Monoclonal Antibodies Reactive With Small Cell Carcinoma of the Lung[1,2,3]

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ABSTRACT—Murine monoclonal antibodies (MoAb) reactive with antigens associated with small cell carcinoma of the lung (SCCL) were prepared and partially characterized. Four were selected for further study on the basis of their lack of reactivity with normal leukocytes and erythrocytes. These MoAb, designated SCCL-41, SCCL-114, SCCL-124, and SCCL-175, are all IgM immunoglobulins. The binding of these MoAb to patient-derived SCCL tumor cells, SCCL cell lines, and non-SCCL cell lines was studied by indirect immunofluorescence and flow cytometry. Considerable heterogeneity in the expression of these cell surface antigens was noted among both the patient-derived tumor cells and the SCCL cell lines. One of the MoAb, SCCL-175, reacted with 7 of 7 patient-derived tumor cell samples and 9 of 10 SCCL cell lines. None of the antigens defined by these MoAb were expressed on non-SCCL lung tumor cell lines. SCCL-175 reacted with cells from both a choriocarcinoma and a colon carcinoma cell line, whereas the other 3 MoAb were unreactive with these and several other tumor cell lines. These MoAb may be useful in the diagnosis and subclassification of SCCL tumors.—JNCI 1984; 72:593-598.

SCCL is a heterogeneous disease in which several morphologic subclasses of tumor cells have been identified (*1, 2*). In addition, SCCL tumors often contain foci of non-SCCL lung tumor cells (*3, 4*). To study these and other parameters of heterogeneity in SCCL with specific probes, as well as to develop improved abilities to diagnose and treat this disease, we have prepared MoAb reactive with cells from SCCL tumors. Cells from patients with SCCL and cell lines derived from patients with SCCL were found to be reactive with these MoAb. In this paper we describe the preparation and specificity of these MoAb.

MATERIALS AND METHODS

Cell lines.—Cell lines studied included DMS 44, 47, 53, 79, 153, 187, 235, 406, 431, and 483, all of which were derived from patients with SCCL and have phenotypic characteristics of SCCL (*5-7*). These cell lines were cultured in either Waymouth's MB 752/1 medium containing 20% FBS (DMS 44, 53, 153, 187, 235, 406, 431, and 483) or RPMI-1640 medium with 20% FBS (DMS 79).

Non-SCCL tumor cell lines studied included IMR 32, a neuroblastoma line (obtained from the ATCC, Rockville, Md.); DMS 351, a malignant melanoma cell line (isolated from lymph node biopsy specimen); Squ Ca, a squamous cell lung carcinoma cell line (provided by K. Havemann and C. Gropp, Marburg, Federal Republic of Germany); DMS 485, a large cell undifferentiated lung tumor cell line (isolated from pleural fluid); A549, an adenocarcinoma lung tumor cell line (obtained from ATCC) (*8*); Ca Lu-1, a squamous cell lung carcinoma line (ATCC); SK-Lu-1, a lung adenocarcinoma cell line (obtained from Dr. J. Fogh, Sloan Kettering Cancer Institute) (*9*); BeWo, a choriocarcinoma cell line (ATCC); DLD-1, a colon carcinoma cell line (provided by D. Dexter, Providence, R.I.) (*10*); and HE-lung, embryonic lung fibroblasts (obtained from M.A. Bioproducts, Walkersville, Md.). Ca Lu-1 was cultured in McCoy's 5-A medium (GIBCO, Grand Island, N.Y.) with 10% FBS. A549, SK-LU-1, IMR 32, and HE-lung were cultured in Dulbecco's modified Eagle minimum essential medium with 10% FBS (HE-lung; 16% FBS); BeWo, DLD-1, DMS 485, and DMS 351 were all cultured in RPMI-1640 with 20% FBS.

The following leukemia cell lines were also studied; HL-60, a promyelocytic leukemia cell line (obtained from R. C. Gallo, National Institutes of Health, Bethesda, Md.) (*11*); K562, an undifferentiated myeloid leukemia cell line (*12*); Daudi, and Epstein-Barr virus-transformed B-cell line (*13*); and CCRF-CEM, a T-cell leukemia line (*14*). These lines were all cultured in RPMI-1640 with 20% FBS.

Patient cells.—Tumor cells from patients with SCCL were obtained from either surgical biopsy specimens (3 patients) or at autopsy performed within 4 hours of death (3 patients). Cells freshly isolated from

ABBREVIATIONS USED: ATCC=American Type Culture Collection; AZ=sodium azide; BSA=bovine serum albumin; FBS=fetal bovine serum; MoAb=monoclonal antibody (antibodies); PBS=phosphate-buffered saline; RIA=radioimmunoassay; SCCL=small cell carcinoma of the lung.

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[3] This study was presented in part at the annual meeting of the American Federation for Clinical Research, Washington, D.C., May 2, 1983, and appeared in abstract form in Clinical Research, Vol. 31, No. 2, p. 402A, 1983.

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primary or metastatic tumors, which in all cases were densely involved with SCCL, were gently teased apart into single-cell suspensions and passed through a stainless-steel filter. Cell viability was assessed by staining with acridine orange and ethidium bromide. Only samples with more than 50% viable cells were included in this study.

Normal cells.—Normal leukocytes were obtained from volunteers and separated into granulocyte, monocyte, and lymphocyte fractions as previously described (*15*). Erythrocytes typed with antisera to Lewis A and B blood group antigens were obtained from the Blood Bank of Mary Hitchcock Memorial Hospital, Hanover, N.H.

Preparation of hybridomas.—BALB/c mice were immunized ip three times over a 3-month period with 2×10^7 cells, which had been dissociated from the primary lung tumor of a patient with SCCL. This tumor was classified as "intermediate" in the modified World Health Organization classification (*2*). Fusion of spleen cells from an immunized mouse with murine myeloma cells of the NS-1 cell line was performed with the use of polyethlene glycol as the fusing agent as previously described (*16*). Hybridomas making MoAb reactive with the immunogen were selected by solid-phase RIA with the use of glutaraldehyde-fixed cells as previously described (*15*). Of these, 4 were selected for more extensive study on the basis of their relative specificity for the immunogen and lack of reactivity with normal leukocytes. These hybridomas were designated SCCL-41, SCCL-114, SCCL-124, and SCCL-175. All 4 of the MoAb produced were IgM antibodies.

Indirect immunofluorescence and cytofluorographic analysis.—The reactivity of these MoAb with SCCL cells was determined by indirect immunofluorescence and flow cytometry. Cells freshly isolated from primary or metastatic tumors were prepared as described above. Adherent cell lines were dissociated from the culture flask after 10-minute incubation with 0.01% EDTA disodium in calcium and magnesium-free Hank's balanced salt solution followed by washing with RPMI-1640. Cells were incubated for 30 minutes at 4°C with 0.5 ml hybridoma supernatant, unbound MoAb were removed by washing with 7 volumes of PBS (pH 7.4) containing 0.1% BSA and 0.05% AZ followed by the addition of fluorescein isothiocyanate-labeled $F(ab')_2$ goat anti-mouse antibody (Boehringer-Mannheim, Indianapolis, Ind.) for an additional 30 minutes. After another wash in PBS-BSA-AZ, the cells were analyzed for fluorescence on the Cytofluorograph 50H (Ortho Instruments, Westwood, Mass.) with the 2150 computer system. Simultaneous gating on both viable and single-cell populations was performed, and the data reported are those obtained from these populations. Positive control antibodies included an MoAb to beta-2-microglobulin, AML-1-201, and a mouse antiserum obtained by immunization with fresh SCCL cells. The negative control antibody was an IgM MoAb, SCC1, reactive with an irrelevant antigen (sheep erythrocytes).

Hapten inhibition studies.—To determine if any of these MoAb react with molecules that possess the terminal pentasaccharide, lacto-*N*-fucopentaose III, to which several reported anti-SCCL MoAb react (*17–19*), we performed inhibition studies. Purified LNF III (provided by V. Ginsburg, National Institutes of Health, Bethesda, Md.) was incubated with each of the MoAb and a known positive control MoAb reactive with LNF III, PM-81 (*20*), at a concentration of 5 mg/ml for 1 hour before addition of these mixtures to SCCL tumor cells from patient B (text-fig. 2). Quantitative comparisons of the binding of each MoAb either in the presence or absence of LNF III was determined by indirect immunofluorescence and flow cytometry.

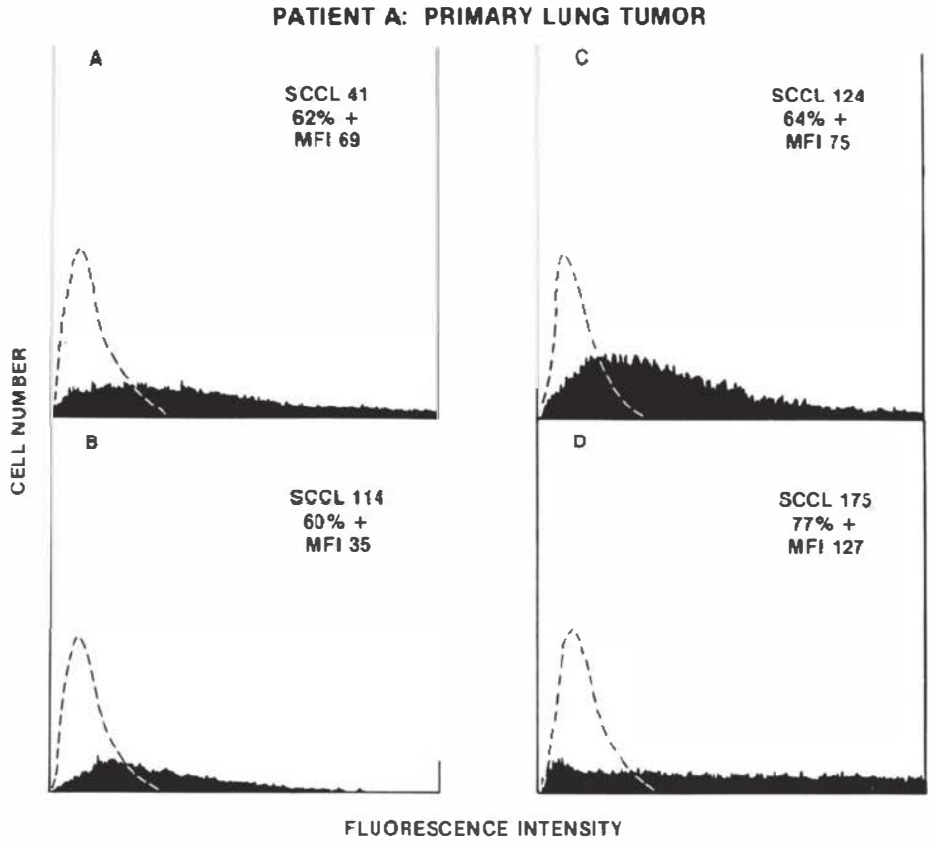
RESULTS

Specificity of hybridomas.—The reactivity of the 4 MoAb with the immunogen is shown in text-figure 1. Intense but variable fluorescence was observed with each MoAb. None of the MoAb reacted at all by indirect immunofluorescence with lymphocytes, monocytes, or granulocytes from the patient from whom the primary tumor was obtained or from 6 normal donors (data not shown), thus indicating that they are probably not reactive with histocompatibility or other common cell surface antigens. In addition, none of the MoAb reacted with erythrocytes from 4 normal donors (2 Lewis B and 1 Lewis A-positive, and 1 Lewis antigen-negative). The reactivities of these MoAb with cells from the primary lung tumor of a second patient with SCCL are shown in text-figure 2. In addition, liver metastases from the same patient were examined and found to have a similar antigenic profile, although staining was less intense in each case. A summary of the reactivities of the MoAb with tumor cells obtained from a total of 7 sites from 6 different patients is shown in table 1.

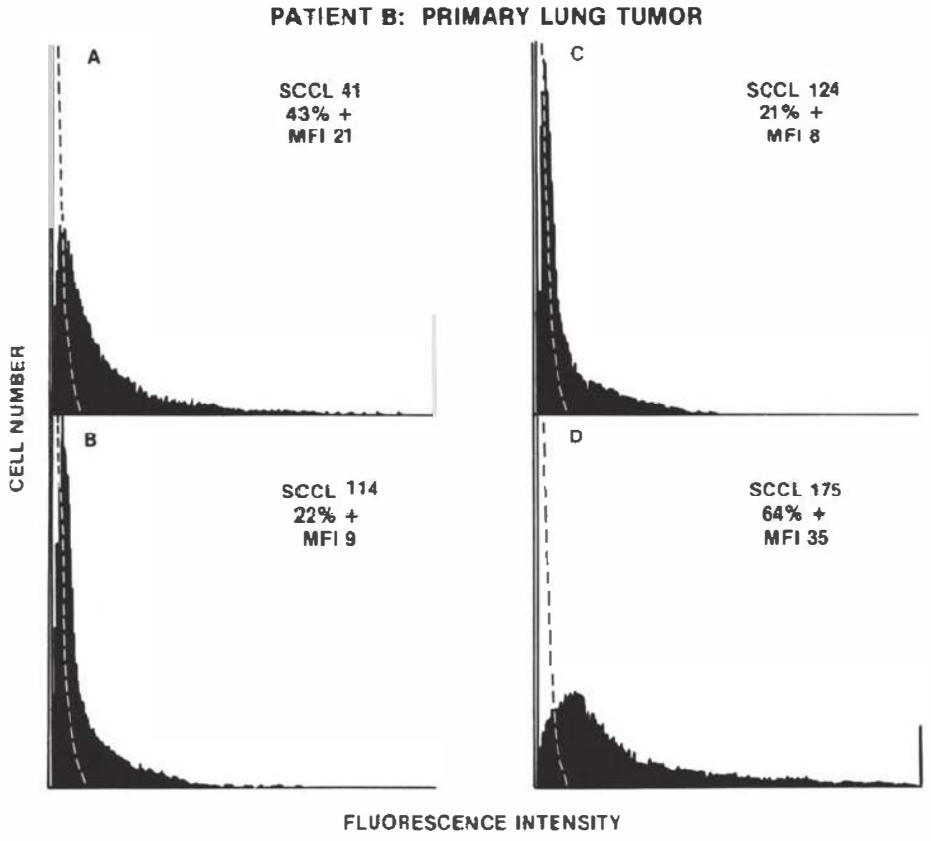
Reactivity with SCCL cell lines.—The expression of these antigens on several SCCL cell lines developed by Pettengill et al. (*5*) was evaluated. Consistent with other evidence of phenotypic heterogeneity in these cell lines, including differential peptide hormone secretion (*6, 21, 22*) and morphology (*5*), we have found considerable heterogeneity in antigen expression. As examples, the reactivities of MoAb SCCL-124 and SCCL-175 with 4 different SCCL cell lines are shown in text-figures 3 and 4. Marked heterogeneity in the expression of antigens defined by each MoAb was noted.

The pattern of reactivity of these MoAb with 10 different SCCL cell lines is shown in table 2. Interestingly, this pattern of reactivity is similar to that obtained with fresh tumor cells from patients. For example, SCCL-175 reacted significantly with all of the samples of fresh tumor tissue as well as the cell lines. In contrast, SCCL-41 reacted with only 4 of the 7 fresh tumor samples and with 3 of the 10 SCCL cell lines.

Reactivity with non-SCCL tumor cell lines.—To further study the specificity of these MoAb, we examined a number of lung tumor cell lines of non-SCCL histology, other solid tumor cell lines, and leukemia cell lines. None reacted with cells from several non-



TEXT-FIGURE 1.—Reactivity of 4 MoAb with patient A-derived SCCL cells. Cells from the primary lung tumor that were used as the immunogen for these hybridomas were studied by cytofluorography. The specific staining of tumor cells (*black area*) for each MoAb is shown in individual panels (A, SCCL-41; B, SCCL-114; C, SCCL-124; D, SCCL-175). The percentage of positive cells, as defined by fluorescence greater than on control MoAb-treated cells (*dotted line*), is shown as well as the mean fluorescence intensity (MFI). The *vertical line at the right of each panel* represents highly fluorescent cells.



TEXT-FIGURE 2.—Reactivity of 4 MoAb with patient B-derived SCCL cells. Cells obtained from the primary lung tumor of a patient with SCCL were studied by cytofluorography. See legend for text-figure 1 for details.

TABLE 1.—*Reactivity of MoAb with SCCL tumor cells freshly isolated from patients*[a,b]

MoAb	Percent positive cells for patient					Patient E	Patient F
	A	B1	B2	C	D		
SCCL-41	62	43	42	16	15	–	+
SCCL-114	60	22	12	5	22	–	+
SCCL-124	64	21	8	22	23	+	+
SCCL-175	77	64	36	36	53	+	+

[a] Reactivity of MoAb with SCCL cells was determined by indirect immunofluorescence and flow cytometry for patients A–D. The numbers reported for these patients' cells are the percentage of cells stained with specific MoAb fluorescing greater than cells stained with control IgM MoAb. Cells from patient E were examined by indirect immunofluorescence of tissue sections and patient F, by RIA. In the latter case, positive (+) indicates a significant reaction over background fluorescence. RIA counts were greater than three times background and similar to those obtained with an anti-SCCL antiserum.

[b] Samples B1 and B2 were primary lung tumor cells (B1) and liver metastases (B2) from the same patient. Cells obtained from patients A, B, and E were obtained at autopsy. Cells from patients C, D, and F were obtained by surgical biopsy. Samples, A, B1, C, and F were obtained from the primary lung tumor, samples B2 and E were liver metastases, and sample D was from a supraclavicular lymph node metastasis.

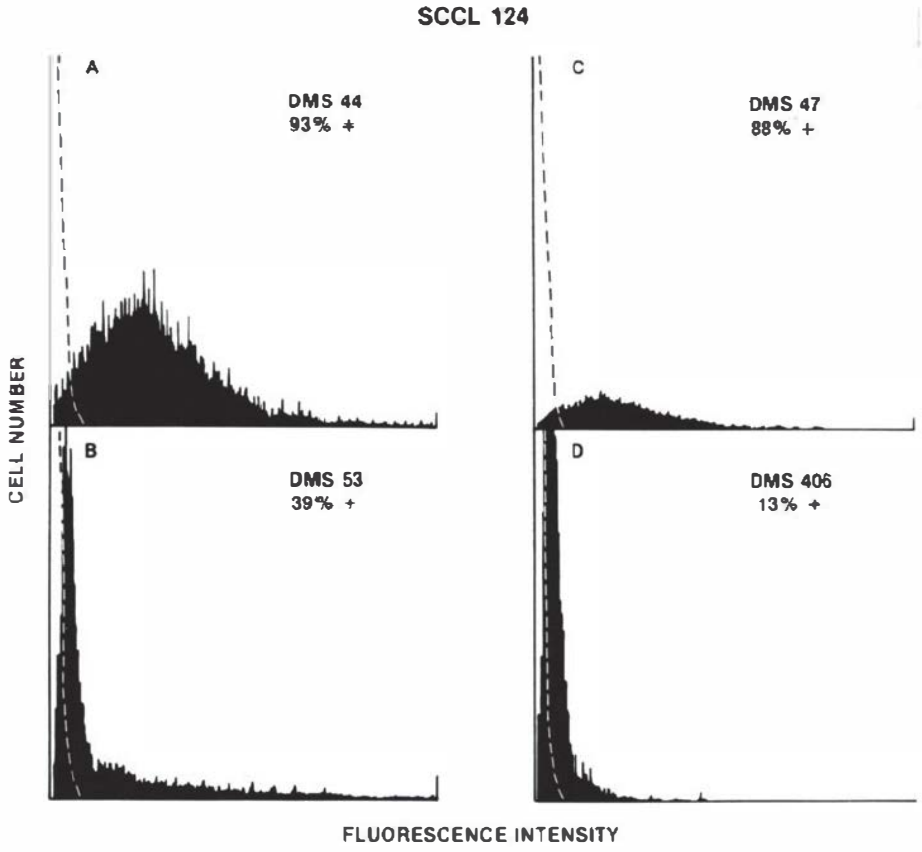
SCCL lung tumor cell lines of epidermoid, adenocarcinoma, and large cell phenotype (table 3). Only SCCL-175 reacted with cells from other tumor cell lines (choriocarcinoma and colon carcinoma, table 3). None of the MoAb reacted with cells from the leukemia cell lines K562, HL-60, Daudi, and CCRF-CEM (table 3). Finally, none of the MoAb reacted with HE-lung fibroblasts.

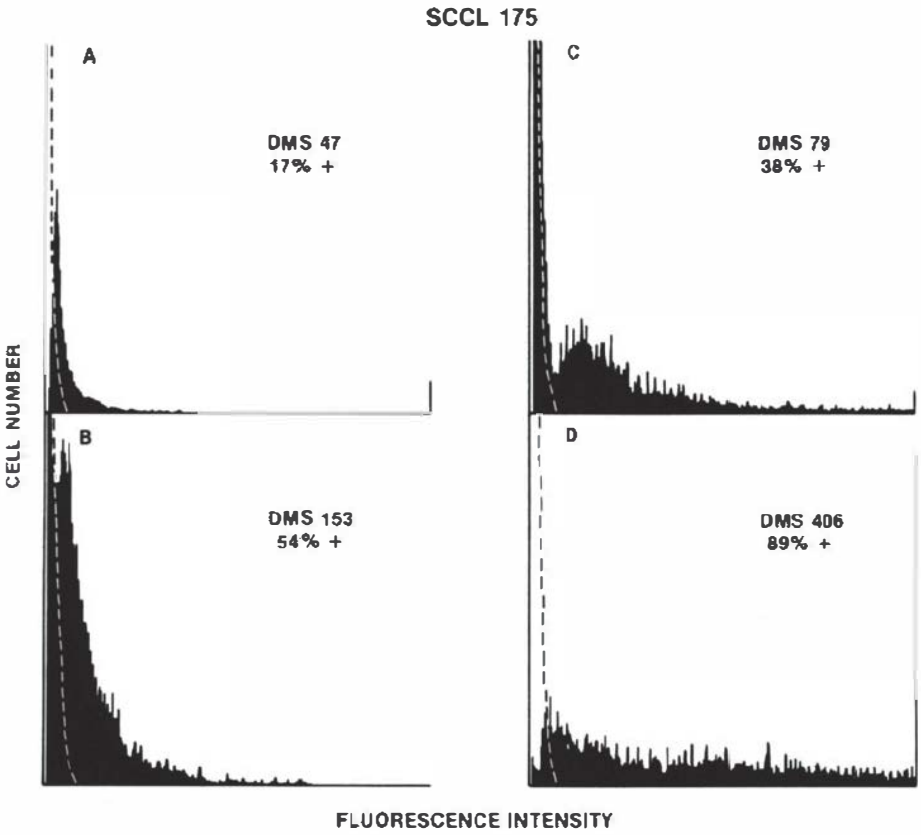
Hapten inhibition studies.—The binding of each MoAb to SCCL tumor cells was not inhibited by concentrations of purified LNF III that have been shown to inhibit completely binding of LNF III-reactive MoAb (*17*) and to abolish completely the binding of the PM-81 MoAb. The percentages of positive cells and their mean fluorescence intensities were nearly identical to those shown in text-figure 2 both in the presence and absence of LNF III.

DISCUSSION

Using the approach of immunizing with fresh SCCL tumor tissue, we have prepared and partially characterized 4 hybridoma-derived MoAb that appear to be relatively specific for SCCL. Our reason for selecting

TEXT-FIGURE 3.—Reactivity of MoAb SCCL-124 with 4 DMS SCCL cell lines as determined by cytofluorography.





TEXT-FIGURE 4.—Reactivity of MoAb SCCL-175 with 4 DMS SCCL cell lines as studied by cytofluorography.

fresh tumor tissue rather than an SCCL cell line as an immunogen was because of the possibility that a cell line(s) might not mirror the antigen profile of an in vivo tumor. Our approach was successful in that we were able to produce MoAb reactive with the cells used as immunogen as well as other patient-derived SCCL tumor cells and SCCL cell lines. We also showed that the MoAb are not reactive with common or histocompatibility antigens on leukocytes from either the patient from whom the tumor immunogen was derived or from normal donors. Moreoever, these MoAb do not react with glycolipids and, in particular, with the glycolipid molecules with which many reported anti-tumor cell, including anti-SCCL, MoAb react (*17–19*). This conclusion is based on the lack of reactivity of our MoAb with lipid extracts of SCCL tumor cells (Ginsburg V: Personal communication) and with human granulocytes, cells known to express molecules with the terminal pentasaccharide lacto-*N*-fucopentaose III to which many other anti-tumor cell and SCCL MoAb are directed (*17–19*). The inability of purified LNF III to block the binding of each MoAb supports this conclusion.

This report demonstrates another aspect of the

TABLE 2.—*Reactivity of MoAb with cells of the DMS SCCL cell lines*[a]

MoAb	DMS SCCL cell lines[b]									
	44	47	53	79	153	187	235	406	431	483
SCCL 41	–	–	+	–	–	–	–	+	–	+
SCCL 114	–	–	+	–	–	–	–	–	+	+
SCCL 124	+++	+++	+	–	–	+	+	–	–	+
SCCL 175	+	–	+	+	++	+	+	+++	+	+

[a] Reactivity of MoAb with SCCL cell lines was determined by flow cytometry.
[b] Reactions were scored as follows: –, <20% of cells positive; +, 20-40% positive; ++, 40-60% positive; +++, >60% of cells positive.

TABLE 3.—*Reactivity of MoAb with non-SCCL tumor cell lines*[a,b]

Cell lines		MoAb			
Tissue origin	Designation	SCCL 41	SCCL 114	SCCL 124	SCCL 175
Lung, squamous	Squ Ca	–	–	–	–
Lung, squamous	Ca Lu-1	–	–	–	–
Lung, large cell	DMS 485	–	–	–	–
Lung, adenocarcinoma	A549	–	–	–	–
Lung, adenocarcinoma	SK-LU-1	–	–	–	–
Neuroblastoma	1MR 32	–	–	–	–
Melanoma	DMS 351	–	–	–	–
Choriocarcinoma	BeWo	–	–	–	++
Colon carcinoma	DLD-1	–	–	–	+
Promyelocytic leukemia	HL-60	–	–	–	–
Myeloid leukemia	K562	–	–	–	–
B-cell leukemia	Daudi	–	–	–	–
T-cell leukemia	CCRF-CEM	–	–	–	–

[a] Reactivity of MoAb with cell lines was determined by flow cytometry.
[b] *See* footnote *b*, table 2.

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