of KL-3 antigens was estimated to be more than 1,000 K because the peak of the antigen content in malignant mesothelioma-derived pleural effusions, as determined by Sandwich ELISA after gel filtration, was seen in only the void fraction. In the case of pleural effusions derived from lung metastasis of pancreatic cancer, the content of KL-3 antigens had peaks not only in the void fraction but also in the 45th and 57th fractions.

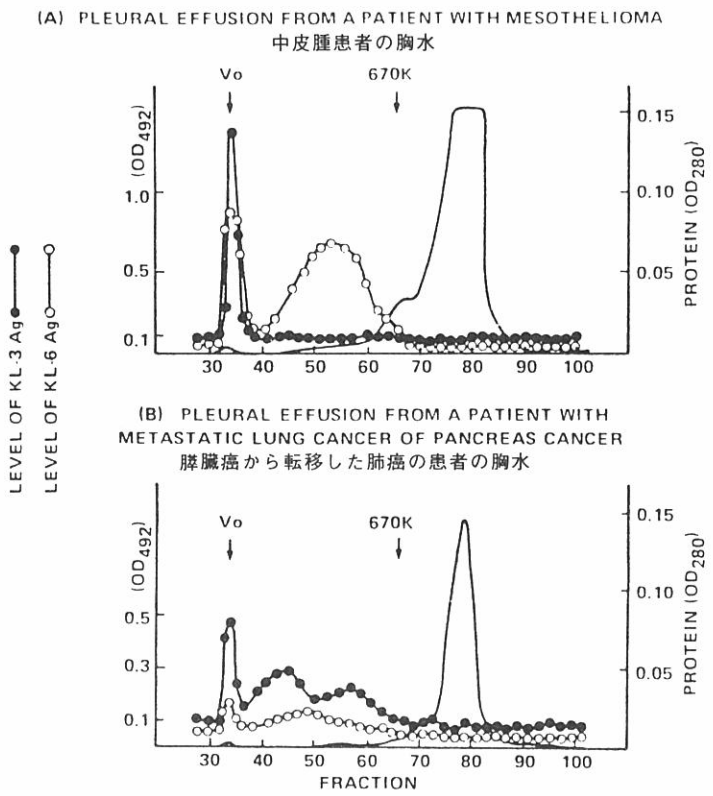
結　果

可溶性抗原の分子量

図1に示すとおり，ゲル濾過の後，Sandwich ELISA法によって測定した悪性中皮腫由来胸水の抗原成分のピークは，void分画にのみ見られるので，KL-3抗原の分子量は1,000K以上と推定した．膵臓癌の肺転移に由来する胸水の場合，KL-3抗原の成分は，void分画だけでなく第45及び第57分画でもピークに達した．

FIGURE 1　ANALYSIS OF MOLECULAR WEIGHTS OF KL-3 AND KL-6 ANTIGENS WITH SEPHAROSE 4B GEL CHROMATOGRAPHY

図1　Sepharose 4Bゲルクロマトグラフィによる KL-3及び KL-6抗原の分子量の解析



KL-6 antigens showed two peaks, in the void fraction and the 53rd fraction, in a malignant mesothelioma case and two peaks, in the void fraction and the 49th fraction, in pleural effusion from a pancreatic cancer patient.

Quantitation of Soluble KL-3 Antigens

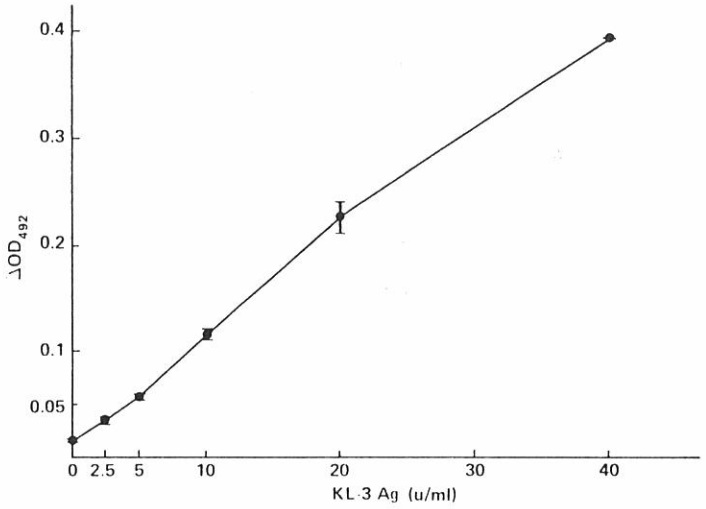
The six standard points were used: 0, 2.5, 5.0, 10, 20, and 40 u/ml, where 40 u/ml is an 80-fold dilution of a certain malignant pleural effusions with a buffered diluent. The standard curve, as shown in Figure 2, was nearly linear. The percent coefficient of variation at each point was less than 10%.

KL-6抗原は，悪性中皮腫の場合，void 分画及び第53分画で各々ピークに達し，膵臓癌患者の胸水では，void 分画と第49分画で各々ピークを示した．

可溶性 KL-3 抗原の定量化

標準検体として6点，すなわち0，2.5，5.0，10，20及び40 u/ml を用いた．40 u/ml は希釈緩衝液によってある悪性胸水を80倍に希釈したものである．図2に示すように，標準曲線はほぼ直線を示した．各点での percent coefficient of variation (% CV) は10%未満であった．

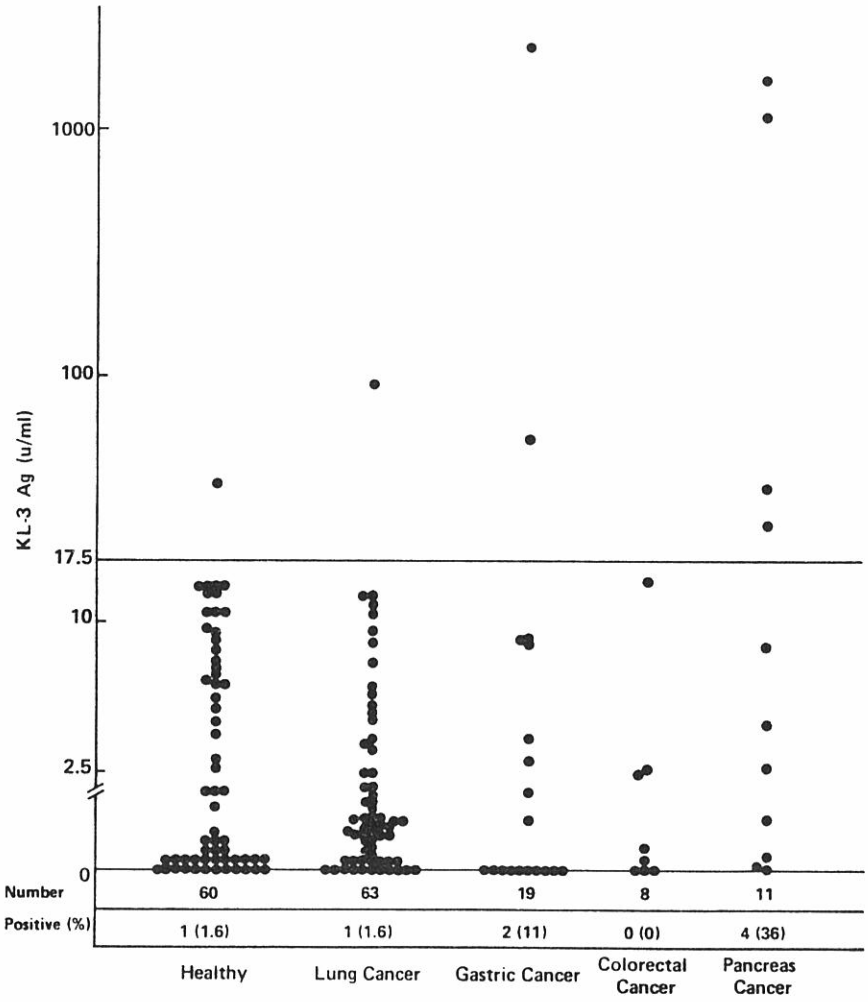
FIGURE 2 STANDARD CURVE OF KL-3 ANTIGEN
図2 KL-3抗原の標準曲線



Serum KL-3 antigen levels are shown in Figure 3. The serum KL-3 antigen level of 60 normal individuals was 4.5± 65 u/ml (mean± SD), the cut-off level being 17.5 u/ml (mean +2 SD). Among 63 cases of lung cancer, only one case showed an abnormally elevated level, and abnormally elevated levels were seen in 11% (2/19) of stomach cancer cases and in 36% (4/11) of pancreatic cancer cases, but all colon cancer cases were negative.

血清 KL-3 抗原値を図3に示している．健常者60人の血清 KL-3抗原値は 4.5±65 u/ml (平均± SD) であり，cut off 値は 17.5 u/ml (平均＋2 SD) とした．肺癌63例のうち1例のみが異常に高い値を示し，胃癌症例では11% (2/19)，膵臓癌症例では36% (4/11) が異常に高い値を示したが，結腸癌症例はすべて陰性の結果であった．

FIGURE 3 LEVELS OF KL-3 ANTIGEN IN SERA
図3 血清中のKL-3抗原値



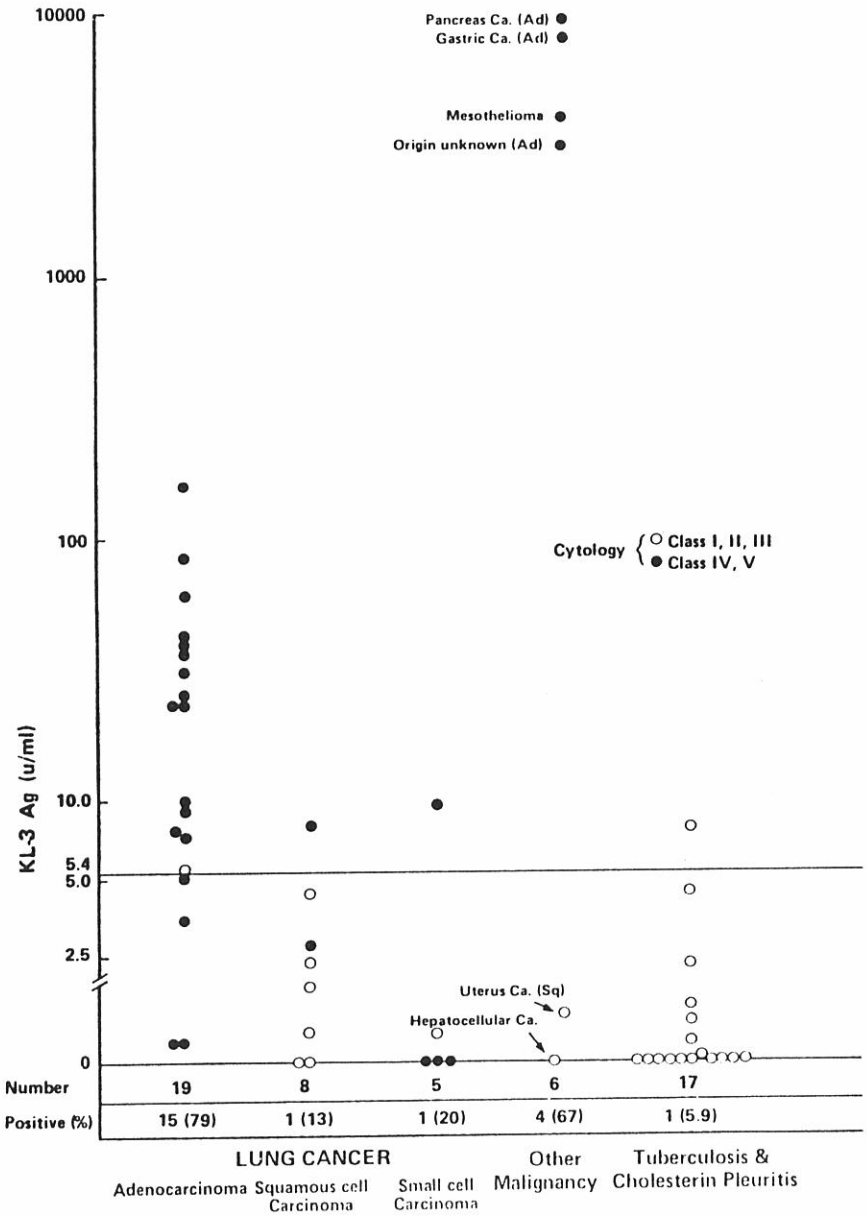
The results of determination of KL-3 antigen levels in pleural effusions are shown in Figure 4. Because the mean value for tuberculous pleuritis and cholesterin pleuritis (tuberculosis group) was 1.0±2.2 u/ml (mean ± SD), the cut-off value of KL-3 antigen levels in pleural effusion was determined to be 5.4 u/ml (mean +2 SD); abnormally elevated levels were present in the pleural effusions of 79% (15/19) of lung adenocarcinoma cases. Positive rates of 13% (1/8) and 20% (1/5) were found in pleural effusions derived from lung squamous cell carcinoma and lung small cell carcinoma, respectively. KL-3 antigen levels in pleural effusions derived from mesothelioma and metastatic lung tumors from gastric and pancreatic cancers had markedly

図4に胸水中のKL-3抗原値の測定結果を示している．結核性胸膜炎及びコレステリン胸膜炎（結核群）の平均値は1.0±2.2u/ml（平均±SD）であったので，胸水中のKL-3抗原値のcut off値は5.4u/ml（平均＋2SD）とした．肺腺癌症例の79％（15/19）の胸水が異常高値を示した．肺扁平上皮癌及び肺小細胞癌由来の胸水では，各々陽性率が13％（1/8）及び20％（1/5）であった．中皮腫及び胃癌又は膵臓癌の肺転移に由来する胸水中のKL-3抗原値は

elevated levels with some higher than 3,000 u/ml, but low levels were present in pleural effusions derived from metastatic lung cancers of hepatocellular carcinoma and uterine squamous cell carcinoma.

著しく高く，3,000 u/ml を超えるものもあったが，肝細胞癌及び子宮扁平上皮癌から転移した肺癌に由来する胸水では低い値であった．

FIGURE 4 LEVELS OF KL-3 ANTIGEN IN PLEURAL EFFUSIONS
図 4 胸水中の KL-3 抗原値



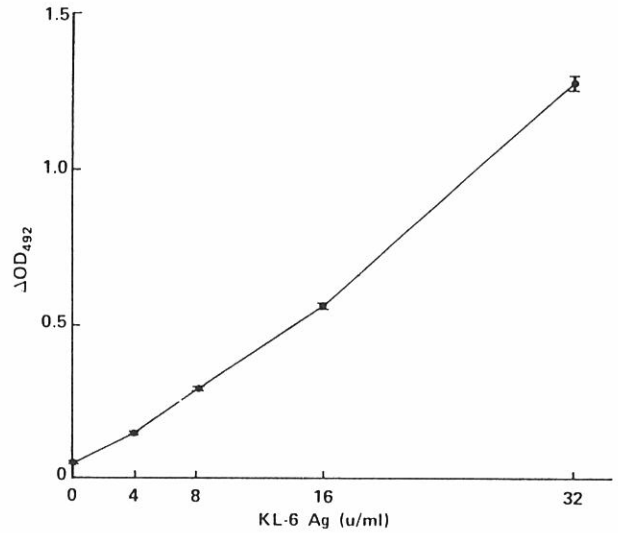
Quantitation of Soluble KL-6 Antigens

Five points, i.e., 0, 4, 8, 16, and 32 u/ml, where 32 u/ml is a 320-fold dilution of malignant pleural effusion, were established as standards for determination of the levels of KL-6 antigens. The standard curve, as shown in Figure 5, was nearly linear. The percent coefficient of variation was less than 10%.

可溶性 KL-6 抗原の定量化

KL-6 抗原値測定の標準値として5点，すなわち，0，4，8，16及び32 u/ml を用いた．32 u/ml は，ある悪性胸水の320倍希釈である．図5に示すとおり，標準曲線はほぼ直線であった．% CV は10%未満であった．

FIGURE 5 STANDARD CURVE OF KL-6 ANTIGEN
図5 KL-6抗原の標準曲線



The KL-6 antigen level in sera is shown in Figure 6. The level was 258 ± 131 u/ml (mean± SD) in 160 normal individuals; 520 u/ml (mean +2 SD) was the cut-off level. The positive rate was 52% (17/33) in lung adenocarcinoma cases, 18% (4/22) in lung squamous cell carcinoma cases, 8% (1/13) in lung small cell carcinoma cases, 13% (1/8) in hepatocellular carcinoma cases, 44% (4/9) in pancreatic carcinoma cases, and 40% (8/20) in breast cancer cases, but the rate was zero in 19 cases of gastric cancer and in eight cases of colon cancer.

In benign diseases of the lung, the positive rate was 14% (3/21) in acute pneumonia, 0% (0/15) in chronic bronchitis, and 43% (9/21) in pulmonary tuberculosis. The positive levels in acute pneumonia were very mildly elevated, and the pulmonary tuberculosis patients who were positive were limited to those who were clinically active and had extensive foci. As regards positive rates in inflammatory diseases

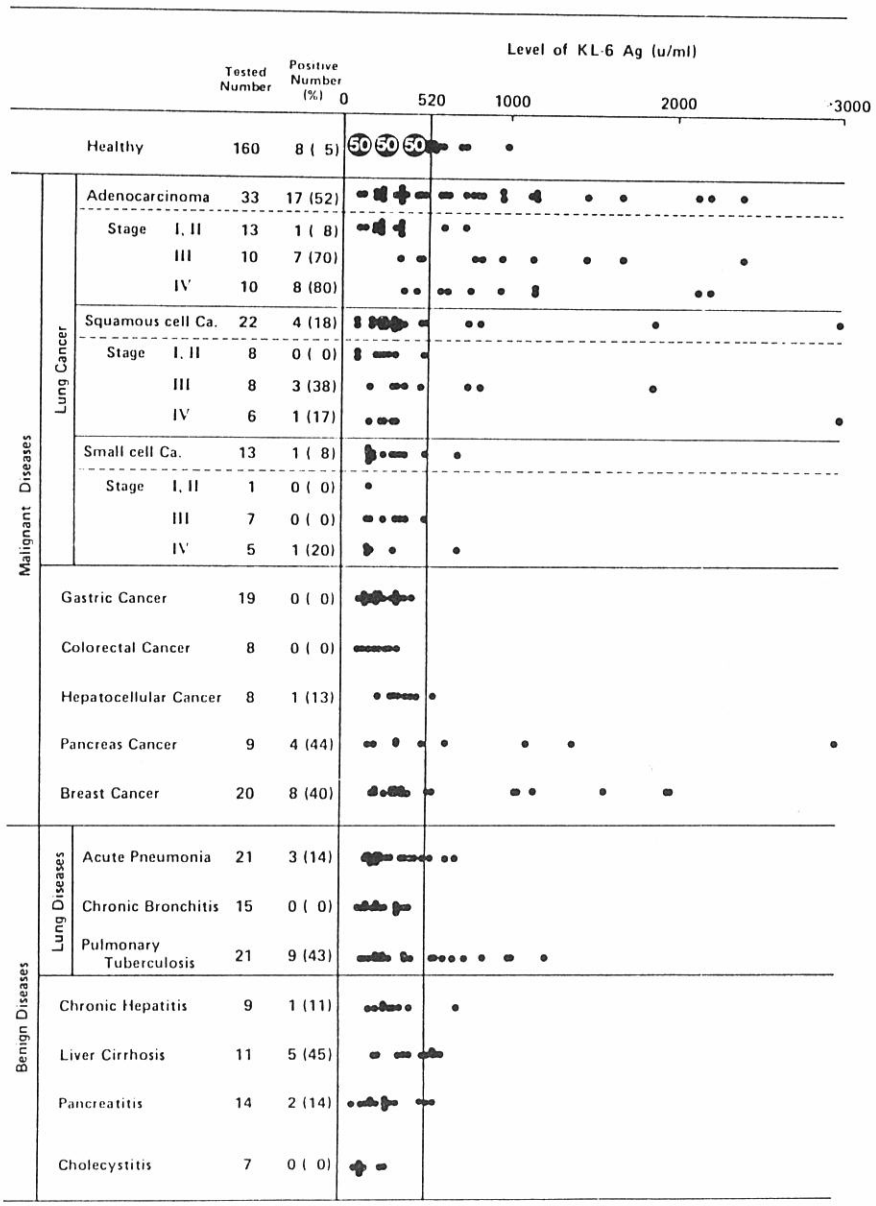
血清中の KL-6 抗原値を図6に示した．健常者160人の値は 258 ± 131 u/ml（平均± SD）であった．cut off 値は 520 u/ml（平均＋2 SD）とした．陽性率は肺腺癌症例では52%（17/33），肺扁平上皮癌症例では18%（4/22），肺小細胞癌症例では 8 %（1/13），肝細胞癌症例では13%（1/8），膵臓癌症例では44%（4/9），乳癌症例では40%（8/20）であったが，胃癌19例及び結腸癌 8 例では 0 %であった．

肺の良性疾患の陽性率については，急性肺炎が14%（3/21），慢性気管支炎が 0 %（0/15），肺結核が43%（9/21）であった．急性肺炎での陽性値は極めてわずか上昇していたのみであったが，陽性を示した肺結核患者は臨床的に活動性であり，かつ広範な病巣を有する者に限られていた．肺以外の臓器の炎症性疾患の陽性率については，慢性肝炎が

of organs other than the lung, the rate was 11% (1/9) in chronic hepatitis, 45% (5/11) in liver cirrhosis, 14% (2/14) in pancreatitis, and 0% (0/7) in cholecystitis, and the positive levels in these diseases were all very mildly elevated.

11%（1/9），肝硬変が45%（5/11），膵炎が14%（2/14），胆のう炎が0%（0/7）で，これらの疾患の陽性値はすべて極めてわずか上昇していたのみであった．

FIGURE 6 LEVELS OF KL-6 ANTIGEN IN SERA
図6 血清中のKL-6抗原値



By clinical stage of lung cancer, as the cases of adenocarcinoma and squamous cell carcinoma exhibiting abnormal levels of KL-6 antigens in sera increased with progression of the clinical stage and especially in the cases of lung adenocarcinoma, the positive rate of stage III and stage IV was 70% (7/10) and 80% (8/10), respectively.

Though the data is not shown, no significant correlation of KL-6 antigen levels in sera with CEA, CA19-9, CRP, and BSR values was observed.

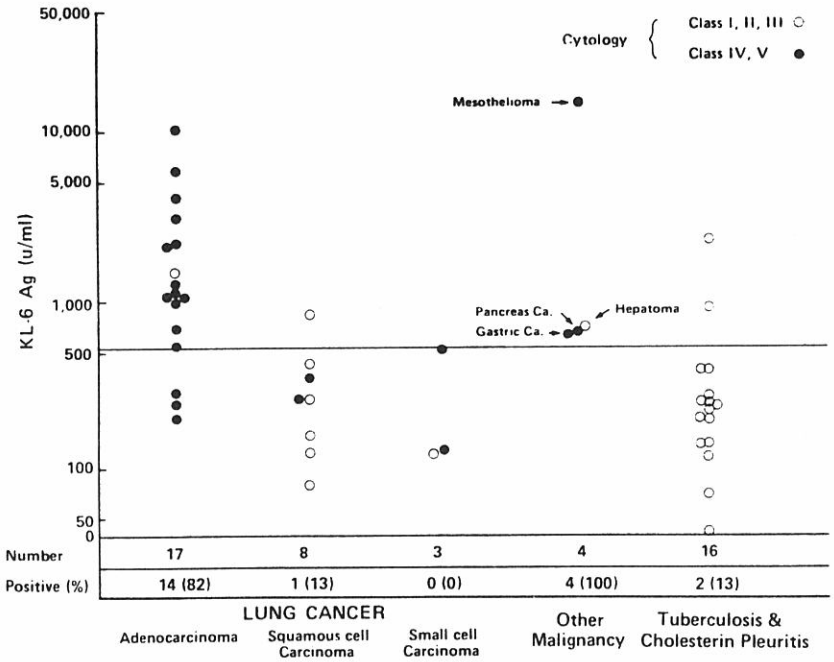
KL-6 antigen levels in pleural effusions are shown in Figure 7. The level in pleural effusion derived from pulmonary adenocarcinoma was 2,165 ± 2,574 u/ml, a notably elevated level compared with 381 ± 547 u/ml in pleural effusions of the tuberculosis group, 309 ± 237 u/ml in pulmonary squamous cell carcinoma, and 254 ± 223 u/ml in pulmonary small cell carcinoma. Although a very high level of KL-6 antigens was present in the pleural effusion from malignant mesothelioma, the levels in the pleural effusions derived from metastatic lung tumors from pancreatic cancer, gastric cancer, and hepatocellular carcinoma were not so high.

肺癌の臨床病期別では，血清中の KL-6 抗原値が異常を示した腺癌及び扁平上皮癌症例は，臨床病期の進行に伴って増加し，特に肺腺癌症例では，臨床病期 III 及び IV の陽性率は各々70％（7/10）と80％（8/10）であった．

データは示していないが，血清中の KL-6 抗原値と CEA，CA19-9，CRP，及び BSR 値との間に有意な相関は認められなかった．

図 7 は胸水中の KL-6 抗原値を示している．肺腺癌に由来する胸水中の値は 2,165 ± 2,574 u/ml で，結核群の胸水中の 381 ± 547u/ml，肺扁平上皮癌の 309 ± 237 u/ml，及び肺小細胞癌の 254 ± 223 u/ml と比べ著しく高かった．悪性中皮腫からの胸水の KL-6 抗原値は極めて高かったが，膵臓癌，胃癌，及び肝細胞癌から転移した肺腫瘍に由来する胸水中の値は，それほど高くなかった．

FIGURE 7 LEVELS OF KL-6 ANTIGEN IN PLEURAL EFFUSIONS
図 7 胸水中の KL-6 抗原値



Combination Assay of KL-3 Antigens and KL-6 Antigens for Detection of Soluble Antigens in Pleural Effusions

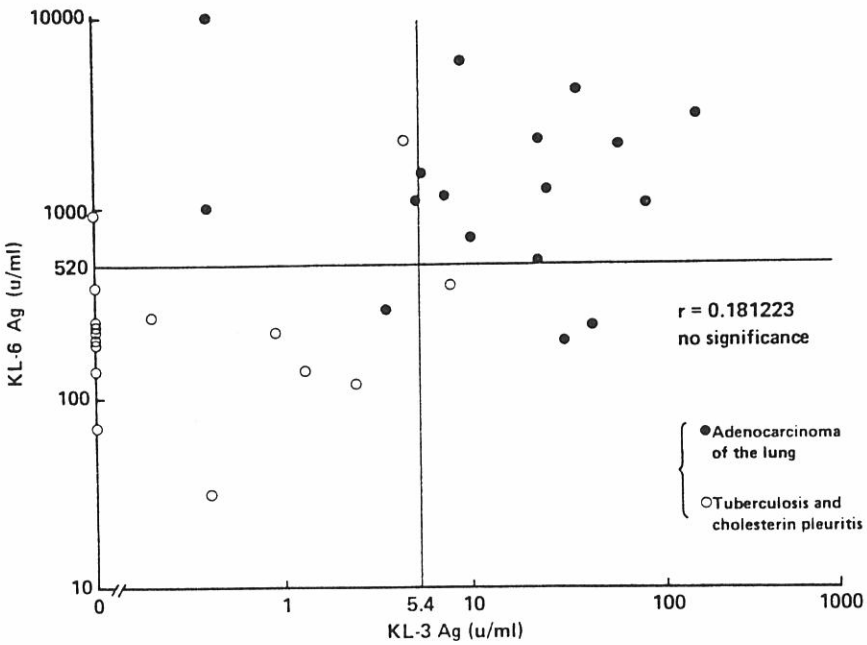
The correlation of KL-3 antigen level and KL-6 antigen level in pleural effusions was studied in 33 cases in total, 17 cases of pulmonary adenocarcinoma and 16 cases of the pulmonary tuberculosis group. As shown in Figure 8, the correlation coefficient was 0.18 and not significant.

KL-3抗原及びKL-6抗原の併用による胸水中の可溶性抗原検出

胸水中のKL-3抗原値とKL-6抗原値の相関を33例について検討した．そのうち17例は肺腺癌で，16例は肺結核群であった．図8に示すとおり，相関係数は0.18で，有意でなかった．

FIGURE 8 CORRELATION BETWEEN KL-3 ANTIGEN AND KL-6 ANTIGEN LEVELS IN PLEURAL EFFUSIONS

図8 胸水中のKL-3抗原値とKL-6抗原値の相関



Among pulmonary adenocarcinoma cases, 76% (13/17) had a KL-3 antigen level higher than 5.4 u/ml in the pleural effusions, and 82% (14/17) had a KL-6 antigen level higher than 520 u/ml. At least one of the two antigens was positive in 94% (16/17) of the cases, thus the positive rate improved remarkably by combining the two. On the other hand, in the pleural effusions of the pulmonary tuberculosis group, one of the two was positive in only 19% (3/16) of the cases, but there were no cases positive for both antigens.

胸水では，肺腺癌症例の76%（13/17）が5.4 u/mlより高いKL-3抗原値を示し，82%（14/17）が520 u/mlより高いKL-6抗原値を示した．症例の94%（16/17）で二つの抗原のうち少なくとも一つが陽性であったので，二つを組み合わせることにより陽性率は著しく高くなった．一方，肺結核群の胸水では，二つのうち一つが陽性であった例は19%（3/16）に過ぎず，両抗原共に陽性の例はなかった．

DISCUSSION

KL-3 and KL-6 antibodies are MoAbs produced by immunization with the human pulmonary adenocarcinoma cell line VMRC-LCR. It was found that both of these antibodies recognize carbohydrate antigens[13] and detect soluble antigens in blood and pleural effusions. The molecular weights of the soluble antigens were all more than 1,000 K. Because the pattern of elution of the soluble antigens by gel filtration very closely resembles the pattern of CA19-9 and ST-439 antigens,[15,16] which are reportedly mucin antigens, the soluble antigens which recognize KL-3 and KL-6 antibodies are assumed to be mucin-type glycoprotein antigens.

The KL-3 antigen content in the sera of lung cancer patients was found not to be different from that in normal individuals. This antigen, however, showed a positive rate of 11% in gastric cancer and 36% in pancreatic cancer indicating that it might be a marker for these tumors. The content of antigen was also observed to be increased in many malignant pleural effusions, and it was abnormally elevated in 79% of pleural effusions from lung adenocarcinoma cases. Determination of KL-3 antigens in pleural effusions therefore may be useful clinically as a method of differential diagnosis of the pleural effusions due to malignant pleuritis and those due to benign disease such as tuberculosis pleuritis.

On the other hand, KL-6 antigens showed abnormal elevation in the sera in lung cancer cases. The positive cases were mostly adenocarcinoma and squamous cell carcinoma, with 52% of adenocarcinoma cases and 18% of squamous cell carcinoma cases being positive. Also, the percentage of cases with high KL-6 antigen levels tended to increse with progression of the clinical stage of the disease. KL-6 antigens showed high rates of positivity in the sera of pancreatic cancer and breast cancer cases as well as in the sera of lung cancer cases, and these antigens might be of clinical use as new tumor markers for these malignancies because they showed no correlation with CEA and CA19-9 levles. No case of gastric cancer or colon cancer was positive for KL-6 antigens; only pancreatic cancer showed a high positive rate among abdominal organ malignant tumors. Since not even CA19-9 antigen nor pancreatic oncofetal antigen,[17,18] which are excellent

考　察

KL-3抗体とKL-6抗体は，ヒト肺腺癌細胞株VMRC-LCRで免疫して作製されたモノクローナル抗体である．これらの抗体が糖鎖抗原を認識し，[13] 血液及び胸水中の可溶性抗原を検出することが明らかになった．可溶性抗原の分子量はすべて1,000K以上であった．ゲル濾過による可溶性抗原の溶出パターンは，ムチン抗原と報告されているCA19-9及びST-439抗原[15,16]のパターンに極めて似ているので，KL-3及びKL-6抗体が認識する可溶性抗原はムチン型糖蛋白抗原と考えられる．

肺癌患者の血清中のKL-3抗原量は，健常者と異ならなかった．しかし，この抗原の陽性率は胃癌では11%，膵臓癌では36%であり，それらの腫瘍マーカーになり得ることが示唆された．また抗原量は多くの悪性胸水で増加しており，肺腺癌症例に由来する胸水の79%で異常高値を示した．したがって胸水中のKL-3抗原の測定は，悪性胸膜炎による胸水と結核性胸膜炎などの良性疾患による胸水の鑑別診断法として臨床的に有用かもしれない．

一方，KL-6抗原は肺癌症例の血清中で異常に増加していた．陽性例のほとんどは腺癌又は扁平上皮癌であり，腺癌症例の52%，扁平上皮癌の18%が陽性であった．また，KL-6抗原値が高い症例の割合は，疾患の臨床病期の進行に伴って増加する傾向を示した．KL-6抗原は肺癌症例のみでなく膵臓癌症例及び乳癌症例の血清中でも高い陽性率を示したが，この抗原値はCEA及びCA19-9値と相関をもたないので，これらの悪性疾患の新しい腫瘍マーカーとして臨床的に有用であるかもしれない．胃癌又は結腸癌症例でKL-6抗原が陽性のものはなく，腹部臓器悪性腫瘍のうち膵臓癌のみが高い陽性率を示した．優れた膵臓癌マーカーであるCA19-9抗原及びpancreatic oncofetal antigen[17,18]でさえ，膵臓癌と他の腹部腫瘍

pancreatic cancer markers, distinguish pancreatic cancer so selectively from other abdominal cancers, KL-6 antigens might be very useful in differential diagnosis of abdominal tumors.

Though it has the characteristics of a tumor marker, KL-6 antigen in sera also was elevated in pulmonary tuberculosis. However, no cases of inflammatory lung diseases such as acute pneumonia and chronic bronchitis nor chronic hepatitis, liver cirrhosis or cholecystitis have shown any notably elevated levels of this antigen, and no correlation with inflammatory parameters such as CRP level and BSR level was observed. Hence, the KL-6 antigen was considered to be a marker of not just inflammation in general but of that due to specific pathological types of inflammatory lung disease. Because pulmonary tuberculosis cases positive for this antigen were confined to those who were in the active stage with extensive lung damage and not to early TB, the antigen might be useful in the differential diagnosis of lung cancer and tuberculoma which present as coin lesions on chest X ray.

KL-6 antigens were markedly elevated in many pleural effusions derived from lung adenocarcinoma, but these antigens were not so elevated in pleural effusions derived from metastatic lung tumors of gastric cancer and pancreatic cancer and this differed altogether from the pattern of KL-3 antigens. Comparison of KL-3 antigens and KL-6 antigens in pleural effusions showed no correlation between the two, and a combination assay of the two antigens might be useful as tumor markers in pleural effusions because it increased the diagnosis rate.

KL-3 and KL-6 antibodies, both of which were produced against the pulmonary adenocarcinoma cell line, reacted with normal lung tissues. Since we conducted our screening with the major aim of establishing MoAbs with which to detect soluble antigens and then analyze their clinical usefulness, we did not necessarily consider that MoAbs reactive with normal lung tissues would be a drawback. It is only necessary that the reaction to lung cancer tissues be stronger than the reaction to normal lung tissues and that these antigens be present in the sera and pleural effusions during specific disease states.

とを明確に区別することはできないので，KL-6抗原は腹部腫瘍の鑑別診断として極めて有用であるかもしれない．

血清中のKL-6抗原は腫瘍マーカーとしての特性を有しているが，肺結核でも増加していた．しかし，急性肺炎及び慢性気管支炎などの炎症性肺疾患，慢性肝炎，肝硬変又は胆のう炎でこの抗原値が著しく増加した症例はなく，CRP値及びBSR値などの炎症性パラメターとの相関も認められなかった．したがってKL-6抗原は一般の炎症だけでなく，特定の病理学的タイプの炎症性肺疾患による炎症のマーカーと考えられた．この抗原が陽性を示した肺結核症例は，広範な肺障害を有する活動性結核患者に限られており，初期の肺結核患者は含まれていないので，この抗原は胸部X線検査でcoin lesionとして現れる肺癌と結核腫の鑑別診断に有用かもしれない．

KL-6抗原は肺腺癌に由来する多くの胸水中で著しく増加していたが，胃癌及び膵臓癌からの転移性肺癌に由来する胸水では増加しておらず，このことはKL-3抗原のパターンとは全く異なっていた．胸水中のKL-3抗原とKL-6抗原を比較したところ両者に相関は見られず，この二つの抗原は併用により診断率を高めたので，胸水中の腫瘍マーカーとして有用かもしれない．

KL-3及びKL-6抗体は肺腺癌細胞株に対して作製されたものであるが，正常肺組織と反応した．可溶性抗原を検出し，その臨床的有用性を解析するためのモノクローナル抗体を作製することを主な目的としてスクリーニングを行ったので，モノクローナル抗体が正常肺組織と反応することは必ずしも問題とは考えなかった．肺癌組織への反応が正常肺組織への反応より強いこと，及びこれらの抗原が特定の病態で血清及び胸水中に存在することのみが必要な条件であると考えたからである．

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