Sialosyllactotetraosylceramide, a novel ganglioside antigen detected in human carcinomas by a monoclonal antibody

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A novel ganglioside was detected in a small cell lung carcinoma by TLC-immunostaining of gangliosides with a monoclonal antibody, the C-50 MAb. Structural characterization showed this ganglioside to be IV^{3}NeuAc-LcOse$_4$Cer, a hitherto unknown ganglioside. This ganglioside has also been detected as a minor component in many different carcinomas using the C-50 MAb. The normally dominant CA-50 ganglioside antigen is IV^{3}NeuAc, III^{4}Fuc-LcOse$_4$Cer. Based upon solid-phase binding to IV^{3}NeuAc, III^4-LcOse$_4$Cer and IV^{3}NeuAc-LcOse$_4$Cer it is concluded that the C-50 MAb recognizes an epitope present in sialylated type I carbohydrate chains.

Carcinoma-associated ganglioside

1. INTRODUCTION

The introduction of the hybridoma technology of Köhler and Milstein [1] has dramatically improved the possibilities for detecting and defining tumour-associated antigens. Marked changes of the glycolipid composition of cells occur during oncogenic transformation, and different tumour-associated glycolipid antigens have been identified using monoclonal antibodies [2–5]. A previous report has described monoclonal antibodies defining carcinoma-associated ganglioside antigens obtained after immunization with the colorectal adenocarcinoma cell line COLO 205 [6]. One of these antibodies, the C-50 MAb, has been studied more extensively and has been shown to detect antigens with generalized carcinoma distribution [7,8]. The CA-50 ganglioside antigen present in the COLO 205 cell line has been characterized as IV^{3}NeuAc, III^{4}Fuc-LcOse$_4$Cer (sialosylfucosyllactotetraosylceramide, Fuciso 3′LM1) (Månsson, J.-E. et al., submitted), which is the sialylated Lewis^a pentaglycosylceramide characterized by Magnani et al. [4]. This paper describes the isolation and characterization of a 'novel' CA-50 antigen.

Abbreviations: The gangliosides have been designated according to [25]

2. EXPERIMENTAL

2.1. *Materials*

Tumour tissue was obtained at autopsy from a 71-year-old smoker who died from small cell lung carcinoma with multiple metastases. Histopathological examination established the diagnosis as oat cell type of small cell carcinoma. Only the primary tumour was available for analysis.

The C-50 monoclonal antibody was obtained by intravenous immunization of mice with the colorectal adenocarcinoma cell line COLO 205 (American Type Culture Collection, Rockville, MD), and fusion between spleen cells from the immunized mice and Sp 2/0 cells [6]. The C-50 MAb was purified from culture medium by ammonium sulphate precipitation, and used in a concentration of approx. 1 μg/ml. The 19-9 MAb [23] was a gift from Dr P. Burtin, Institut de Recherches Scientifiques, Villejuif, France; it was used at a concentration of approx. 3 μg/ml. Affinity purified goat anti-mouse IgM immunoglobulin was obtained

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from Kirkegaard and Perry, Gaithersburgh, MD, and ^{125}I-labelled to a specific activity of 20–60 μCi/μg using the Iodogen^{R} method [9]. ^{125}I-labelled anti-mouse F(ab)$_2$ fragment was obtained from The Radiochemical Centre, Amersham. Silica gel 60, 230–400 mesh; glass-backed TLC and HPTLC plates, silica gel G; and alumina-backed HPTLC plates, silica gel G, were all from Merck, Darmstadt, FRG. Sephadex G-25 fine was from Pharmacia, Uppsala, Sweden. The anion exchange resin Spherosil-DEAE-dextran was a gift from Institut Merieux, Lyon, France [10]). *Vibrio cholerae* sialidase (EC 3.2.1.18) was from Behringwerke, Marburgh-Lahn, FRG. All organic solvents and other chemicals were of analytical quality and used without further purification. Gangliosides used as standards and references were all isolated at the Department of Psychiatry and Neurochemistry, University of Göteborg.

2.2. *Isolation of gangliosides*

The tumour tissue (75 g) was homogenized in a scissor homogenizer in 3 vols (w/v) distilled water, and extracted twice with 20 vols chloroform/methanol/water (C/M/W; 4:8:3, (v/v) [11]. The total lipid extract was evaporated to dryness and dissolved in C/M/W (60:30:4.5). Low molecular mass contaminants were removed by gel filtration on Sephadex G-25 [12]. Gangliosides were isolated and separated into mono- and oligosialogangliosides by eultion from the anion exchange resin Spherosil-DEAE-dextran with a discontinuous gradient of potassium acetate in methanol [10]. The monosialoganglioside fraction was purified by alkaline methanolysis in 0.5 M KOH in 50% methanol, followed by column chromatography on Silica Gel 60, 230–400 mesh. The column was eluted with C/M/W, 65:25:4, until all gangliosides with a TLC migration corresponding to GM3 and GM2 were eluted, as monitored by HPTLC analysis of the eluate. The solvent was then changed and the column eluted with 10 vols C/M/W, 60:35:8; the eulate was subsequently collected as one fraction. Individual gangliosides in the 60:35:8 fraction were isolated by preparative TLC using *n*-propanol/2.0 M ammonia in 0.25% aq. KCl, 7:3 (v/v) as developing solvent.

The isolation of the CA-50 ganglioside antigen was monitored by TLC-immunostaining, essentially as described by Brockhaus et al. [13].

2.3. *Structural analyses of the CA-50 ganglioside antigen*

Sialic acid was determined with the resorcinol assay [14], and sphingosine bases were assayed with a modified methyl orange method [15]. Sialidase hydrolysis and acidic hydrolysis were performed as described in [16]. The carbohydrate composition was quantitatively determined by GLC of the corresponding alditol acetates [17]. The ganglioside was permethylated according to Hakomori [18], and the individual sugars analysed as their corresponding partially methylated alditol acetates by GC-MS [19].

2.4. *Determination of C-50 and 19-9 reactivity*

The reactivity of the C-50 MAb against different gangliosides was determined by a solid-phase double antibody radioimmunoassay with the gangliosides adsorbed to the wells of polyvinyl microtiter plates [20]. The reactivity of the C-50 MAb against the CA-50 ganglioside antigen fraction isolated from this case was compared with the C-50 reactivity against sialosylfucosyllactotetraosylceramide (IV^{3}NeuAc-III^{4}Fuc-LcOse$_4$Cer) isolated from COLO 205 cells and with 3'LM1 (IV^{3}NeuAc-nLcOse$_4$Cer) isolated from erythrocytes [21]. The reactivity of the 19-9 MAb was also tested against sialosylfucosyllactotetraosylceramide and the CA-50 ganglioside fraction isolated from this tumour.

3. RESULTS AND DISCUSSION

TLC-immunostaining of gangliosides with the C-50 MAb showed that this tumour contained a CA-50 ganglioside antigen with a faster TLC migration than that present in the COLO 205 cells (fig. 1). A ganglioside with the same chromatographic mobility can be detected as a minor CA-50 ganglioside antigen in various carcinomas, but this is the first case analysed thus far in which the fast migrating CA-50 ganglioside antigen was the quantitatively dominant CA-50 ganglioside antigen.

The ganglioside was isolated to apparent homogeneity by combined silica column chromatography and preparative TLC, based upon C-50 TLC-immunostaining and TLC-analysis (fig.1,2). The isolated ganglioside contained sphingosine, *N*-acetylneuraminic acid, glucose, galactose and glucosamine in the molar ratio 1:1:1:2:1. Hydro-

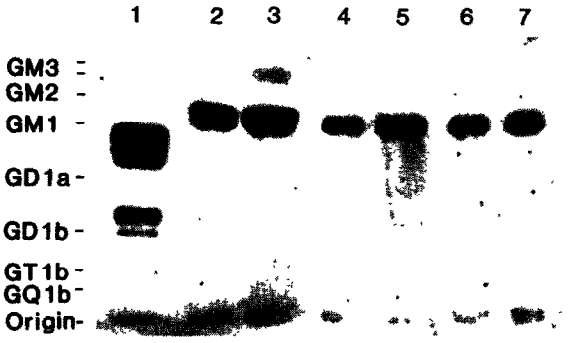
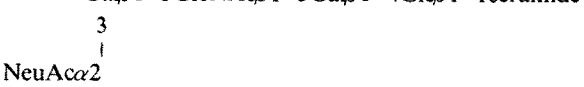


Fig.1. C-50 TLC-immunostaining of monosialogangliosides isolated from a small cell lung carcinoma. The gangliosides were separated on alumina-backed HPTLC-plates developed C/M/0.25% KCl (5:4:1). The immunostaining was performed as in [13]. Lane 1, 25 pmol of total monosialogangliosides isolated from COLO 205 cells; upper band IV^3NeuAc,III^4Fuc-LcOse$_4$Cer, lower band IV^3NeuAc,V^3Fuc-LcOse$_6$Cer; lanes 2,3, total monosialogangliosides from the small cell lung carcinoma corresponding to 5 and 10 mg tissue, respectively; lanes 4,5 C/M/W (60:35:8) fraction from the silica column, 5 and 10 mg tissue, respectively; lanes 6,7, purified CA-50 ganglioside, 5 and 10 mg tissue, respectively.

lysis with sialidase or weak acid yielded a tetraglycosylceramide with a similar TLC-migration as neolactotetraosylceramide. Prolonged acid hydrolysis produced compounds with the same mobility on TLC as lactosylceramide and glucosylceramide.

The results of the methylation analysis of the intact ganglioside and the desialylated substance are shown in table 1. The finding of both 4,6-Me$_2$-GlcNAcMe and 3,6-Me$_2$-GlcNAcMe indicated that the isolated ganglioside was not homogeneous, but contained gangliosides related to both the lactotetraose and the neolactotetraose series. The relative ratio of 4,6-Me$_2$-GlcNAcMe and 3,6-Me$_2$-GlcNAcMe was approx. 4:1. When the desialylated substance was analyzed, 2,3,4,6-Me$_4$-Gal appeared, which indicated that the sialic acid was attached in a terminal NeuAcα2–3Gal linkage. A combination of the structural analyses suggests that the C-50 reactive ganglioside isolated from this tumour contained as the dominant constituent, IV^3NeuAc-LcOse$_4$Cer (sialosyllactotetraosylceramide, 3′-isoLM1) and IV^3NeuAc-nLcOse$_4$Cer



IV^3NeuAc-LcOse$_4$Cer, sialosyllactotetraosylceramide.

(sialosylneolactotetraosylceramide, 3′LM1). Sialosyllactoteraosylceramide has not been isolated and characterized previously. However, Falk et al. [22] had indications of the existence of this ganglioside in a pancreatic adenocarcinoma. This compound represents the second ganglioside based on the lactotetraose structure characterized to date.

The C-50 reactivity against the CA-50 ganglioside fraction isolated from this tumour; IV^3NeuAc,III^4Fuc-LcOse$_4$Cer isolated from COLO 205 cells; and 3′LM1 isolated from human erythrocytes [21], is shown in fig.3A. As the C-50 MAb did not react with the 3′LM1, it is concluded that the CA-50 ganglioside antigen isolated from this tumour was IV^3NeuAc-LcOse$_4$Cer. Thus the C-50 MAb recognized as epitope present both in IV^3NeuAc-LcOse$_4$Cer and in IV^3NeuAc,III^4Fuc-LcOse$_4$Cer. The sialic acid was an absolute requirement for antigenicity, as sialidase hydrolysis of the ganglioside completely abolished the binding of the C-50 MAb (Nilsson, O. et al., in preparation), while the fucose residue was not necessary for binding (fig. 3A). The reac-

Table 1

Results of the permethylation analysis of the CA-50 ganglioside antigen isolated from a case of small cell lung carcinoma and of the corresponding desialylated glycolipid

	2,3,4,6-Me$_4$ Gal	3,6-Me$_2$ GlcNAcMe	4,6-Me$_2$ GlcNAcMe	2,4,6-Me$_3$ Gal	2,3,6-Me$_3$ Glc
Ganglioside	0	(+)	+	+	+
Desialylated glycolipid	+	(+)	+	+	+

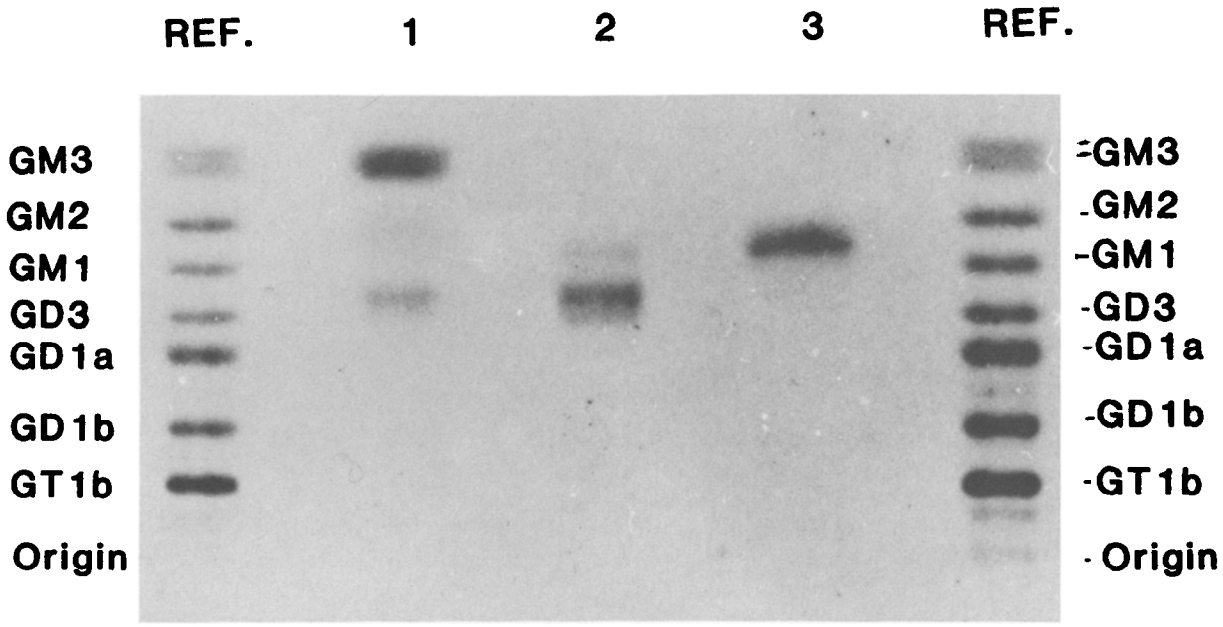


Fig.2. Chemical staining of monosialogangliosides isolated from a small cell lung carcinoma. The gangliosides were isolated as described in section 2. They were separated in C/M/W, 5:4:1, and visualized with resorcinol spray. Lane 1, total monosialogangliosides; lane 2, C/M/W (60:35:8) fraction; lane 3, purified CA-50 ganglioside (see fig.1, lanes 6,7).

tivity of the C-50 MAb was approx. 10-times higher for IV^{3}NeuAcIII^{4}Fuc-LcOse$_4$Cer compared with IV^{3}NeuAc-LcOse$_4$Cer determined with the solid-phase assay used here.

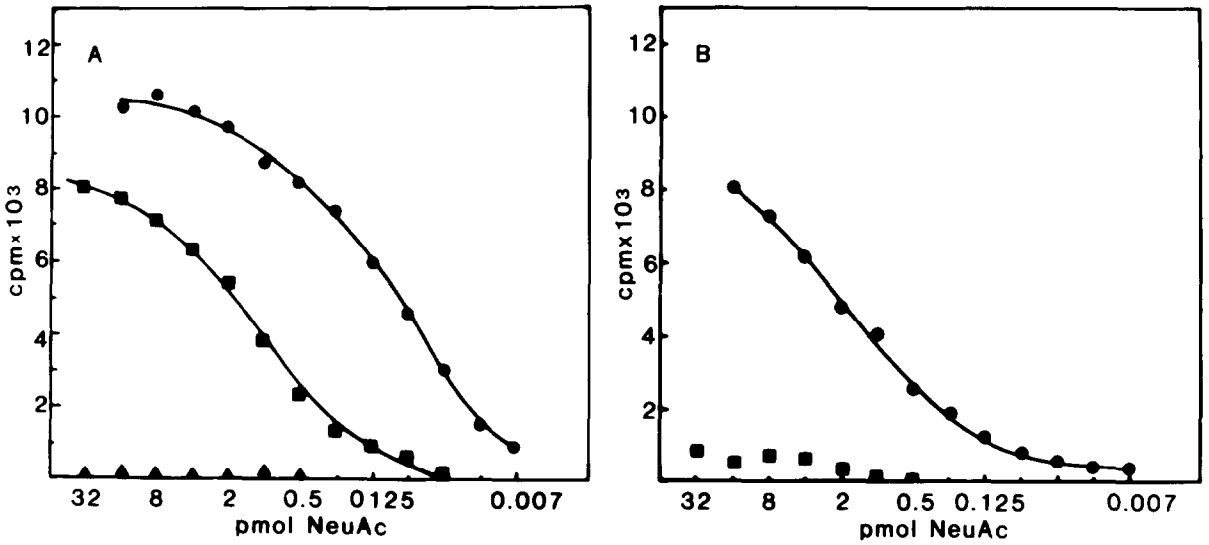


Fig.3. Solid-phase binding of (A), C-50 MAb, (B) 19-9 MAb, to gangliosides. The assay was performed as described in section 2. (●) IV^{3}NeuAc,III^{4}Fuc-LcOse$_4$Cer isolated from COLO 205 cells; (■) CA-50 ganglioside fraction isolated from the carcinoma described in this paper; this fraction contained ~80% IV^{3}NeuAc-LcOse$_4$Cer and 20% 3′LM1; (▲) 3′LM1 isolated from erythrocytes.

Koprowski et al. [23] described a monoclonal antibody, the 19-9 MAb, which defines the $IV^3NeuAcIII^4Fuc\text{-}LcOse_4Cer$ (S-Le^a pentaglycosylceramide) [4]. The fucose and the sialic acid residue have been established to be involved in the recognition structure of the 19-9 MAb [24], which was also indicated by the negligible reactivity of the 19-9 MAb with the sialosyllactotetraosylceramide (fig.3B). The 19-9 MAb would then seem to define an epitope different from that defined by the C-50 MAb.

The CA-50 ganglioside antigen, present in the COLO 205 cell line (Månsson, J.-E. et al., submitted and the normally dominant CA-50 ganglioside in carcinomas ($IV^3NeuAc,III^4Fuc\text{-}LcOSe_4Cer$) is related to the expression of the Lewis gene and would not be expressed in Lewis negative individuals. Lewis negative subjects lack the fucosyl transferase transferring the fucose residue to the glucosamine, but can synthesize lactotetraosylceramide. The Lewis status of the present case was not determined, but one explanation for the presence of sialosyllactotetraosylceramide as the only CA-50 ganglioside antigen could be that the subject was Lewis negative.

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