Immunohistological Detection of Fucosyl-G<sub>m1</sub> Ganglioside in Human Lung Cancer and Normal Tissues with Monoclonal Antibodies

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ABSTRACT

With the aid of a highly specific murine monoclonal antibody, F12, an immunofluorescence method was elaborated that allowed sensitive and specific detection of the ganglioside antigen fucosyl-G<sub>m1</sub> (IV<sup>FucIIP</sup>NeuAcG<sub>0</sub>se4Cer) in different types of human lung cancer and normal tissues. Nineteen of 21 cases of small cell lung cancer were positive with the F12 immunofluorescence method as compared to 2 of 10 squamous epithelial cell lung cancers and 1 of 5 large cell lung cancer specimens. Specimens of lung adenocarcinoma (8 cases) and bronchioloalveolar carcinoma (3 cases) were all negative, as were 2 examined cases of neuroblastoma. No fucosyl-G<sub>m1</sub> could be detected in normal lung and bronchus. However, in thymus, spleen, and lamina propria of the small intestine sparsely distributed clusters of small round cells were stained as well as intramural ganglionic cells of the small intestine and islet cells of the pancreas. All other normal tissues tested were negative. Results obtained with immunofluorescence closely agreed with immunochemical determination of fucosyl-G<sub>m1</sub> in lipid extracts of tissues. Our findings suggest that fucosyl-G<sub>m1</sub> is strongly associated with small cell cancer of the lung and demonstrate that this tumor-associated antigen can be detected with high sensitivity and specificity with an immunofluorescence method based on the use of the F12 monoclonal antibody.

INTRODUCTION

SCCL<sup>3</sup> is a highly malignant tumor that comprises about 20% of all lung cancer cases (2). It differs from the other major lung cancer types by having a more rapid proliferation and a higher propensity for metastatic spread (3, 4), which in most instances renders it surgically incurable. On the other hand, compared with SQCL, LCCL, and ADCL, SCCL has a higher sensitivity for cytostatic drugs and is currently treated by combination chemotherapy with or without radiotherapy and prophylactic brain irradiation. However, despite impressive initial responses to such treatment in 50–80% of all patients the long-term prognosis of SCCL remains poor with a 5-year survival rate of 5–10% in unselected clinical materials (5).

The hybridoma technique by Köhler and Milstein (6) for the preparation of MAbs has revolutionized the possibilities for the identification of new tumor-associated antigens. MAbs specific for such antigens may be of great value in the diagnosis, treatment, and follow-up of malignant diseases (7). Several SCCL-associated antigens defined by MAbs have recently been described (8–14). One of these is the membrane-bound antigen fucosylsialosylgangliotetraose (Fucα1 → 2Galβ1 → 3GalNAcβ1 → 4(NeuAcα2 → 3)Galβ1 → 4Glc) as present in fucosyl-G<sub>m1</sub> gangliosides (13–15). We observed the presence of a large amount of fucosyl-G<sub>m1</sub>, with an unusual ceramide portion containing 2-hydroxylated fatty acids in one case of SCCL (13) and demonstrated subsequently by immunobiochemical methods using F-MAbs that this antigen was present in more than 90% of the SCCL specimens tested (14).

We now describe a sensitive and specific immunofluorescence method for the detection of fucosyl-G<sub>m1</sub> in tissue sections based on the use of the best of several tested F-MAbs designated F12. Using this method we have examined the distribution of fucosyl-G<sub>m1</sub> in normal and lung cancer tissues and compared the results with those obtained in parallel immunochemical analyses of fucosyl-G<sub>m1</sub> in glycolipid extracts from the same tissue specimens.

MATERIALS AND METHODS

Monoclonal Antibodies. The production of the 7 F-MAbs used in this study, F1, F3, F4, F6, F9, F10, and F12, has been described by us previously (14). The reactivities of these MAbs with a panel of solid-phase immobilized glycolipids of defined structures have been studied (15). The results suggest that these MAbs, which were raised against highly purified fucosyl-G<sub>m1</sub> ganglioside, may be divided into 3 classes with regard to specificity: Class 1, specific for fucosyl-G<sub>m1</sub> (F12); Class 2, cross-reactive with GM<sub>0</sub> (F4); and Class 3, reacting with a variety of glycolipids carrying determinants present on the terminal part of the carbohydrate structure of fucosyl-G<sub>m1</sub> (F1, F3, F6, F9, and F10). Unless stated otherwise undiluted culture supernatants from hybridomas cloned twice were used. The F12 MAb was concentrated by precipitation of hybridoma culture supernatants with 50% saturated ammonium sulfate solution, the precipitate redissolved in one-tenth of the starting volume and dialyzed against PBS. After addition of merthiolate to 0.01% (w/v) final concentration aliquots of the concentrated F12 preparation were stored at −70°C until use.

Cell Lines. The rat hepatoma cell line, H4-II-E (American Type Culture Collection, Rockville, MD), was cultured in Iscove's-medium supplemented with 10% fetal calf serum, trypsinized, washed, and suspended in PBS. Small cell lung cancer cell lines U-1285, U-1690, U-1906, U-2020, and U-2050 (16, 17) were cultured in suspension or semiadherently and were harvested by pipetting, washed, and suspended in PBS.

Human Cells. RBC were prepared from blood of healthy donors of all ABH blood groups. Freshly prepared suspensions of T- and B-cells and monocytes from heparinized blood samples (18, 19) were a gift from Dr. N. Lycke, Göteborg, Sweden. Nucleated bone marrow cells were prepared from aspirates with a normal cytological appearance by centrifugation on Lymphoprep (Nyegaard, Oslo, Norway).

Preparation of Cells for Immunofluorescence. Cells (10<sup>6</sup>) in PBS in a sample volume of 100 µl were prepared on glass slides by centrifugation in a Cytospin 2 (Shandon) at 1500 rpm for 3 min. Slides were then air dried, stored at −20°C, and used within 1 month.

Human Tissues. Tissue specimens obtained at autopsy were fixed in...
4% formaldehyde in PBS and then frozen in liquid nitrogen and stored at −70°C. Some autopsy and all biopsy specimens were directly frozen without previous formaldehyde fixation. Cryostat sections, 4-6 μm thick, were put on glass slides precoated with 0.3% gelatin (to prevent tissue sections from falling off during the staining procedure). Sections from all tumor tissues were histochemically stained to verify tumor cell presence and diagnoses by comparison with routinely stained diagnostic tissue specimens from the same patients. Lung tumors were classified according to the scheme recommended by WHO (20).

**Immunofluorescence Staining Procedure.** Air-dried cryostat sections of tissues were used. Different fixating agents were tested: acetone, 10 min; 95% ethanol, 30 min; 5% paraformaldehyde in PBS, 30 min; all at room temperature. PBS incubation instead of fixative for 30 min was also tested. Treatment with 7.5% sucrose in PBS after fixation was performed overnight at 4°C. The optimal dilutions of MAbs with SwAR-FITC followed by RAM-FITC (DAKO; Dakopatts, Copenhagen, Denmark) were done for 30 min each. An additional incubation step with RAM-FITC (DAKO) for 30 min was also tested. Normal serum homologous to the species of the tissue tested was mixed with the fluorescein-conjugated antiserum in equal volumes for adsorption. All dilutions of antiserum were made with 0.1% BSA in PBS. Optimal dilutions were determined by checkerboard titrations. Each incubation step during the staining procedure was followed by washing for 15 min with three changes of PBS. Slides were finally mounted with 87% glycerol in PBS.

**Immunofluorescence Scoring.** Immunofluorescence intensity was scored from 0 to 4; 0, total absence of fluorescence; 1, weak; 2, moderate; 3, strong; and 4, very strong. The percentage of the tumor cells that were scored 2-4 was estimated.

**Extraction and Immunohistochemical Detection of Fucosyl-GM<sub>1</sub>.** Total lipids from tumor tissues, normal tissues, and cell lines were extracted twice with 20 volumes of chloroform/methanol/water, 4/8/3 (v/v/v) (21). Monosialogangliosides were isolated by anion-exchange chromatography on Spherosil-DEAE-dextran as described previously (22). Detection of fucosyl-GM<sub>1</sub> in the monosialoganglioside fraction was performed by HPTLC immunostaining as described by Brockhaus et al. (23) using the F12 MAb. Aluminum-backed HPTLC plates, Silica Gel 60, were purchased from Merck, Darmstadt, Federal Republic of Germany. Bound F12 was detected with 1125-labeled goat anti-mouse antiserum (Radiochemical Centre, Amersham, United Kingdom) and visualized by exposure to X-AR5 X-ray film (Eastman-Kodak, Rochester, NY) for 12-24 h. A semiquantitative determination of fucosyl-GM<sub>1</sub> was achieved by comparison of the staining intensity obtained with samples from tissues with that of a standard preparation of fucosyl-GM<sub>1</sub> tested simultaneously in a series of known concentrations.

**RESULTS**

**Methodological Studies**

**Staining Procedure.** A standard immunofluorescence staining procedure, consisting of paraformaldehyde fixation and subsequent incubation with F12 MAb and RAM-FITC, resulted in staining with only moderate fluorescence intensity (score 2) of sections of a SCCL specimen despite a known high content of fucosyl-GM<sub>1</sub> in the tissue. In order to raise the sensitivity of the method we tested different fixatives, addition of sucrose incubation, and an extra step of fluorescein-conjugated antiserum. The best fixative turned out to be paraformaldehyde which provided for a homogeneous ring-shaped membrane-associated fluorescence. Ethanol fixation or PBS incubation only for 30 min resulted in a total abolishment of the fluorescence seen with paraformaldehyde fixation. Acetone on the other hand gave a fluorescence intensity equal to that seen with paraformaldehyde fixation, but the pattern was granular instead of ring shaped. The addition of an extra SwAR-FITC step after RAM-FITC clearly enhanced the fluorescence intensity further without any increase in background staining. Sucrose incubation overnight after fixation had only a marginal effect on the staining intensity but enhanced the quality of the staining by making the fluorescing structures more distinct. Whether or not the tissue had been fixed in formaldehyde before freezing and cutting did not influence the intensity or the quality of the staining to an appreciable extent. The final procedure adopted for the immunofluorescence detection of fucosyl-GM<sub>1</sub> in tissues was as follows: (a) paraformaldehyde fixation (5% in PBS for 30 min at room temperature); (b) sucrose treatment (7.5% in PBS for 4°C overnight); (c) MAb; (d) RAM-FITC; and finally (e) SwAR-FITC incubation (30 min each at room temperature). The dilutions of RAM-FITC and SwAR-FITC used, arrived at after checkerboard titrations, were 1/10 and 1/20, respectively, and the dilution of concentrated F12 MAb was 1/10.

**Reactivity of F-MAbs with H4-II-E Cells.** The rat hepatoma cell line H4-II-E contains large amounts of membrane-associated fucosyl-GM<sub>1</sub> as determined by HPTLC immunostaining of ganglioside extracts from cell pellets. The ability of the different F-MAbs to detect membrane-associated antigen in these cells was evaluated by immunofluorescence after staining with the optimal method. Two of the 7 tested F-MAbs, F1 and F6, did not react with H4-II-E cells. All the other MAbs stained this cell line, F12 giving the strongest reactions (Fig. 1). No F-MAb gave detectable staining of a fucosyl-GM<sub>1</sub>-negative colorectal carcinoma cell line, Colo 205 (Fig. 2a), which was included as a negative control. The Colo 205 cell line was strongly stained with MAb C-50 (Fig. 2b) directed against the CA-50 ganglioside antigen (7).

**Reactivity of Different F-MAbs with Human Tissues.** The reactivities of the different F-MAbs with SCCL tissues, small intestine, adrenal gland, and pancreas were then compared in order to select the most sensitive and specific MAb. All the tissues examined except adrenal gland contain fucosyl-GM<sub>1</sub> gangliosides as detected by immunochemical analyses of glycolipid extracts. The results are presented in Table 1. All F-MAbs tested react with fucosyl-GM<sub>1</sub> in the enzyme-linked immunosorbent assay. MAb F12, with the greatest specificity for fucosyl-GM<sub>1</sub> as assessed by previous studies (15), gave the most consistent staining reactions with the fucosyl-GM<sub>1</sub>-containing tissues and was therefore chosen for the following studies.

**Staining of Lung Tumor Tissues**

Both pulmonary lesions and metastases of lung cancer in other organs were tested (Table 2). Of specimens from 21 different patients with SCCL, 19 (90%) were stained by F12. In 10 of these cases all tumor cells were stained (Fig. 3). In 5 cases more than 50% of the tumor was stained whereas in 4
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Table 2 Immunofluorescence staining of SCCL tissues using the F12 MAb
For scoring refer to "Materials and Methods."

<table>
<thead>
<tr>
<th>SCCL specimen</th>
<th>Score</th>
<th>Fraction of tumor cells stained (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3003</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>0710</td>
<td>2</td>
<td>&gt;50</td>
</tr>
<tr>
<td>1210</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>0373-84</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>S862-84</td>
<td>3</td>
<td>&lt;50</td>
</tr>
<tr>
<td>S899-84</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>S950-84</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>S1087-84</td>
<td>2</td>
<td>&lt;50</td>
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<tr>
<td>S1119-84</td>
<td>3</td>
<td>&gt;50</td>
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<tr>
<td>S183-85</td>
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<td>3</td>
<td>&gt;50</td>
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<tr>
<td>3604</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>K120-84</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>S375-84</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>S863-84</td>
<td>4</td>
<td>&gt;50</td>
</tr>
<tr>
<td>S941-84</td>
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<tr>
<td>S975-84</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>S1093-84</td>
<td>3</td>
<td>&lt;50</td>
</tr>
<tr>
<td>S132-85</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>S185-85</td>
<td>2</td>
<td>&lt;50</td>
</tr>
</tbody>
</table>

Fig. 2. a. This human colon adenocarcinoma-derived cell line, Colo 205, does not contain any fucosyl-GM₁ and is unstained with MAB F12 by immunofluorescence. × 100. b. MAB C-50 (7) gives a strong immunofluorescence staining of Colo 205 cells. × 100.

Table 1 Immunofluorescence staining of different tissues with F-MAbs
For scoring refer to "Materials and Methods."

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Monoclonal antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCCL 1210</td>
<td>F1 F3 F4 F6 F9 F10 F12</td>
</tr>
<tr>
<td>SCCL 3003</td>
<td>0 3 2 0 2 3 3</td>
</tr>
<tr>
<td>Small intestine*</td>
<td>0 0 2 0 2 0 4</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>0 0 0 2 0 0 0</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0 0 2 0 2 2 3</td>
</tr>
</tbody>
</table>
| * Both adult and infant small intestine was tested.
| * Lamina propria cells and ganglionic cells.
| * Mesenchymal and smooth muscle.
| * Islet cells.

Reactivity with Normal Human Tissues

Immunofluorescence studies with F12 indicate that fucosyl-GM₁ is sparsely distributed in normal tissues outside the central nervous system. Tissues that did not stain with F12 included lung and bronchi, liver, kidney, lymph node, thyroid, parathyroid, adrenal, gastric mucosa and submucosa, large artery, and striated, smooth, and cardiac muscle. Bone marrow and peripheral blood cells, including preparations of B- and T-cells and monocytes, were also negative. However, in spleen, thymus, and small intestine clusters of small round cells were stained by F12. In spleen and thymus these cell populations constituted approximately 1 and 10% of the total cell content, respectively. In the small intestine the cells were located in the lamina propria. In this tissue the submucosal and myenteric ganglionic cells were also stained by F12. In pancreas more than 50% of the cells in each islet were positive.

Fucosyl-GM₁ in SCCL Cell Lines

Five established SCCL cell lines propagated in vitro were tested for their content of fucosyl-GM₁ using immunofluorescence with F12. In one cell line, U-2050, all cells were intensely stained with F12 (Fig. 5). In another cell line, (U-1690), 20–30% of the cells were positive. The remaining three cell lines all were completely negative (U-1285, U-1906, and U-2020). We also tested early passages of in vitro cultures from these...
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Fig. 4. a. In this specimen of a squamous epithelial cell lung cancer no cells are stained with MAb F12 by immunofluorescence. × 100. b. Immunofluorescence staining with MAb C-50 (7) of the same squamous epithelial cell lung cancer specimen as in a. Malignant cells are positive. × 100.

Table 3 Summary of F12 immunofluorescence staining of various tumor tissues

<table>
<thead>
<tr>
<th>Tumor histiotype</th>
<th>No. positive/ no. tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small cell lung cancer</td>
<td>19/21</td>
</tr>
<tr>
<td>Squamous cell lung cancer</td>
<td>2/10</td>
</tr>
<tr>
<td>Lung adenocarcinoma</td>
<td>0/8</td>
</tr>
<tr>
<td>Large cell lung cancer</td>
<td>1/5</td>
</tr>
<tr>
<td>Adenosquamous cell lung cancer</td>
<td>0/1</td>
</tr>
<tr>
<td>Bronchial carcinoid</td>
<td>0/3</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>0/2</td>
</tr>
<tr>
<td>Malignant schwannoma</td>
<td>0/1</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>0/1</td>
</tr>
</tbody>
</table>

Fig. 5. Immunofluorescence staining with MAb F12 of a human small cell lung carcinoma-derived cell line, U-2050. Almost 100% of all cells are positive, indicating that this cell line homogeneously expresses the ganglioside antigen fucosyl-GMI. × 100.

Fig. 3. a. In this specimen of a squamous epithelial cell lung cancer no cells are stained with MAb F12 by immunofluorescence. × 100. b. Immunofluorescence staining with MAb C-50 (7) of the same squamous epithelial cell lung cancer specimen as in a. Malignant cells are positive. × 100.

HPTLC Immunostaining of Monosialoganglioside Extracts

Monosialoganglioside fractions were isolated from 14 cases of SCCL, 4 SQCL, 4 ADCL, and 1 LCCL as well as from normal pancreas, spleen, thyroid, liver, and lung. The content of fucosyl-GMI in each case was then examined by HPTLC immunostaining with F12. Cell lines H4-II-E and U-2050 were also tested in this way. The results were compared with those obtained by F12 immunofluorescence staining of these cases. The results were concordant in all but one of the tumor tissue cases. In this SQCL specimen small foci of moderately stained cells were found in F12 immunofluorescence, but no fucosyl-GMI could be detected in the immunohistochemical staining of the ganglioside extract from this tumor. In normal tissues there was total agreement between the two methods. Fucosyl-GMI from SCCL tumors and U-2050 migrated in double bands parallel to reference preparations of fucosyl-GMI containing 2-hydroxylated fatty acids in the lipid portion. Fucosyl-GMI detected in the normal tissues and H4-II-E migrated in a single band parallel to a fucosyl-GMI reference containing only non-hydroxylated fatty acids.

DISCUSSION

In the present work we have developed an optimized immunofluorescence method, using the fucosyl-GMI specific monoclonal antibody F12, for the specific detection of fucosyl-GMI in sections of human lung tumor and normal tissues. The carbohydrate sequence of fucosyl-GMI has to date been found only in glycolipids. Like other glycolipids this antigen is readily eluted from paraffin-embedded tissues by organic solvents making it difficult to use this kind of material for the immunohistochemical detection of fucosyl-GMI. We therefore tested cryostat sections of tissues in combination with various fixatives. In our hands, the best fixative tested was paraformaldehyde. It was also necessary to include an additional secondary step of fluorescein-conjugated antiserum in order to enhance the sensitivity. Introduction of sucrose incubation (24) of tissue sections after paraformaldehyde fixation also improved the quality of the staining.

Using the optimized staining procedure we found that 90% of the SCCL cases tested contained fucosyl-GMI. In 4 of 19 positive SCCL cases only a minor fraction of the tumor cells was stained indicating a heterogeneity with respect to fucosyl-GMI expression between different areas within these tumors. Heterogeneity of tumor-associated antigen expression in tumors has also been reported by others (25). The majority of the SCCL cases studied by us were metastatic lesions presented at advanced stages of disease, and since there may be considerable morphological changes in these tumors (26–28) this may explain the heterogeneity of antigen expression observed. Discrepancies in antigen expression between different tumor locations within one patient have also been observed by us in cases where both pulmonary and hepatic lesions were tested. In one of the cases the lung tumor was positive and the liver metastasis was negative in F12 immunofluorescence, whereas in another case the reverse was found. Heterogeneity of fucosyl-GMI expression was also seen in one cell line derived from a clinical case of SCCL, where only 20–30% of the cells were positive. A difference was also seen between the clinical specimens and the cell lines in the percentage of the SCCL cases containing cells expressing fucosyl-GMI. More than 90% of the clinical tumor...
cases but only 2 of 5 cell lines were positive. On the other hand, when early passages of the negative cell lines were tested staining could clearly be seen in 5–10% of the cells in one case. Our results demonstrate that fucosyl-G<sub>M1</sub> is expressed at a high frequency in SCCL, but they also suggest potential instability with loss of this antigen during progressive tumor cell proliferation.

Current views suggest that SCCL and non-SCCL lung cancers represent a differentiation continuum with a common entodermal origin (27, 29–31). Our observation that some SQCL specimens contain foci of F12-positive cells is in accord with a lineage relationship between SCCL and SQCL. Expression of fucosyl-G<sub>M1</sub> in SQCL may thus indicate transition of SQCL to a more malignant state with a worse prognosis than for the fucosyl-G<sub>M1</sub>-negative tumor. If this prediction holds true it may have implications for the choice of therapy in these “borderline” cases.

The distribution of fucosyl-G<sub>M1</sub> in normal tissues outside the central nervous system seems to be quite restricted. Stained small round cells were seen in lymphoid tissues such as spleen, thymus and lamina propria of the small intestine. These cells may constitute a distinct subpopulation of lymphocytes with a specific role at their sites of localization, since no positive cells could be found in preparations of peripheral blood or bone marrow samples. Work is in progress to characterize these fucosyl-G<sub>M1</sub>-expressing cells and their functions. The other normal tissues showing staining with F12 are represented by the pancreas, where islet cells were stained, and the small intestine, where ganglionic cells also were positive.

In our earlier works we have described that all monoclonal antibodies raised against fucosyl-G<sub>M1</sub> react with purified fucosyl-G<sub>M1</sub> immobilized on plastic surfaces or silica gel plates (14, 15). In our present work we show that several of these F-MAbs (i.e., F1, F3, F6, F9, and F10) did not react with fucosyl-G<sub>M1</sub> in all sections that contained enough of this antigen to be detected with F12. Moreover, F4 does not seem to react with tissue-bound G<sub>M1</sub> ganglioside, which is ubiquitous in most human tissues, the staining with F4 being as restricted as that with F12. These results indicate that the ability of a MAb to detect specific antigens should be defined within each assay system.

The F12 MAb demonstrates a high capacity to differentiate SCCL from other lung tumors. Other MAbs that show a preferential reactivity with SCCL have also been described, e.g., SCLC 5023 (8), SM1 (9), B10/12 (10), TSF-4 (11), and NE-25 (12). A principal difference between these MAbs and the F12 MAb is the restricted glycolipid association of the carbohydrate epitope recognized by F12, the others reacting primarily with protein-associated antigens. They all also differ in their reactivity with carcinoid, neuroblastoma, and normal tissues, especially endocrine tissues. Glycolipid-reactive MAbs have been used by another group (32) to study the glycolipid antigen expression in human lung cancer. None of these MAbs, however, seems to be significantly reactive with fucosyl-G<sub>M1</sub> based on the results presented (32).

Tumor-reactive monoclonal antibodies can be of great value in diagnosis and staging of malignant disease. They may help the pathologist to a rapid and perhaps more adequate histogenetic classification of tumors. Especially in SCCL, crushing artifacts and small size of endobronchial biopsies make the diagnostic work difficult when using routine histopathological methods (33, 34). The use of F12 immunohistochemistry in these cases may reduce some of these problems. The use of immunoscintigraphy based on monoclonal antibodies may become a sophisticated and rapid method for the detection and staging of tumor disease. Finally, monoclonal antibodies may become very potent agents in the treatment of cancer. Preliminary results from investigations of the therapeutic capacity of MAb on experimental tumors in vitro and in vivo demonstrate a potential role for the F12 MAb in the treatment of SCCL.

In conclusion, fucosyl-G<sub>M1</sub> seems to be an antigen highly associated with SCCL. F12 represents a monoclonal antibody with a high specificity for fucosyl-G<sub>M1</sub> and is able to detect this antigen in tissue sections of SCCL using an immunofluorescence method. Immunohistochemical detection of fucosyl-G<sub>M1</sub> with the aid of the MAb F12 therefore may become a valuable tool in the diagnosis and management of small cell cancer of the lung.

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