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MONOCLONAL ANTIBODIES KL-3 AND KL-6 AGAINST HUMAN PULMONARY ADENOCARCINOMA: 1. CHARACTERIZATION OF THE ANTIBODIES AND THEIR APPLICATION IN DETECTION OF TUMOR CELLS IN 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
1．反応特異性の検討と胸水中の腫瘍細胞検出への応用

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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 1．反応特異性の検討と胸水中の腫瘍細胞検出への応用

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SUMMARY

Two monoclonal antibodies, KL-3 (IgM) and KL-6 (IgG_1), were produced by the fusion of murine myeloma NS1 cells with splenocytes from a BALB/c mouse immunized with human pulmonary adenocarcinoma VMRC-LCR cells. Both antibodies recognized carbohydrate antigens. Immunohistochemical analysis of tissue sections showed that KL-3 antibodies preferentially react with lung adenocarcinomas and small cell carcinomas, whereas KL-6 antibodies react with these as well as squamous cell carcinomas of the lung. Both of these antibodies also reacted with many different types of cancer. Immunofluorescence was used to demonstrate that both antibodies were reactive only with tumor cells but not with nonmalignant blood cells and mesothelial cells in pleural effusions. These antibodies may be clinically useful in immunocytochemical diagnosis of tumor cells in pleural effusions.

INTRODUCTION

The incidence of lung cancer continues to increase, and it is a major medical problem. The histological picture of lung cancer is varied, and there are as yet no satisfactory methods of early diagnosis and treatment. As an approach to improve early diagnosis, many monoclonal antibodies (MoAbs) against lung cancer have

要 約

ヒト肺腺癌由来VMRC-LCR細胞で免疫したBALB/cマウス由来の脾細胞とマウスの骨髄腫由来NS1細胞とを融合させることにより，KL-3 (IgM) とKL-6 (IgG_1) という二つのモノクローナル抗体を産生した．両抗体共に糖質抗原を認識した．組織切片を免疫組織化学的に解析したところ，KL-3抗体は肺腺癌及び小細胞癌と特に反応し，KL-6抗体はこれらのほかに肺扁平上皮癌とも反応した．両抗体は，その他多くの異なる病型の癌とも反応した．免疫蛍光法により，胸水中の細胞に対しては両抗体が腫瘍細胞のみと反応し，非悪性血球及び中皮細胞とは反応しないことが示された．これらの抗体は，胸水中の腫瘍細胞の免疫組織化学的診断において，臨床的に有用であろうと考えられた．

緒 言

肺癌の発生率は上昇し続けており，医学上重要な問題である．また，肺癌の組織像は多様であって，早期診断及び治療に対する満足な方法は依然として開発されていない．早期診断を促進する方法として，肺癌細胞及び組織の免疫組織学的研究，並びに

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been produced for immunohistological study of lung cancer cells and tissues and for potential clinical application in the diagnosis and treatment of this malignancy.[1-10] We also have produced MoAbs against lung cancer cells for the same purposes and have previously reported on LuCa3 and LuCa4 which show a high degree of specificity for squamous cell carcinoma.[11] Besides these antibodies, two additional MoAbs with possible applicability in the cytological examination of pleural effusions were produced. These antibodies are the subject of this report as their potential utility in clinical diagnosis is thought to be high.

MATERIALS AND METHODS

Cell Lines

Lung adenocarcinoma cell line VMRC-LCD, lung squamous cell carcinoma cell line SK-MES-1, lung small cell carcinoma cell line SK-AK-LCL, urinary bladder cancer cell line T-24, uterocervical cancer cell line ME-180 and malignant melanoma cell line MeWo were furnished by Dr. Michael A. Bean (Virginia Mason Research Center, Seattle, Wash); lung adenocarcinoma cell line Luci 3 and Luci 10, lung squamous cell carcinoma cell line PC-1, lung small cell carcinoma cell line QG-90, renal cancer cell line Scattola and sarcoma cell line U-20-S, by Dr. Ryuzo Ueda (Aichi Cancer Center); lung adenocarcinoma cell line ABC-1 and lung small cell carcinoma cell line SBC-3 and SBC-5, by Dr. Shunkichi Hiraki (Okayama University School of Medicine); stomach cancer cell line AZ-521, by Dr. Kozo Imai (Sapporo Medical College); liver cancer cell line SK-HEP1 and colon cancer cell line SW1222, by Dr. Tadashi Watanabe (Nagoya University School of Medicine); pancreatic cancer cell line PK-1-66, by Dr. Hirotake Hisano (Tohoku University School of Medicine); breast cancer cell line YMB-1, by Dr. Motoi Yamane (Hiroshima University School of Medicine); and lung squamous cell carcinoma cell line RERF-LC-AI was established in our laboratory. Lung fibroblast cell line CCD-18Lu, nasopharyngeal cancer cell line K.B., laryngeal cancer cell line HEp-2, and pancreatic cancer cell line Panc-1 and MIA PaCa-2 were obtained from American Type Culture Collection. These cell lines were propagated in serial culture using complete RPMI medium [RPMI 1640 medium (Grand Island Biological Co., Grand Island, NY), 10% fetal calf serum (FCS; Hyclone,

肺癌の臨床的診断や治療に応用する目的で，肺癌に対する多くのモノクローナル抗体が産生されてきた.[1-10] 我々も同様の目的で，肺癌細胞に対するモノクローナル抗体を産生し，扁平上皮癌に高度の特異性を示すLuCa 3及びLuCa 4抗体については以前に報告した.[11] これらの抗体のほかに，胸水の組織学的検査に応用可能な二つのモノクローナル抗体を産生した．これらの抗体は臨床診断に極めて有用であると考えられるので，以下に報告する．

材料及び方法

細胞株

本調査に使用した細胞株とその提供者は次のとおりである．肺線癌細胞株 VMRC-LCD，肺扁平上皮癌細胞株 SK-MES-1，肺小細胞癌細胞株 SK-AK-LCL，膀胱癌細胞株 T-24，子宮頸部癌細胞株 ME-180，及び悪性黒色腫細胞株 MeWo — Dr. Michael A. Bean（Virginia Mason 研究センター，Seattle，Washington）；肺腺癌細胞株 Luci 3及び Luci 10，肺扁平上皮癌細胞株 PC-1，肺小細胞癌細胞株 QG-90，腎臓癌細胞株 Scattola，並びに肉腫細胞株 U-20-S — 上田龍三博士（愛知がんセンター）；肺腺癌細胞株 ABC-1並びに肺小細胞癌細胞株 SBC-3及び SBC-5 — 平木俊吉博士（岡山大学医学部）；胃癌細胞株 AZ-521 — 今井浩三博士（札幌医科大学）；肝臓癌細胞株 SK-HEP 1及び結腸癌細胞株 SW1222 — 渡辺 正博士（名古屋大学医学部）；膵臓癌細胞株 PK-1-66 — 久野弘武博士（東北大学医学部）；乳癌細胞株 YMB-1 — 山根 基博士（広島大学医学部）．肺扁平上皮癌細胞株 RERF-LC-AI は当研究室で産生された．肺線維芽細胞株 CCD-18Lu，鼻咽頭癌細胞株 K.B.，喉頭癌細胞株 HEp-2，並びに膵臓癌細胞株 Panc-1及び MIA PaCa-2は American Type Culture Collection から入手した．完全 RPMI 培地〔RPMI 1640培地（Grand Island Biological 社，Grand Island，New York），10%ウシ胎児血清（FCS；

Sterile Systems, Inc., Utah), 4 mM HEPES [4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid], streptomycin (100 μg/ml), penicillin (100 units/ml) and 2 mM L-glutamine]. The 8-azaguanine-resistant mouse myeloma cell line P3-NS 1-Ag 4/1 (NS-1) which was used as the partner in hybridoma fusions was cultured in complete RPMI medium to which 100 μM of 8-azaguanine (Sigma Chemical Co., St. Louis, Mo) had been added.

Hybridoma-production Method

An eight-week-old BALB/c mouse was immunized subcutaneously with 5×10^6 VMRC-LCR cells and then intraperitoneally with 8×10^6 VMRC-LCR cells twice at an interval of two weeks. Three days after the final immunization the spleen was removed, and splenocytes were fused with NS1 cells according to the method of Köhler and Milstein.[12] That is, splenocytes and NS1 cells were mixed at the ratio of 2 to 1 and centrifuged. To the sediment 1 ml of 42% polyethylene glycol 6000 (Eastman Kodak, Rochester, NY) was added and stirred slowly for five minutes at 37°C. After washing, the mixed cell solution was suspended in complete RPMI medium, and 0.1 ml of the suspension was dispensed into a total of 126 wells at the rate of 10^6 cells per well in 96-well plastic microplates (Costar, Cambridge, Mass). After 24 hours, 0.1 ml of HAT medium (complete RPMI medium containing 100 μM of hypoxanthine, 0.4 μM of aminopterin, and 16 μM of thymidine) was added to each well. On days 2, 3, 5, 7 and 11 after commencement of culture, 0.1 ml of the culture supernatant was discarded and 0.1 ml of the HAT medium was added. Hybridomas reaching the limit of proliferation were transferred into wells of a 24-well culture plate into which 5×10^6 feeder cells (BALB/c mouse thymocytes) had been seeded beforehand and cultured in an HT medium (complete RPMI medium containing 100 μM of hypoxanthine and 16 μM of thymidine). When the hybridomas reached the limit of proliferation, antibody activity in the culture supernatant against VMRC-LCR cells was detected by the enzyme-labeled antibody technique. Hybridomas exhibiting antibody activity were cloned by the limiting dilution method. The clones secreting antibodies that reacted with VMRC-LCR cells but not with CCD-18Lu lung fibroblasts were selected and cloned again.

Hyclone, Sterile Systems社, Utah), 4 mM HEPES〔4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid〕, ストレプトマイシン (100 μg/ml), ペニシリン (100 units/ml) 及び2 mM L-グルタミン〕を用いて, 上述の細胞株を連続培養した. ハイブリドーマ融合に用いた8-アザグアニン耐性のマウス骨髄細胞株 P3-NS 1-Ag 4/1 (NS-1) は, 100 μM の8-アザグアニン (Sigma Chemical 社, St. Louis, Missouri) 添加完全 RPMI 培地で培養した.

ハイブリドーマの産生方法

8週齢の BALB/c マウスを 5×10^6 個の VMRC-LCR 細胞で皮下免疫し, 次に, 8×10^6 個の同細胞で2週間おいて2回腹腔内に免疫した. 最終免疫の3日後に脾臓を摘出し, Köhler と Milstein[12] の方法に従って脾細胞を NS1 細胞と融合させた. すなわち, 脾細胞と NS1細胞を2対1の割合で混和し, 遠心分離した. 得られた沈渣に42%ポリエチレングリコール 6000 (Eastman Kodak, Rochester, New York) 1 ml を添加し, 37℃で5分間ゆっくりと撹拌した. この混合細胞溶液を洗浄し, 完全 RPMI 培地に浮遊した後, 0.1 ml の浮遊液を, 96穴プラスチック・マイクロプレート (Costar, Cambridge, Massachusetts) に合計して126穴ほど, 1穴当たりの細胞数が 10^6 個になるように分注した. 24時間後, 0.1 ml の HAT 培地 (ヒポキサンチン 100 μM, アミノプテリン 0.4 μM 及びチミジン 16 μM を含む完全 RPMI 培地) を各穴に添加した. 培養開始後2, 3, 5, 7及び11日目に, 培養上澄液 0.1 ml を捨て, HAT 培地 0.1 ml を添加した. フィーダー細胞 (BALB/c マウスの胸腺細胞) 5×10^6 個をあらかじめ播種し, HT 培地 (ヒポキサンチン 100 μM 及びチミジン 16 μM を含む培地) で培養しておいた24穴培養皿に, 増殖限界に達したハイブリドーマを移した. このハイブリドーマが増殖限界に達した時点で, VMRC-LCR 細胞に対する培養上澄の抗体活性を酵素抗体法で検出した. 抗体活性を示すハイブリドーマを限界希釈法を用いてクローン化した. VMRC-LCR 細胞とは反応するが, CCD-18Lu 肺線維芽細胞とは反応しない抗体を分泌するクローンを選択し, 再度クローン化した.

Enzyme Immunoassay for Determining Antibody Activity in Hybridoma Culture Supernatant Against Cell Lines

Target cell lines were cultured to maximum density in 96-well plastic plates and fixed for five minutes with 0.25% glutaraldehyde, after which they were washed with phosphate-buffered saline (PBS). To each culture well 0.1 ml of hybridoma culture supernatant was added and incubated for one hour at room temperature and then washed. After this, 50 μl of peroxidase-labeled goat antimouse immunoglobulin (Cappel Laboratories, Cochranville, Penn) diluted 100-fold with 10% FCS solution was added, and the solution was incubated for one hour at room temperature. After washing, 100 μl of ABTS solution [150 μg/ml azinobis-3-ethylbenzothiazoline-6, 6-sulfonate (Nakarai Kagaku, Japan), 50mM citrate buffer pH 4.0, 1.1% H_2O_2] was added and allowed to react for five minutes at room temperature. Then, the reaction was stopped by adding 50 μl of 10% oxalic acid. The absorbance at OD_{414} was determined using a microplate photometer (Corona Denki, Japan). Values over 0.02 were defined as positive.

Immunoperoxidase Staining

The reactivity of MoAbs against tissues was determined by the immunoperoxidase technique (ABC technique) using Vectastain (Vector Laboratories, Burlingame, Calif). That is, 4 μm-thick frozen tissue sections were used after they were acetone-fixed for 10 minutes at −20°C and dried. The tissues were allowed to react with hybridoma culture supernatant for 30 minutes at room temperature after the nonspecific reaction was at first obstructed using horse sera. After being washed for 10 minutes with PBS, the tissue sections were allowed to react with biotinized horse antimouse IgG for 30 minutes at room temperature and washed, after which avidin-biotin-labeled peroxidase was allowed to react with them for one hour. Following this, the tissues were washed and allowed to react for five minutes with DAB solution (0.5 mg/ml Diaminobenzidine, 0.01% H_2O_2, 50 mM Tris-HCl buffer pH 7.0).

Immunofluorescent Staining of Pleural Effusion Cells and Blood Cells

Pleural effusion cells and peripheral blood mononuclear cells were obtained from the middle layer by density centrifugation using

各細胞株に対するハイブリドーマ培養上澄の抗体活性を測定するための酵素免疫測定法

96穴のプラスチック・プレートで，標的細胞株を最大濃度になるまで培養し，5分間0.25%グルタルアルデヒドで固定した後，リン酸緩衝食塩水（PBS）で洗浄した．各培養穴に，ハイブリドーマ培養上澄0.1 ml を添加し，室温で1時間培養した後，洗浄した．次に，10%FCS 溶液で100倍に希釈したペルオキシダーゼ標識ヤギ抗マウス免疫グロブリン（Cappel Laboratories, Cochranville, Pennsylvania）50 μl を添加し，この溶液を室温で1時間培養した．これを洗浄した後，100 μl の ABTS 溶液〔150μg/ml の azinobis-3-ethylbenzothiazoline-6, 6-sulfonate（半井化学，日本），50mM クエン酸緩衝液 pH 4.0，1.1% H_2O_2〕を加え，室温で5分間反応させた．この後，10%シュウ酸を50 μl 加えて反応を停止させた．マイクロプレート光度計（コロナ電器，日本）を用いて，OD_{414}での吸光度を測定した．0.02以上の値を陽性と判定した．

免疫ペルオキシダーゼ染色

組織に対するモノクローナル抗体の反応を Vectastain（Vector Laboratories, Burlingame, California）を用いて，免疫ペルオキシダーゼ法（ABC 法）によって測定した．すなわち，厚さ4 μm の凍結切片を−20℃で10分間アセトンで固定し，乾燥させたものを用いた．非特異的反応をウマ血清を用いて最初に阻害した後，この組織を室温で30分間ハイブリドーマ培養上澄と反応させた．切片を PBS で10分間洗浄し，室温で30分間ビオチン化ウマ抗マウス IgG と反応させた後，アビジン−ビオチン標識ペルオキシダーゼと1時間反応させた．次に，組織を洗浄し，5分間 DAB 溶液（0.5 mg/ml Diaminobenzidine, 0.1% H_2O_2, 50 mM Tris-HCl 緩衝液 pH 7.0）と反応させた．

胸水細胞及び血球の免疫蛍光染色

Ficoll-Hypaque を用いた密度勾配遠心法〔比重：1.007± 0.001（標準偏差）〕により，胸水細胞及び

Ficoll-Hypaque [specific gravity 1.007±0.001 (SD)]. Granulocytes were obtained by the dextran sedimentation method from the bottom layer formed after density centrifugation of peripheral blood. That is, after 3.0% dextran was added to the centrifuged preparation and it was left to stand, the top granulocyte layer was collected and granulocytes were further purified hemolyzing the contaminating red blood cells by adding 0.83% ammonium chloride and 20 mM tris-hydrochloric acid and washing.

末梢血単核球を中間層より採取した．末梢血の密度勾配遠心後に形成された下層から，デキストラン沈降法を用いて顆粒球を収集した．すなわち，3.0%のデキストランを遠心分離標本に添加し，これを静置した後，上部の顆粒球層を採取し，0.83%塩化アンモニウムと20 mM tris-hydrochloric acid を加えて洗浄することにより，混入した赤血球を溶血させて顆粒球を更に精製した．

Cells (5×10^5) obtained by the above-described methods and 0.5 ml of hybridoma culture supernatant were allowed to react for one hour and washed, after which the preparation was allowed to react for 30 minutes with 10 μl of fluorescein isothiocyanate (FITC)-labeled goat antimouse IgG $F(ab')_2$ fragments (Tago Inc., Burlingame, Calif). After washing, the cells were fixed with 1% paraformaldehyde and analyzed using a fluorescent microscope (Nihon Kogaku, Japan) and a cell sorter (FACS 420, Becton Dickinson).

上述したように採取された5×10^5個の細胞と0.5 mlのハイブリドーマ培養上澄とを1時間反応させて洗浄した．次に，この標本を，10 μlのイソチオシアン酸フルオレッセイン（FITC）標識ヤギ抗マウスIgG $F(ab')_2$分画（Tago社，Burlingame，California）と30分間反応させた．洗浄後，細胞を1%パラホルムアルデヒドで固定し，蛍光顕微鏡（日本光学，日本）とセル・ソーター（FACS 420，Becton Dickinson）を用いて解析した．

Biochemical Study of Antigens Reacting with Monoclonal Antibodies

モノクローナル抗体と反応する抗原の生化学的検討

Test of sensitivity to proteinase. The 10^6 VMRC-LCR cells were allowed to react with 0.25% trypsin or 0.25% pronase for 30 minutes and washed with PBS, after which the cells were studied by the aforementioned fluorescent antibody technique.

蛋白分解酵素に対する感受性の検査．10^6個のVMRC-LCR細胞を0.25%トリプシン，又は0.25%プロナーゼと30分間反応させ，PBSで洗浄した後，前述の蛍光抗体法で調べた．

Test of sensitivity to periodic acid and neuraminidase. VMRC-LCR cells were cultivated to the limit of proliferation in a 96-well plastic microplate and fixed with 0.25% glutaraldehyde, after which 50 μl of periodic acid or neuraminidase was added and allowed to react for 30 minutes. After washing with PBS, hybridoma culture supernatant was allowed to react with the cells for one hour. The cells were washed and 1.0×10^5 cpm of ^{125}I-labeled goat antimouse immunoglobulin (New England Nuclear, Boston, Mass) was added and incubated for 60 minutes after which they were washed seven times. Then, the cells were rendered soluble with 100 μl of 2.0 N sodium hydroxide and transferred to a polystyrene tube, and their radioactivity was determined with a gamma counter. Periodic acid and neuraminidase were used with three different concentrations, 1, 10 and 100 mM and 0.01, 0.1 and 1.0 μ/ml, respectively. As

過ヨウ素酸及びノイラミニダーゼに対する感受性の検査．VMRC-LCR細胞を96穴プラスチック・マイクロプレートで増殖限界まで培養し，0.25%グルタルアルデヒドで固定した後，50 μlの過ヨウ素酸，又はノイラミニダーゼを加え，30分間反応させた．PBSで洗浄した後，ハイブリドーマ培養上澄とこの細胞を1時間反応させた．この細胞を洗浄し，1.0×10^5 cpmの^{125}Iで標識したヤギ抗マウス免疫グロブリン（New England Nuclear，Boston，Massachusetts）を添加し，60分間培養した後，7回洗浄した．次に，この細胞を100 μlの0.2N水酸化ナトリウムで溶解し，ポリスチレン試験管に移した後，その放射活性をガンマ・カウンターで測定した．過ヨウ素酸及びノイラミニダーゼは，三つの異なる濃度，すなわち，1，10及び100 mMと0.01，0.1及び1.0 μ/mlで用いた．

negative controls, the culture supernatants of MOPC 21 (IgG_1) and MOPC 104E (IgM) mouse myeloma cells were used.

RESULTS

Production and Screening of Hybridomas

After cell fusion, 17 wells whose supernatants showed reactivity against VMRC-LCR cells were studied. Upon cloning those wells, 268 clones were obtained. Of these clones, 89 had supernatants reactive with VMRC-LCR cells and 27 of those 89 did not react with the normal lung fibroblast cell line CCD-18Lu. The reactivity of the supernatants of these 27 clones to seven lung cancer cell lines including the immunizing cell line VMRC-LCR cells was tested, and 6 clones showing distinct patterns of reactivity to the cell lines were selected for further study. Further, two of these six clones, KL-3 and KL-6, were found by immunoperoxidase staining to react strongly with many lung cancer tissues. The Ouchterlony method showed that the KL-3 antibodies belong to the IgM class and the KL-6 antibodies to the IgG_1 class.

Biochemical Properties of Antigens

Expression of the KL-3 antigens on the surface of VMRC-LCR cells was not changed by treatment with trypsin and pronase. KL-6 antigens were resistant to tripsin but were mildly sensitive to pronase treatment. The effects of periodic acid and neuraminidase treatment were studied in the ^{125}I-immunoglobulin binding assay. KL-3 antigens showed sensitivity to periodic acid, but the binding of KL-3 antibody increased after neuraminidase treatment. KL-6 antigens showed sensitivity to both periodic acid and neuraminidase. Thus, KL-3 antigens appear to be carbohydrate antigens without terminal sialic acid, and KL-6 antigens appear to be glycoprotein antigens having a terminal sialic acid, and KL-6 antibodies recognize the carbohydrate portion.

Reactivity to Cell Lines

The reactivity to various cell lines derived from the lung was studied by the enzyme-labeled antibody method, with results as shown in Table 1. KL-3 antibodies reacted with lung adenocarcinoma and small cell carcinoma cells, but not with squamous cell carcinoma. On the other hand, KL-6 antibodies reacted with cell lines derived from these three histological types of lung cancer.

陰性の対照として，MOPC 21 (IgG_1) と MOPC 104E (IgM) のマウス骨髄細胞の培養上澄を用いた．

結　果

ハイブリドーマの産生とスクリーニング

細胞融合を行った後，上澄が VMRC-LCR 細胞に対して反応した17個の穴を調べた．これらの穴に対してクローニングを行ったところ，268個のクローンが得られた．これらのクローンのうち，89個の上澄は VMRC-LCR 細胞と反応し，そのうち27個は正常肺線維芽細胞株 CCD-18Lu と反応しなかった．免疫に使った細胞株 VMRC-LCR 細胞など七つの肺癌細胞株に対する，これら27個のクローンの上澄の反応性を検査した．これらの細胞株に対して特異な反応パターンを示した6個のクローンを，更に検査するために選んだ．更に，免疫ペルオキシダーゼ染色によって，これら6個のクローンのうちの2個，すなわち，KL-3 と KL-6 は多くの肺癌組織と強く反応することが判明した．また，Ouchterlony 法により，KL-3抗体は IgM クラスに，KL-6抗体は IgG_1 クラスに属することがわかった．

抗原の生化学的特徴

VMRC-LCR 細胞上での KL-3抗原の発現は，トリプシン及びプロナーゼによる処理によって変化しなかった．KL-6抗原はトリプシンに対して耐性を示したが，プロナーゼ処理に対しては軽度の感受性を示した．過ヨウ素酸及びノイラミニダーゼによる処理の影響を ^{125}I-免疫グロブリン結合測定法を用いて調べた．KL-3は過ヨウ素酸に対して感受性を示したが，KL-3の結合度はノイラミニダーゼ処理の後増大した．KL-6抗原は，過ヨウ素酸とノイラミニダーゼの両方に感受性を示した．したがって，KL-3抗原は，末端シアル酸を含まない糖質抗原と思われ，KL-6抗原は，末端シアル酸を有する糖蛋白抗原と思われる．また，KL-6抗体は糖質部分を認識する．

細胞株に対する反応性

肺由来の様々な細胞株に対する反応性を酵素抗体法で調べた．その結果は表1に示すとおりである．KL-3抗体は肺腺癌細胞及び小細胞癌細胞と反応したが，扁平上皮癌とは反応しなかった．一方，KL-6抗体は，肺癌のこれら三つの組織型に由来する細胞株と反応した．

TABLE 1 REACTIVITY OF MONOCLONAL ANTIBODIES TO CELL LINES BY ENZYME IMMUNOASSAY

表 1 酵素抗体法によって測定した各細胞株に対するモノクローナル抗体の反応性

Origin	Cell Line	Reactivity[a]	
		KL-3	KL-6
Lung adenocarcinoma	VMRC-LCR	+++	+++
	VMRC-LCD	−	−
	Luci 3	+	+++
	Luci 10	+	++
	ABC-1	−	+++
Lung squamous cell carcinoma	RERF-LC-AI	−	+
	SK-MES 1	−	−
	PC-1	−	+
Lung small cell carcinoma	QG-90	+	−
	SBC-5	−	+
	SK-AK-LCL	+++	−
	SBC-3	−	−
Lung normal fibroblast	CCD-18Lu	−	−
Nasopharyngeal carcinoma	K.B.	−	+++
Laryngeal carcinoma	HEp-2	−	+
Gastric carcinoma	AZ-521	−	−
Hepatoma	SK-HEP 1	−	−
Pancreatic carcinoma	Panc-1	++	+++
	PK-1-66	++	−
	MIA PaCa-2	−	−
Colorectal carcinoma	SW 1222	+++	−
Renal carcinoma	Scattola	−	+
Bladder carcinoma	T-24	+	+++
Cervical carcinoma	ME-180	+++	++
Breast carcinoma	YMB-1	+++	+++
Melanoma	MeWo	+	−
Sarcoma	U-20-S	+	++

[a]The reactivity was scored as follows: −, ΔOD=~0.019; +, ΔOD=0.020~0.049; ++, ΔOD=0.050~0.099; +++, ΔOD=0.100~

As regards reactivity to cell lines derived from malignant tumors other than lung cancer, both antibodies were found to react with various malignant tumor cell lines including sarcoma cells although their reactivity differed.

肺癌以外の悪性腫瘍から得られた細胞株に対する反応性については，両抗体共に肉腫細胞など様々な悪性腫瘍細胞株と反応することがわかった．ただし，反応の特異性は異なっていた．

Antigen Distribution in Tissues

The distribution of antigens in frozen sections of various cancer tissues was studied by immunoperoxidase staining, the results of which are shown in Table 2. In lung cancer tissues, as with lung cancer-derived cell lines, KL-3 antigens were

組織における抗原の分布

様々な癌組織の凍結切片における抗原の分布を免疫ペルオキシダーゼ染色を用いて調べた．その結果を表 2 に示した．肺癌由来細胞株と同様，肺癌組織に

TABLE 2 REACTIVITY OF MONOCLONAL ANTIBODIES WITH FROZEN TISSUES BY IMMUNOPEROXIDASE STAINING

表 2 免疫ペルオキシダーゼ染色による凍結切片に対するモノクローナル抗体の反応性

Organ	Histology	Reactivity[a] (No. positive/No. tested)	
		KL-3	KL-6
	Malignant		
Lung	Adenocarcinoma	+ (12/16)	++ (15/15)
	Squamous cell carcinoma	− (0/ 6)	++ (7/ 7)
	Small cell carcinoma	+ (4/ 5)	+ (4/ 5)
Stomach	Adenocarcinoma	++ (3/ 3)	+ (3/ 3)
Ampulla vater	Adenocarcinoma	+ (1/ 1)	+ (1/ 1)
Bile duct	Adenocarcinoma	++ (1/ 1)	++ (1/ 1)
Pancreas	Adenocarcinoma	++ (3/ 3)	++ (3/ 3)
Colon	Adenocarcinoma	+ (1/ 2)	+ (1/ 2)
Rectum	Adenocarcinoma	+ (1/ 1)	+ (1/ 1)
Thyroid	Papillary adenocarcinoma	++ (1/ 1)	+ (1/ 1)
	Meddulary carcinoma	− (0/ 1)	− (0/ 1)
Breast	Meddulary tubular carcinoma	+ (1/ 2)	+ (2/ 2)
Kidney	Transitional cell carcinoma	+ (1/ 1)	+ (1/ 1)
Esophagus	Squamous cell carcinoma	++ (1/ 2)	++ (1/ 2)
Cervix	Squamous cell carcinoma	− (0/ 1)	− (0/ 1)
Liver	Hepatocellular carcinoma	− (0/ 1)	− (0/ 1)
	Normal		
Lung	Alveoli	− (0/11)	+ (11/11)
	Bronchioles	+ (3/ 3)	++ (3/ 3)
	Bronchus	− (0/ 1)	− (0/ 1)
	Bronchial gland	− (0/ 1)	+ (1/ 1)
Thyroid	Follicle epithelium	+ (1/ 1)	++ (1/ 1)
Esophagus	Epithelium	− (0/ 1)	++ (1/ 1)
	Duct of esophageal gland	+ (1/ 1)	++ (1/ 1)
Stomach	Surface mucous cells	+ (1/ 2)	− (0/ 2)
	Fundic gland	− (0/ 1)	++ (1/ 1)
	Pyloric gland	+ (2/ 2)	− (0/ 2)
Duodenum	Epithelium	+ (2/ 2)	− (0/ 2)
Colon	Epithelium	+ (2/ 2)	− (0/ 2)
Rectum	Epithelium	+ (1/ 1)	− (0/ 2)
Liver	Hepatic cells	− (0/ 1)	− (0/ 1)
Pancreas	Gland	− (0/ 2)	− (0/ 2)
	Secretary duct	++ (2/ 2)	+ (2/ 2)
Kidney	Glomerulus	− (0/ 1)	− (0/ 1)
	Renal tubule	+ (1/ 1)	+ (1/ 1)
Bladder	Transitional epithelium	+ (1/ 1)	++ (1/ 1)
Uterus	Cervix	− (0/ 1)	− (0/ 1)
	Endometorium	− (0/ 1)	+ (1/ 1)
Skin	Epidermis	− (0/ 1)	− (0/ 1)
	Hair follicle	+ (1/ 1)	− (0/ 1)

[a]The staining was scored as follows: −, negative; +, positive; ++, strong positive

found only in adenocarcinoma and small cell carcinoma but they were not detected in squamous cell carcinoma. KL-6 antigens appeared in all three histological types of lung cancer. A photograph of an immunoperoxidase-stained case of lung adenocarcinoma is shown in Figure 1. Neither of the two antibodies react with the interstitium. KL-3 antibodies stain the entire circumference of the cell membrane of cancer cells and, KL-6 antibodies strongly stain their lumen side.

おいても，KL-3抗原は腺癌と小細胞癌のみに認められ，扁平上皮癌には検出されなかった．KL-6抗原は，肺癌の三つの組織型すべてに観察された．肺腺癌を免疫ペルオキシダーゼで染色したものの写真を図1に示した．両抗体共に間質とは反応していない．KL-3抗体は癌細胞の表面の全周径を染色し，KL-6はその内腔側を強く染色している．

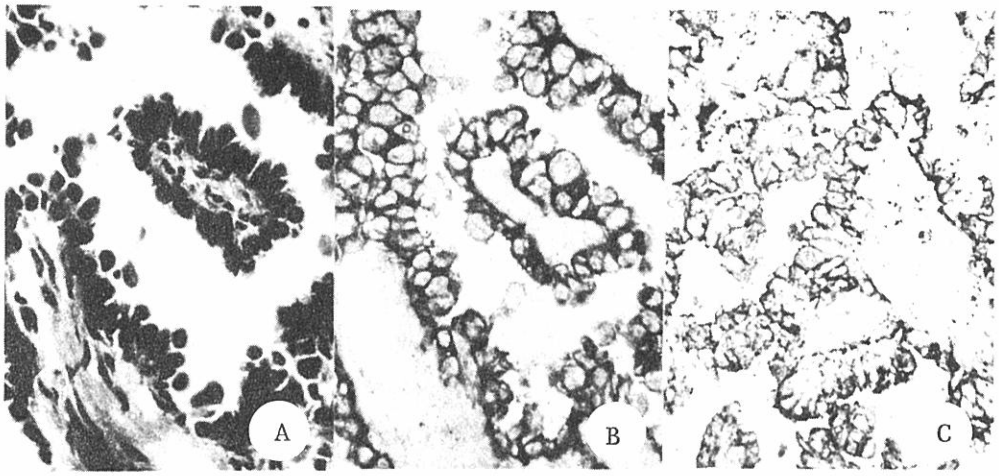

Figure 1. Immunoperoxidase staining of a well-differentiated papillary (alveolar bronchiole)-type lung adenocarcinoma. Frozen sections were stained with A. hematoxylin-eosin, B. KL-3 antibodies, and C. KL-6 antibodies.

図1．免疫ペルオキシダーゼで染色した高分化型乳頭（肺胞細気管支）型肺腺癌．凍結切片は，A．ヘマトキシリン－エオシン，B．KL-3抗体，及び C．KL-6抗体で染色した．

The distribution patterns of KL-3 and KL-6 antigens in the cancer tissues of organs other than the lung are very similar, with these antigens being detected in most of the different adenocarcinomas and in squamous cell carcinoma of the esophagus. KL-3 antigens were found only in the bronchiolar epithelia in the normal lung, and they were not detected in the pulmonary alveoli, bronchial epithelia or bronchial glands. KL-6 antigens were found on type II alveolar pneumocytes, bronchiolar epithelia, and bronchial gland cells.

肺以外の臓器の癌組織におけるKL-3及びKL-6抗原の分布パターンは酷似しており，これらの抗原は，種々の腺癌及び食道の扁平上皮癌で検出された．KL-3抗原は，正常肺の細気管支上皮にのみ認められ，肺胞，気管支上皮及び気管支腺には検出されなかった．KL-6抗原は，Ⅱ型肺胞上皮，細気管支上皮及び気管支腺細胞に観察された．

Study of the distribution of the two antigens in various epithelial cells other than those of the

肺の上皮細胞以外の様々な上皮細胞におけるこれら2種の抗原の分布を調べたところ，両抗原共に広く

lung showed that while both antigens were found extensively, the patterns of expression of the two, insofar as they were studied, differed in the following respects. KL-3 antigens were present in the epithelia of the gastric mucosa, pyloric gland, duodenum, colon, and rectum, but KL-6 antigens were not found in these tissues. They were detected in the esophageal epithelium, fundus gland, and endometrium.

Though not shown in the table, no antigens with which the two antibodies reacted were present on either normal peripheral blood mononuclear cells or granulocytes, nor were such antigens detected on O and AB type erythrocytes.

Reactivity of Monoclonal Antibodies to Cells from Pleural Effusions

The reactivity by the fluorescent antibody technique of KL-3 and KL-6 antibodies to pleural effusion cells is shown in Table 3. The KL-3 antibodies, differing from their reactivity to frozen tissue sections, did not react with lung adenocarcinoma-derived effusion cells. KL-6 antibodies reacted with the cells of lung adenocarcinoma (7/8) and of small cell carcinoma of class V cytology and did not react with the cells of squamous cell carcinoma. As regards metastatic lung tumors, although only one case each of adenocarcinoma originating in the stomach and pancreas were studied, KL-3 antibodies reacted with both cancers and KL-6 antibodies reacted only with that originated in the pancreas. Both antibodies reacted strongly with malignant mesothelioma. On the other hand, neither of the two antibodies reacted with cells derived from patients with class I or II pleural effusion cytodiagnosis, nor did they react with pleural effusion cells derived from noncancer patients. Numerous inflammatory mesothelial cells were present in the pleural effusion of case PL-33 (class II) in particular, but neither of the antibodies showed any reaction with these cells. Incidentally, it is interesting that cells strongly reactive with KL-6 antibodies were found in the pleural effusion of case PL-5 identified as class III on pleural effusion cytodiagnosis, and this case was diagnosed as lung adenocarcinoma with carcinoma invasion to pleura as a result of lung biopsy at thoracotomy performed three months later. The cells diagnosed as class III on cytodiagnosis are believed to have been adenocarcinoma cells.

分布しているが，検討した限りにおいて，両者の発現様式は次の点で異なっていた．KL-3抗原は，胃粘膜，幽門腺，十二指腸，結腸及び直腸の上皮に存在したが，KL-6抗原はこれらの組織には認められなかった．後者は，食道上皮，胃底腺及び子宮内膜で検出された．

表には示さなかったが，正常末梢血単核球や顆粒球のいずれにも両抗体と反応する抗原は存在せず，また，O及びAB型の赤血球にはこのような抗原は検出されなかった．

胸水由来細胞に対するモノクローナル抗体の反応性

胸水細胞に対するKL-3及びKL-6抗体の反応性を蛍光抗体法で測定した結果を表3に示す．KL-3抗体は，凍結組織切片に反応した場合とは異なり，肺腺癌由来胸水細胞とは反応しなかった．KL-6抗体は，肺腺癌（7/8）及び細胞診クラスVの小細胞癌の細胞と反応したが，扁平上皮癌細胞とは反応しなかった．転移性肺癌に関しては，胃及び膵臓由来の腺癌を1例ずつ検討したのみであるが，KL-3抗体は両方の癌と反応し，KL-6抗体は膵臓由来の癌のみと反応した．両抗体共に悪性中皮腫と強く反応した．一方，両抗体のいずれも，細胞診クラスⅠ又はクラスⅡの胸水細胞を有する患者から得られた細胞とは反応しなかったし，また，癌以外の患者から得られた胸水細胞とも反応しなかった．多数の炎症性中皮細胞が，特に症例PL-33（クラスⅡ）の胸水に認められたが，両抗体のいずれも，これらの細胞とは反応しなかった．ついでながら，胸水の細胞診でクラスⅢと診断された症例PL-5の胸水に，KL-6抗体と強く反応する細胞が検出されたことは興味深い所見である．3か月後に実施された開胸手術での肺生検の結果，この症例は胸膜への浸潤を伴う肺腺癌と診断された．細胞診でクラスⅢと診断された細胞は腺癌細胞であると考えられる．

TABLE 3 REACTIVITY OF MONOCLONAL ANTIBODIES WITH TUMOR CELLS IN PLEURAL EFFUSIONS BY IMMUNOFLUORESCENCE STAINING

表３　免疫蛍光法による胸水中の腫瘍細胞に対するモノクローナル抗体の反応性

Diagnosis	Case	Cytology Class	Reactivity[a]	
			KL-3	KL-6
Lung adenocarcinoma	PL- 5	III	–	++
	PL- 8	V	–	+
	PL-18	V	–	–
	PL-20	V	–	++
	PL-25	V	–	++
	PL-32	V	–	++
	PL-35	V	–	++
	PL-37	V	–	++
Lung squamous cell carcinoma	PL- 7	V	–	–
	PL- 9	II	–	–
	PL-10	III	–	–
Lung small cell carcinoma	PL-21	I	–	–
	PL-24	V	–	W
Metastatic lung cancer				
Pancreas carcinoma	PL-19	V	+	+
Gastric carcinoma	PL-22	V	+	–
Mesothelioma	PL-31	V	++	++
Nonmalignant diseases	PL-28	I	ND[b]	–
	PL-29	I	ND	–
	PL-33	II	–	–
	PL-42	I	–	–
	PL-44	I	–	–
	PL-45	I	–	–

[a]The staining was scored as follows: –, negative; W, weak; +, positive; ++, strong positive

[b]ND, not done

Figure 2 shows pleural effusion cells derived from case PL-35 (lung adenocarcinoma, class V) fluorescently stained with KL-6 antibodies. The surface of the cell membrane is strongly fluorescent. Figure 3 shows the results of analysis with a cell sorter of pleural effusion cells of case PL-25 (lung adenocarcinoma, class V) fluorescently stained with KL-6 antibodies. Compared with nonimmune mouse IgG used as a negative control, the intensity of fluorescence of the cells stained with KL-6 antibodies is remarkably increased

DISCUSSION

Two MoAbs, KL-3 and KL-6, generated by immunization with the lung adenocarcinoma-derived cell line VMRC-LCR, appear to be clinically useful. The antigen determinants

図２に，症例 PL-35（肺腺癌，クラスV）から得られた胸水細胞をKL-6抗体で蛍光染色したものを示した．細胞膜の表面は強い蛍光を示している．図３には，KL-6抗体で蛍光染色した症例 PL-25（肺腺癌，クラスV）の胸水細胞をセル・ソーターで分析した結果を示した．陰性対照として用いた非免疫マウスIgGと比較して，KL-6抗体で染色した細胞の蛍光強度は著しく増大している．

考　察

肺腺癌由来細胞株 VMRC-LCR を用いた免疫処置によって産生された，２種のモノクローナル抗体，KL-3及びKL-6は，臨床的に有用と思われる．この二つの

which the two antibodies recognize are believed to be carbohydrates, and KL-6 antibodies appear to recognize terminal sialilated carbohydrate chains.

抗体が認識する抗原決定基は糖質と考えられ，また，KL-6抗体は末端のシアル化された糖鎖を認識すると思われる．

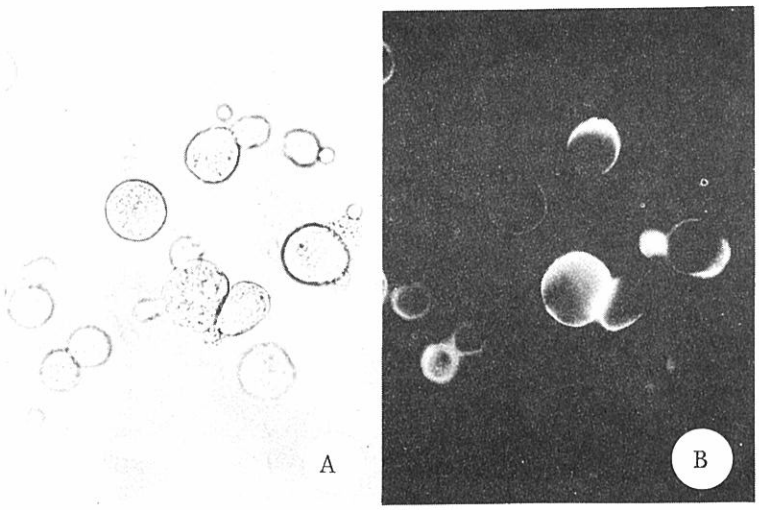

Figure 2. Immunofluorescence staining of cells in a pleural effusion of lung adenocarcinoma patients; A. bright field and B. fluorescence.

図２．免疫蛍光染色した肺腺癌患者の胸水細胞；A．明視野及び B．蛍光

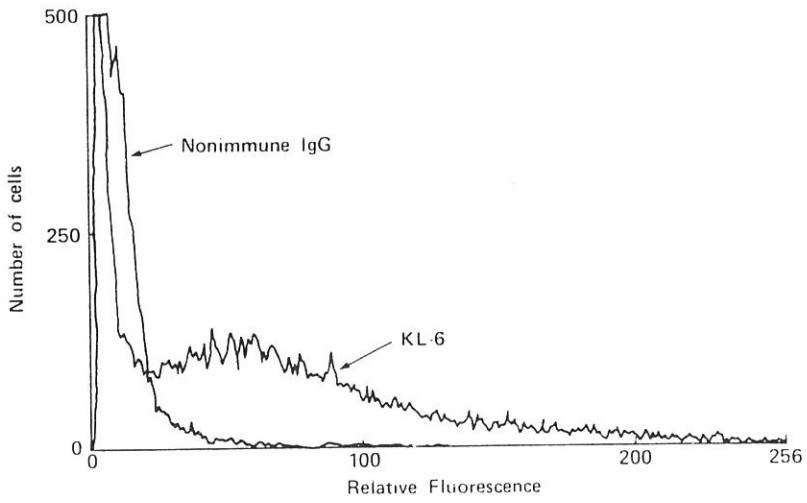

Figure 3. Flow cytometric analysis of the reactivity of KL-6 antibodies to cells in a pleural effusion of lung adenocarcinoma.

図３．肺腺癌の胸水細胞に対する KL-6抗体の反応性に関するフロー・サイトメトリーによる解析

Since the technique for continuous mass production of MoAbs was reported by Köhler and Milstein[12] in 1975, numerous MoAbs have been produced which recognize cancer-associated antigens, including lung cancer-associated antigens.[1-10] For most of them, however, tumor specificity has been established only by reactivity to cell lines and the number of antibodies whose clinical usefulness has actually been confirmed are few. The few MoAbs reported to have a high immunohistological specificity for lung cancer tissues include LuCa3 and LuCa4 antibodies against squamous cell carcinoma[11] reported by us, KS1/9 antibodies against adenocarcinoma reported by Varki et al,[13] and SM1 antibodies against small cell carcinoma reported by Bernal and Speak.[14] Only a few MoAbs such as the aforementioned LuCa3 and LuCa4 antibodies,[11] Ca1 antibodies against the laryngeal cancer cell line HEp2 reported by Woods et al,[15] and the B 72.3 antibodies against breast cancer cell membrane reported by Johnston et al[16] have been studied for their usefulness in the cytodiagnosis of malignant pleural effusion.

KL-3 antibodies appear to be useful in differentiating the histological types of lung cancer tissues because they reacted with adenocarcinoma and small cell carcinoma but not with squamous cell carcinoma. KL-6 antibodies, on the other hand, showed a high rate of reactivity with tissue sections of each of these histological types of lung cancer. However, since the antigens with which these antibodies react are also widely distributed in normal tissues and are found in most malignant tumors other than lung cancer, their tumor specificity and organ specificity are not high. However, these two antibodies showed, when experimentally applied in the cytodiagnosis of pleural effusions, a high tumor specificity limited to cells exudated in pleural effusions because they reacted with cancer cells and not with other cells in pleural effusions such as erythrocytes, lymphocytes, granulocytes, macrophages, and mesothelial cells. KL-3 antibodies were useful in detecting malignant cells of lung small cell carcinoma and metastasis to lung of other tumors, and KL-6 antibodies recognized malignant cells of lung adenocarcinoma in addition to metastasis to lung of pancreatic cancer.

We have suggestive evidence that the antigen determinants which the two antibodies recognize

モノクローナル抗体の連続大量産生方法が1975年にKöhler と Milstein[12] によって報告されて以来，肺癌関連抗原などの癌関連抗原を確認するモノクローナル抗体が大量に産生された．[1-10] しかし，その大部分において，腫瘍特異性は細胞株に対する反応性によって確認されたにすぎず，臨床的有効性が実際に認められた抗体の数は少ない．肺癌組織に対して高い免疫組織学的特異性をもつと報告された数少ないモノクローナル抗体は，我々が報告した扁平上皮癌に対するLuCa 3とLuCa 4抗体，[11] Varki ら[13] が報告した腺癌に対するKS1/9抗体，並びにBernal とSpeak[14] が報告した小細胞癌に対するSM1抗体である．悪性胸水の細胞診における有用性について検討が行われたのは，前述のLuCa 3及びLuCa 4抗体，[11] Woods ら[15] が報告した喉頭癌細胞株 HEp 2に対するCa 1抗体，並びにJohnston ら[16] が報告した乳癌細胞膜に対するB 72.3抗体など少数のモノクローナル抗体のみである．

KL-3抗体は腺癌及び小細胞癌と反応したが扁平上皮癌とは反応しなかったので，肺癌組織の組織型を鑑別するのに有効であると思われる．一方，KL-6抗体は，肺癌のこれらの組織型の組織切片各々に対して高い反応性を示した．しかし，これらの抗体が反応する抗原は正常組織にも広く分布し，また，肺癌以外の多くの悪性腫瘍に認められるので，その腫瘍特異性及び臓器特異性は高くない．しかし，これら二つの抗体は，胸水の細胞診に実験的に適用してみると，胸水中に滲出している細胞に対しては高い腫瘍特異性を示した．すなわち，両抗体は胸水中の癌細胞と反応し，赤血球，リンパ球，顆粒球，マクロファージ及び中皮細胞とは反応しなかったのである．KL-3抗体は肺小細胞癌の悪性細胞やほかの腫瘍の肺への転移を検出するのに有用であり，また，KL-6抗体は，肺腺癌の悪性細胞並びに膵臓癌の肺への転移に由来する悪性細胞を認識した．

両抗体が認識する抗原決定基が糖質であることを示唆する所見が得られた．抗原分子の生化学的特徴

are possibly carbohydrates. The biochemical properties of the antigen molecules are not known in detail at present and will have to be studied further. Although application of the two antibodies in cytodiagnosis of pleural effusion is considered possible, additional cases will have to be studied to confirm the initial results.

は現在のところ詳しくはわかっておらず，今後の研究をまたなければならない．胸水の細胞診に両抗体を用いることは可能と考えられるが，この最初の結果を確認するために更に症例研究を行うべきである．

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