Generation of Two Murine Monoclonal Antibodies That Can Discriminate N-Glycolyl and N-Acetyl Neuraminic Acid Residues of GM2 Gangliosides

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ABSTRACT

Since the preliminary analyses of the glycolipids of small cell carcinomas of the lung showed an increase of GM2 ganglioside, we generated new murine monoclonal antibodies directed to GM2 to identify the molecular species of the glycolipid. The monoclonal antibodies MK2-34 and MK1-16 (both IgM), which specifically detect N-glycolyl GM2 and N-acetyl GM2, respectively, were generated by immunizing mice with liposomes containing monophosphoryl lipid A, trehalose dicycolate, and the antigenic ganglioside. Among the glycolipid preparations extracted from the lung tissues of 39 patients with lung cancer, a significant amount of N-acetyl GM2 was detected with MK1-16 antibody in 70% of the squamous cell carcinoma cases, 50% of the lung adenocarcinoma cases, and 33% of the large cell carcinoma cases, and 100% of the cases of small cell carcinoma of the lung. The monoclonal antibody MK2-34 was found not in any of the glycolipid fractions prepared from the lung cancer tissues examined in this study. Immunohistochemical studies of the lung cancer tissues with the MK1-16 antibody showed that the N-acetyl GM2 was present not only in small cell carcinoma tissues as one of the antigens related to tumors of neuroectodermal origin, but also in the squamous cell carcinoma and adenocarcinoma of the lung with a comparable frequency. The appearance of the N-acetyl GM2 antigen correlated well with the degree of differentiation of the cancer cells in patients with squamous cell carcinoma and adenocarcinoma of the lung.

INTRODUCTION

Certain glycolipids having sialic acid residues, the gangliosides, have been reported to be associated with human cancers (1–3). GM2 is one of such gangliosides which frequently appear in malignant tumors of neuroendocrine or neuroectodermal origin such as melanoma and neuroblastoma (4). Sialic acid residues in normal human tissues are known to be solely of the N-acetyl species, but it has been shown that a significant amount of sialic acid residues of the N-glycolyl species, the Hanganutzui-Deicher antigen, is present in a variety of human cancer tissues (6–9). In preliminary experiments, we have analyzed the ganglioside fractions prepared from small cell carcinomas of the lung and found an increase in the amount of a glycolipid, which was later identified tentatively as the ganglioside, GM2.

Although some murine and human anti-GM2 monoclonal antibodies have been reported (4, 5), a monoclonal anti-GM2 antibody which can discriminate between the sialic acid residues of N-acetyl and N-glycolyl species has not been introduced yet. In this study, we generated two murine monoclonal antibodies, MK2-34, which specifically detects N-glycolyl GM2, and MK1-16, which specifically detects N-acetyl GM2, by using the liposomes containing MPL, TDM, and the antigenic glycolipid for immunization. With these monoclonal antibodies, we studied the distribution of the GM2 ganglioside in lung cancers of various histological types, and examined the molecular species of the GM2 antigen. We also tested whether or not the expression of the GM2 antigen in lung cancers is really confined to the cancers of the neuroendocrine origin, such as small cell carcinomas of the lung.

MATERIALS AND METHODS

Preparation of Standard Glycolipids. CDH, CTH, GM1 (NeuAc), GM2 (NeuAc), GM3 (NeuAc), GM4, and G010 were purchased from Supelco Inc. (Bellefonte, PA). Synthetic GM1 (NeuGc) was provided by the Meet Pharmaceutical Company (Tokyo, Japan). N-Glycolyl GM2 and N-glycolyl sialosylparagloboside (HD2) were obtained from C57BL/6 mouse liver and bovine erythrocyte as reported (10, 11). Asialo GM2, asialo GM3, and sialosylparagloboside were prepared by the mild acid hydrolysis of GM2, GM3, and sialosylparagloboside, respectively (12, 13). GM2 was prepared by treating GM230, with Jack Bean α-galactosidase (Sigma, St. Louis, MO) as described by Taki et al. (14).

Monoclonal Antibody Procedures. The monoclonal antibody specific for N-glycolyl GM2 (MK2-34) was established as follows: BALB/c mice were immunized with N-glycolyl GM2 adsorbed to MPL + TDM emulsion by the procedure described in the instruction of the kits provided by RIBI (Hamiltom, MT). Every mouse received the following i.p. injections of the N-glycolyl GM2-MPL-TDM complex: 10 μg N-glycolyl GM2 on Day 0, 20 μg on Day 5, 20 μg on Day 10. After a rest period of 2 weeks, the mouse received a final booster injections with 30 μg N-glycolyl GM2. Three days later the spleen cells were harvested and fused according to the method described by Köhler and Milstein (15), using polyethylene glycol 4000 (Sigma, St. Louis, MO) with the BALB/c mouse myeloma P3X63-Ag8U1 (P3XU1). The antibodies produced by the hybridoma were assayed for their binding to N-glycolyl and N-acetyl GM2 by the solid-phase enzymoimmunoassay as described below. The hybridoma MK2-34, whose culture supernatant showed a positive reaction only with N-glycolyl GM2, was selected and cloned twice by the limiting dilution method. The hybridoma MK1-16, which secreted the monoclonal antibody specific to N-acetyl GM2 was established by essentially the same method using N-acetyl GM2 for the immunization and cloning procedure. The monoclonal antibody specific to N-acetyl GM2 was selected and cloned twice by the limiting dilution method. The hybridoma MK1-16, which secreted the monoclonal antibody specific to N-acetyl GM2 was established by essentially the same method using N-acetyl GM2 for the immunization and cloning procedure.

Assessment of Reactivity of Monoclonal Antibodies with Various Glycolipids by a Solid-Phase Enzymoimmunoassay. The enzymoimmunoassay was performed using various glycolipid antigens which were immobilized at the bottom of 96-well culture plates, by a standard method described previously (16). Peroxidase-conjugated goat anti-mouse IgG (heavy and light chain specific) and anti-mouse IgM (μ chain specific) were obtained from Cappel Inc., (Malvern, PA).

Preparation of the Ganglioside Fractions from Lung Cancer Tissues. Cancer tissues taken surgically from patients with various kinds of lung cancer tissues were prepared as described previously (17, 18).

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3. The abbreviations used are: GM2 (NeuAc), II NeuAcGgOseCer or GalNAc2-4 NeuAc2-3 Gal2-4GlcCer; PBS, phosphate buffered saline; BSA, bovine serum albumin; MPL, monophosphoryl lipid A; TDM, trehalose dimycolate; TLC, thin-layer chromatography; GD3, II NeuAcLacCer or NeuAc2-8 NeuAc2-3 Gal2-4 GlcCer; GM2 (NeuAc), II NeuAcLacCer or NeuAc2-3 Gal2-4GlcCer; GD3, II NeuAcGgOseCer or GalNAc2-4 NeuAc2-8 NeuAc2-3 Gal2-4GlcCer. For other abbreviations and the structures for glycolipids used in this study, see Table 1.

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carcinoma were obtained at the Chest Disease Research Institute Hospital of Kyoto University, the Katsura Hospital (Kyoto, Japan), and the Kitano Hospital (Osaka, Japan). Ganglioside fractions of these cancer tissues were prepared as follows (13): Glycolipids were extracted from cancer tissues with isopropanol/hexane/water (55/20/25, v/v/v) and partitioned according to Folch. The glycolipids in the upper layer fraction of the Folch’s partition were subjected to DEAE-Sephadex A-25 (Pharmacia, Uppsala, Sweden) column chromatography. After the neutral glycolipids were eluted with chloroform/methanol/water (30/60/8, v/v/v), gangliosides were eluted with chloroform/methanol/0.8 N sodium acetate (30/60/8, v/v/v). The ganglioside mixture in the latter fraction was used in the TLC-immunostaining method without further purification.

TLC Immunostaining of Various Glycolipids of Lung Cancers with Monoclonal Antibodies. TLC immunostaining was performed with the gangliosides extracted from cancer tissues according to the method originally introduced by Magnani et al. (17), with minor modifications (18). Briefly, glycolipids were developed on a Baker high-performance TLC plate (Si-HPF plate; 7011-3, Baker, Phillipsburg, NJ) with the solvent system of chloroform/methanol/water (60/35/8, v/v/v). The plate was air dried, blocked with PBS containing 5% BSA for 2 h and exposed overnight to the culture fluid containing respective monoclonal antibody. After washing five times with PBS containing 0.5% BSA, the plate was exposed for 1 h to the solution of rabbit anti-mouse IgM (n chain specific, ICN Immunobiologicals, Lisle, Israel). After five washes with PBS containing 0.5% BSA, the plate was then exposed for 1 h to the PBS which contained 125I-Protein A (Du Pont, Boston, MA) and washed 10 times with PBS. The plate was then air dried and subjected to the autoradiography.

Immunohistochemical Techniques. The avidin-biotin complex technique for the immunohistochemical study of lung cancer was performed with formalin-fixed tissue sections of lung cancers according to a method described in the instructions of the kits (Vectastain) provided by Vector, (Burlingame, CA).

RESULTS

Specificity of the Monoclonal Antibodies (MK2-34 and MK1-16) Directed to N-Glycolyl and N-Acetyl GM2. When the specificity of the monoclonal antibody MK2-34 was tested by the solid-phase enzymoimmunoassay using various standard glycolipids as antigens, a significant reactions was obtained only with N-glycolyl GM2, and no reactivity was observed with N-acetyl GM2, CDH, CTH, GM3, GM1, GD2, asialo-GM2, asialo-GM1, GD1b, GD1a, globoside, paragloboside, or sialosylparagloboside (Fig. 1a). The binding of MK2-34 to N-glycolyl GM2 on the thin-layer plate was dependent on the amount of N-glycolyl GM2 used and was highly restricted to the N-glycolyl neuraminyl residue of GM2 (Fig. 2a). These results indicate that the antibody is specific to the N-glycolyl GM2 ganglioside. In the immunodiffusion test, this antibody was shown to be IgM.

Similarly, the monoclonal antibody MK1-16 (IgM) was specific to N-acetyl GM2, as ascertained by both the solid-phase enzymoimmunoassay (Fig. 1b) and TLC-immunostaining method (Fig. 2b). The specificities of these two antibodies and the structure of the glycolipid antigen tested in this study are summarized in Table 1.

TLC Immunostainings of Gangliosides Extracted from Lung Cancer Tissues by Monoclonal Antibodies Specific to N-Acetyl GM2 and N-Glycolyl GM2. When the presence of GM2 antigen in the glycolipids extracted from lung cancer tissues of various histological types was tested by the TLC-immunostaining methods, the results showed that the GM2 antigen of the N-acetyl species was present in some cases of every type of lung cancer tissue including squamous cell carcinoma of the lung (Fig. 3, a–d), pulmonary adenocarcinoma (Fig. 4, a and b), large cell carcinoma (Fig. 4, c and d), and small cell carcinoma (Fig. 4, e and f). Ganglioside fractions prepared from three nonmalignant lung tissues contained no detectable amount of N-acetyl GM2 (data not shown).

As summarized in Table 2, the positive incidence of N-acetyl GM2 was highest in small cell carcinoma of the lung (100%), followed by squamous cell carcinoma (70%), and adenocarcinoma (50%). Large cell carcinoma had the lowest incidence for
Fig. 2. Specificities of monoclonal antibodies MK2-34 and MK1-16 towards various glycolipid antigens as ascertained by the TLC-immunostaining method. In each figure, left, TLC stained with orcinol/H$_2$SO$_4$; right, TLC immunostained with respective monoclonal antibody. Lane 1, the ganglioside mixture extracted from human type 0 erythrocytes serving as a control; Lane 2, 3 µg of pure N-glycolyl G$_{M2}$; Lane 3, 10 µg of pure N-glycolyl G$_{M2}$; Lane 4, 30 µg of pure N-glycolyl G$_{M2}$; Lane 5, 3 µg of pure N-acetyl G$_{M2}$; Lane 6, 10 µg of pure N-acetyl G$_{M2}$; Lane 7, 30 µg of pure N-acetyl G$_{M2}$.

Table 1 Carbohydrate structure of glycolipids used in this study and the summary of the reactivities of the monoclonal antibodies MK2-34 and MK1-16 as ascertained by the solid-phase enzymeimmunoassay and TLC-immunostaining method

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
<th>MK2-34 ELISA</th>
<th>MK2-34 TLC</th>
<th>MK1-16 ELISA</th>
<th>MK1-16 TLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDH</td>
<td>Galβ1→4Glc-Cer</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CTH</td>
<td>Galα1→4Galβ1→4Glc-Cer</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Globoside</td>
<td>GalNAcβ1→3Galα1→4Galβ1→4Glc-Cer</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Asialo-GM$_2$</td>
<td>GalNAcβ1→4Galβ1→4Glc-Cer</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Asialo-GM$_3$</td>
<td>Galβ1→3GalNAcβ1→4Galβ1→4Glc-Cer</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Paragloboside</td>
<td>Galβ1→4GlcNAcβ1→3Galβ1→4Glc-Cer</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G$_{M2}$ (NeuAc)</td>
<td>NeuAcβ2→3Galβ1→4Glc-Cer</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G$_{M2}$ (NeuGc)</td>
<td>NeuGcβ2→3Galβ1→4Glc-Cer</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G$_{M2}$ (NeuAc)</td>
<td>GalNAcβ1→4Galβ1→4Glc-Cer</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>G$_{M2}$ (NeuGc)</td>
<td>NeuGcβ2→3Galβ1→4Glc-Cer</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Paragloboside</td>
<td>Galβ1→4GlcNAcβ1→3Galβ1→4Glc-Cer</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sialosylparagloboside</td>
<td>NeuAcβ2→8NeuAcβ2</td>
<td>3</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sialosylparagloboside</td>
<td>NeuAcβ2→8NeuAcβ2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* ELISA, enzyme-linked immunosorbent assay.

the antigen among the four major histological types of lung cancer (33%).

Judging from the intensity of the orcinol/H$_2$SO$_4$ staining of the G$_{M2}$ antigen on the TLC plate, it was obvious that the small cell carcinoma of the lung contained a large chemical amount of N-acetyl G$_{M2}$, much more than any other histological type of pulmonary carcinomas. Some cases of squamous cell carcinoma and adenocarcinoma also contained a significant amount.
[Image 19x9 to 593x783]

of N-acetyl GM₂, which was clearly detected by the orcinol/H₂SO₄ staining. In other cancer tissues including one out of three large cell carcinoma tissues, the amount of N-acetyl GM₂ antigen was very small; the antigen was hardly visualized chemically by orcinol/H₂SO₄ staining, and it was only detected by the TLC-immunostaining method, because of the higher sensitivity of the method employing ¹²⁵I-labeled protein A. This discrepancy is perhaps due partly to the masking of the antigen by other membrane components in tissue sections (19), and partly to the difference in sensitivity between TLC immunostaining and immunohistochemistry. In the TLC immunostaining study employing MK2-34 did not show any clear positive evidence of N-glycolyl GM₂ in pulmonary carcinoma tissues, the extensive immunohistochemical study was performed only with the antibody MK1-16 specific to N-acetyl GM₂.

Fig. 5, a–d, shows examples of the immunohistochemical distribution of N-acetyl GM₂ antigen in the four major kinds of pulmonary carcinomas, stained through the avidin-biotin complex technique. As summarized in Table 3, N-acetyl GM₂ antigen was detected in 63.0% of squamous cell carcinoma tissues, 50.0% of adenocarcinoma, and 52.2% of small cell carcinoma tissues. By the immunohistochemical study, large cell carcinoma was again characterized as having the lowest incidence of the antigen among the four major histological types of lung cancer (37.1%). The positive incidences obtained by the immunohistochemical examination were more or less lower than those which had been obtained by the TLC immunostaining in all kinds of pulmonary carcinomas, and this tendency was most remarkable in the results for small cell carcinoma of the lung. This discrepancy is perhaps due partly to the masking of the antigen by other membrane components in tissue sections (19), and partly to the difference in sensitivity between TLC immunostaining and immunohistochemistry. In the TLC immunostaining study employing MK2-34 did not show any clear positive evidence of N-glycolyl GM₂ in pulmonary carcinoma tissues, the extensive immunohistochemical study was performed only with the antibody MK1-16 specific to N-acetyl GM₂.

Table 2 Result of TLC-immunostaining study of ganglioside fractions prepared from lung cancers using MK1-16 antibody detecting N-acetyl GM₂

<table>
<thead>
<tr>
<th>Histology</th>
<th>Positive rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous cell carcinoma</td>
<td>78% (14/18)</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>50% (6/12)</td>
</tr>
<tr>
<td>Large cell carcinoma</td>
<td>33% (1/3)</td>
</tr>
<tr>
<td>Small cell carcinoma</td>
<td>100% (6/6)</td>
</tr>
<tr>
<td>Total</td>
<td>69% (27/39)</td>
</tr>
</tbody>
</table>

Detection of N-Acetyl GM₂ Antigen in Tissues of Pulmonary Carcinomas by Immunohistochemical Examination. As N-glycolyl GM₂ was not found in the glycolipid fraction of any kinds of pulmonary carcinoma, and a pilot immunohistochemical study employing MK2-34 did not show any clear positive evidence of N-glycolyl GM₂ in pulmonary carcinoma tissues, the extensive immunohistochemical study was performed only with the antibody MK1-16 specific to N-acetyl GM₂.

Fig. 3, Presence of the N-acetyl GM₂ antigen in the acidic glycolipid fractions extracted from the cancer tissues of patients with squamous cell carcinoma. In each figure: left, TLC stained with orcinol/H₂SO₄; right, TLC immunostained with the monoclonal antibody MK1-16. Lanes 1 and 12, the ganglioside mixture extracted from human type 0 erythrocytes serving as a control; Lanes 2–11 and 13–20, the ganglioside fractions extracted from the cancer tissues of 18 patients with squamous cell carcinoma of the lung. The amount of the extract applied in each lane corresponds to the glycolipids prepared from 200 mg (wet weight) of cancer tissues.

Fig. 4. Presence of the N-acetyl GM₂ antigen in the acidic glycolipid fractions extracted from cancer tissues of patients with adenocarcinoma (a and b), large cell carcinoma (c and d) and small cell carcinoma of the lung (e and f). a, c, and e, TLC stained with orcinol/H₂SO₄; b, d, and f, TLC immunostained with the monoclonal antibody MK1-16. Lanes 1, 14, and 18, ganglioside mixture extracted from human type 0 erythrocytes; Lanes 19–24, acidic glycolipid fractions extracted from three patients with large cell carcinoma of the lung; Lanes 15–17, acidic glycolipid fractions extracted from six patients with small cell carcinoma of the lung. The amount of the extract applied in each lane corresponds to the glycolipids prepared from 200 mg (wet weight) of cancer tissues.
Fig. 5. Distribution of the N-acetyl GM2 antigen in the lung cancers detected immunohistochemically by the avidin-biotin complex technique using the monoclonal antibody MK1-16. a, squamous cell carcinoma; b, adenocarcinoma; c, large cell carcinoma; and d, small cell carcinoma (intermediate type) of the lung. Original magnification, × 200.

Table 3 Result of immunohistochemical study of lung cancers using the MK1-16 antibody detecting N-acetyl GM2

<table>
<thead>
<tr>
<th>Histology</th>
<th>Positive rate</th>
<th>Differentiation grading or subtype</th>
<th>Positive rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous cell carcinoma</td>
<td>63.0% (46/73)*</td>
<td>Well</td>
<td>83.3% (20/24)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moderately</td>
<td>56.2% (18/32)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Poorly</td>
<td>47.1% (8/17)*</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>50.0% (36/72)</td>
<td>Well</td>
<td>78.3% (18/23)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moderately</td>
<td>35.7% (10/28)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Poorly</td>
<td>38.1% (8/21)</td>
</tr>
<tr>
<td>Large cell carcinoma</td>
<td>37.1% (13/35)*</td>
<td>Well</td>
<td>38.7% (10/26)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moderately</td>
<td>35.7% (10/28)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Poorly</td>
<td>38.1% (8/21)</td>
</tr>
<tr>
<td>Small cell carcinoma</td>
<td>52.2% (12/23)</td>
<td>Classic</td>
<td>50.0% (4/8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intermediate</td>
<td>53.3% (8/15)</td>
</tr>
<tr>
<td>Total</td>
<td>52.7% (107/203)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Difference between the incidence of antigen in squamous cell carcinoma and the incidence of antigen in large cell carcinoma is significant at $P < 0.05$ by the $\chi^2$ test.

* Differences between the incidence of antigen in well-differentiated squamous cell carcinoma and that in moderately or poorly differentiated squamous cell carcinoma are significant at $P < 0.05$ by the $\chi^2$ test.

* Differences between the incidence of antigen in well-differentiated adenocarcinoma and the incidence of antigen in moderately or poorly differentiated adenocarcinoma are significant at $P < 0.01$ by the $\chi^2$ test.

Inmunostaining method we applied radioisotopic detection which is known as having the higher sensitivity than the enzymoimmunochemical detection used in the immunohistochemical study. Another possibility is that we used formalin-fixed tissue sections in the immunohistochemical study, which gives usually weaker staining than freshly frozen tissue sections. The discrepancy of the positive incidences detected by TLC immunostaining and immunohistochemistry can also be due to the presence of glycoproteins which have a carbohydrate side chain very similar to GM2; such glycoprotein antigens can be detected by the immunohistochemical method but not by the TLC-immunostaining method.

When the appearance of N-acetyl GM2 in the lung tissue was correlated with the degree of differentiation of the cancer cells in squamous cell carcinoma and adenocarcinoma of the lung, the results clearly indicated that the incidence of the GM2 antigen was significantly higher in well-differentiated cancers than in cancers having a lower degree of differentiation (Table 3).

**DISCUSSION**

Gangliosides which belong to the ganglioseries glycolipids have long been known to be present specifically in the normal...
the HD antigen has recently been found frequently in human
neutrophils and other neural tissues of humans and other animals,
and the cancer-associated change of the gangliosides glyco-
lipids has been reported to occur mainly in tumors of the
neuroectodermal or neuroendocrine origin (4, 5, 20–24). For
example, human melanoma have been reported to contain large
amounts of the gangliosides glycolipids such as GM3 (20, 21)
and GD3 (23, 24), and the human monoclonal antibodies di-
tected to GM3 and GD3 have been generated from patients with
melanoma using the lymphocytes transformed by the Epstein-
Barr virus (5, 22). A remarkable amount of GD2 has been
detected in human neuroblastoma (25), and the amount of GM3
has been shown to increase significantly in astrocytoma (4).
Among the lung cancers, small cell carcinoma has been sup-
posed to occur from neuroendocrine origin, and reported to
contain an increased level of the gangliosides glycolipids, such
as GD3, and fucosyl GM1 (26–28). It is not surprising that we
could find a significant amount of the GM2 antigen, ganglio-
series glycolipid, in small cell carcinomas of the lung.

However, our results clearly indicate that the appearance of
GM2 is not strictly confined to cancers of neuroendocrine origin
in lung. Adenocarcinoma and squamous cell carcinoma of the
lung, which are usually referred to as tumors of endodermal
origin, contained GM2 antigen with high frequencies which were
comparable with the incidence of the antigen in the small cell
carcinoma of the lung. The abnormal occurrence of gangli-
series glycolipids in lung cancers except small cell carcinoma
has not been reported. It was reported that the cultured human
epithelial cancer cells only rarely showed GM2 expression, and
only one out of five lung cancer cell lines was positive for GM2
(4); but this conclusion seems to be based on too small a number
of samples.

Moreover, our immunohistochemical data shows that N-
acetyl GM2 appears to be a differentiation grating antigen in
pulmonary squamous cell carcinoma and adenocarcinoma. The
positive incidence of GM2 in cancer tissues correlated very well
with the differentiation grades of squamous cell carcinoma and
adenocarcinoma. The positive incidence of GM2 in large cell
carcinoma of the lung was the lowest among the four major
histological types of lung cancer, as was ascertained by both the
TLC immunostaining of extracted glycolipids and the immu-
nohistochemical examination of tissue sections. These findings,
taken together, strongly suggest that, as far as the lung is
concerned, GM2 is not a specific marker for the cancers of
neuroendocrine or neuroectodermal origin, but is rather ubiqui-
Tously present in various histological types of lung cancer and
has a significance as differentiation-associated antigen. Accord-
ing to Yesner's "Y" concept theory, large cell carcinoma of the
lung represents the more undifferentiated type among lung
cancers, and adenocarcinoma and squamous cell carcinoma are
both supposed to differentiate from large cell carcinoma (29). Small
cell carcinoma of the lung are also claimed to stem from the
large cell carcinoma, albeit resulting from differentiation in the
different direction. Our data on the distribution of GM2 among
the various histological types of lung cancers do not
contradict Yesner's hypothesis.

Gangliosides are known to contain two species of sialic acid,
N-acetyl and N-glycolyl sialic acid residues. The sialic acid
residues found in normal human tissue are solely of N-acetyl
species, and N-glycolyl sialic acid is a foreign antigen to hu-
mans. It was found to be an essential part of the immunodeter-
minant of the antigenic glycolipids and glycoproteins called
Hanganutziu-Deicher (HD) antigen (12). The antigen is cur-
cently regarded as one of the tumor-associated antigens, since
the HD antigen has recently been found frequently in human
cancer tissues, sera from patients with various tumors, and
established human cell lines (6–9). It is of interest to know the
molecular species of sialic acid residues in GM2, antigen appear-
ing in various histological types of lung cancers. For this
purpose, we generated monoclonal antibodies which can dis-
criminate N-acetyl and N-glycolyl GM2, as no such specific
antibodies have been established so far. Some monoclonal
antibodies directed to GM3 have been reported, but they cannot
discriminate the molecular species of the sialic acid residues in
the antigen molecule (4).

By TLC immunostaining of extracted glycolipids and immu-
nohistological examination using these specific monoclonal
antibodies, it was disclosed that the GM2 antigen present in
pulmonary small cell carcinoma, adenocarcinoma, and squa-
mous cell carcinoma was solely of N-acetyl-type GM2, which
was specifically detected by the monoclonal antibody MK1-16.
No significant amount of N-glycolyl GM2 was detected in any
of the 39 pulmonary carcinoma cases when MK2-34, the spec-
ific anti-N-glycolyl GM2 antibody, was used. Since there was
no appreciable difference in the sensitivity for the detection of
the antigen by these two antibodies in TLC-immunostaining
methods, we conclude that only few, if any, carcinoma of the
lung contain N-glycolyl GM2.

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ADDENDUM

During the course of preparation of this manuscript, a paper ap-
peared (30) which describes the generation of a specific monoclonal
antibody, Y-2-HD-1, against N-glycolyl GM3, the antibody having a
very similar specificity to our MK2-34.

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Generation of Two Murine Monoclonal Antibodies That Can Discriminate N-Glycolyl and N-Acetyl Neuraminic Acid Residues of Gm2 Gangliosides

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