A monoclonal antibody reacting specifically with ganglioside GD1b in human brain

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A mouse monoclonal antibody directed against one of the major human brain gangliosides, GD1b, has been produced. The antibody binds specifically to the carbohydrate structure of GD1b as it does not react with structurally related gangliosides like GM1, GD2, GT1b or Fuc-GM1, or any other ganglioside of human brain. The results further indicate that terminal galactose as well as the disialosyl group linked to the inner galactose moiety are involved in binding to the antibody.

Monoclonal antibody *Brain ganglioside* *Ganglioside GD1b-specificity*

1. INTRODUCTION

Gangliosides are localized to the outer surface of mammalian cell membranes. Their concentration and structural composition undergo marked changes during development and transformation. Gangliosides in the nervous system have been extensively studied with regard to structural composition, but little is known about their function and exact topographical location [2]. Monoclonal antibodies detecting specific gangliosides might be a useful tool for the elucidation of such problems but gangliosides have generally been poor immunogens [3,4]. To our knowledge no specific monoclonal antibody directed against any of the 4 major brain gangliosides has been produced. This paper reports the production and specificity of a monoclonal antibody to one of these gangliosides, GD1b.

Abbreviations: Ganglioside abbreviations follow the nomenclature system of Svennerholm, IUPAC-IUB Commission on Biochemical Nomenclature CBN, The Nomenclature of Lipids [1]. GM3, II^3NeuAc-LacCer; GD3, $II^3(NeuAc)_2$-LacCer; GM2, II^3NeuAc-$GgOse_3$-Cer; GD2, $II^3(NeuAc)_2$-$GgOse_3$Cer; GM1, II^3NeuAc-$GgOse_4$Cer; GD1a, IV^3NeuAc,II^3NeuAc-$GgOse_4$Cer; GD1b, $II^3(NeuAc)_2$-$GgOse_4$Cer; Fuc-GD1b, IV^2Fuc, $II^3(NeuAc)_2$-$GgOse_4$Cer; GT1b, IV^3NeuAc,$II^3(NeuAc)_2$-$GgOse_4$Cer; and GQ1b, $IV^3(NeuAc)_2$,$II^3(NeuAc)_2$-$GgOse_4$Cer

2. MATERIALS AND METHODS

2.1. *Materials*

The gangliosides used in this study were all prepared from human brain and the extraction and isolation were performed as described [5,6]. Rabbit anti-mouse immunoglobulin, Ig, conjugated with horseradish peroxidase (HRP) was purchased from Dakopat a/s (Glostrup, Denmark). Affinity-purified goat antibody to mouse IgM (μ) from Kirkegaard and Perry Laboratories (Gaithersburg, MD) was iodinated by the Iodo-gen method [7] to a specific activity of 30–60 μCi/μg protein.

2.2. *Production and screening of monoclonal antibodies*

Purified antigen, ganglioside GD1b, was adsorbed to acid washed *Salmonella minnesota*, the ganglioside to bacteria ratio being 1:10 by wt as described [8,9]. The antigen-bacteria complex, corresponding to 50 μg bacteria, in phosphate-buffered saline (PBS, 0.01 M phosphate buffer, pH 7.4, 0.14 M NaCl) mixed with an equal volume

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of Freund's complete adjuvant was injected subcutaneously. Booster doses, with the same amount of the complex in an equal volume of Freund's incomplete adjuvant, were administered on days 14 and 23 and fusion with Sp 2/0 cells was performed on day 26. The antibodies produced were assayed for their binding to GD1b with the ELISA-method described below. The positive hybrid was cloned by limiting dilution and the antibody was found to be of the IgM-type.

2.3. *Binding assays*

2.3.1. Ganglioside-ELISA

A modification of the ganglioside-ELISA method described by Svennerholm and Holmgren [10] was used and all steps were performed at room temperature. Serial dilutions of gangliosides, dissolved in 20 μl methanol, were added to the wells of a polyvinyl chloride microtiter plate (Titertek® Immuno Assay-Plate, Flow Laboratories, Ayshire, Scotland) and the solution was evaporated. Unspecific adsorption was blocked by preincubation, 30 min, with 1% bovine serum albumin, dissolved in phosphate-buffered saline (PBS-BSA), after which the wells were incubated for 3–4 h with 50 μl monoclonal antibody and then with HRP-conjugated anti-mouse immunoglobulin, diluted in PBS containing 0.05% Tween 20. Bound conjugate was assayed by addition of 100 μl of substrate (10 ml of 0.1 M citrate buffer (pH 4.5), 5 mg *o*-phenylenediamine, OPD, and 4 μl of 30% H_2O_2). After 10 min the absorbance was read in a spectrophotometer at 450 nm.

2.3.2. Radioimmunodetection of ganglioside antigen on thin-layer plates

Radioimmunodetection of ganglioside antigens separated on thin-layer plates was performed as described by Magnani et al. [11]. The gangliosides were chromatographed on aluminium-backed high performance silica gel plates (Silica gel 60, Merck AG, Darmstadt) and overlaid with anti-GD1b monoclonal antibody. Bound antibody was detected by ^{125}I-labelled anti-mouse IgM and the thin-layer plate was exposed to X-ray film (XR5, Eastman-Kodak, Rochester, NY).

3. RESULTS AND DISCUSSION

The fusion of spleen cells from 2 immunized

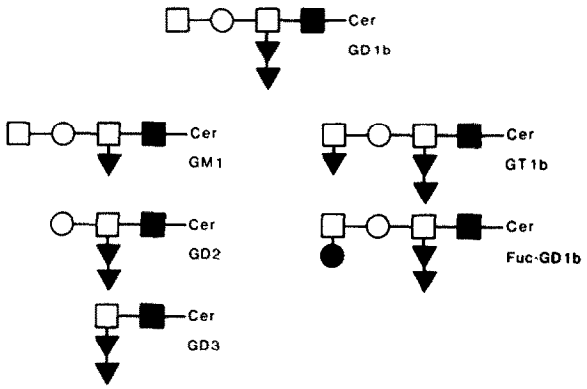

Fig.1. Schematic drawing of the ganglioside structures tested. (□) Galactose, (○) *N*-acetylgalactosamine, (■) glucose, (▼) *N*-acetylneuraminic acid, (Cer) ceramide.

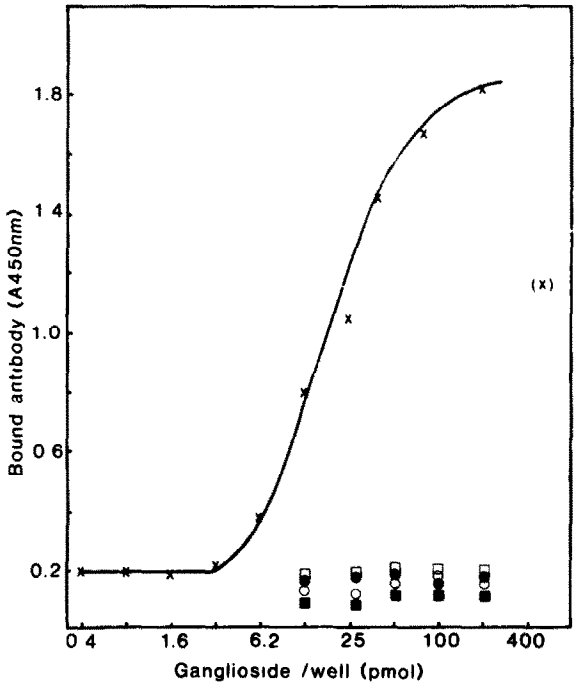

Fig.2. Ganglioside-ELISA assay of the binding of the anti-GD1b antibody to gangliosides. Isolation of gangliosides and the assay procedure are described in section 2. (×) GD1b, (□) Fuc-GD1b, (■) GT1b, (○) GM1 and (●) GD2. Gangliosides GD3, GQ1b and GD1a were also tested (not shown) and found to give negative results as GT1b, GM1 and GD2. The values represent the mean of triplicate determinations.

mice with mouse myeloma Sp 2/0 cells resulted in a hybrid that produced a high affinity monoclonal antibody to ganglioside GD1b (figs 2 and 3). The specificity of the antibody was tested by comparing its binding to purified gangliosides with closely related structures (see fig.1) and to ganglioside mixtures from brain tissue. In the ganglioside-ELISA assay (fig.2) half-maximum binding was obtained with approx. 25 pmol GD1b, while no binding occurred to other gangliosides even when 200 pmol was added to the wells. Radioimmunodetection of the antigen in the ganglioside fractions of human normal grey tissue (fig.3) revealed no binding to any ganglioside other than GD1b which in the lowest concentration corresponded to 4 pmol (fig.3, lane 1). The proportion of other gangliosides in these mixtures is seen in the orcinol stained part of fig.3.

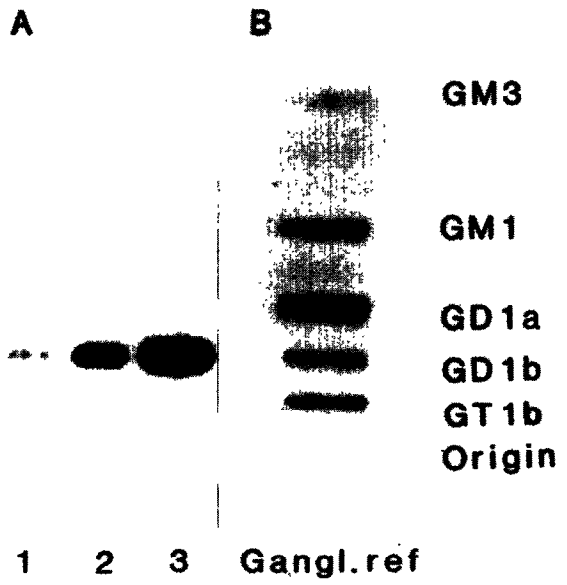

Fig.3. Radioimmunodetection of the binding of the anti-GD1b antibody to gangliosides from human brain grey matter. Isolation of gangliosides and the assay procedure are described in section 2. (A) Binding of the anti-GD1b antibody to gangliosides from (1) 20 μg, (2) 100 μg and (3) 500 μg wet wt grey matter, containing 4, 20 and 100 pmol GD1 ganglioside, respectively. (B) Orcinol staining of the gangliosides from 1 mg wet wt grey matter.

The anti-GD1b monoclonal antibody appears to be specific for the carbohydrate moiety of GD1b. The binding activity is completely eliminated by removal of any terminal sugar residue such as sialic acid (to give GM1) or galactose (GD2) or by the addition of sialic acid or fucose to the terminal galactose (GT1b) and Fuc-GD1b, respectively (fig.1). In addition, GD3 had no binding activity, which indicates that a disialosyl group bound to galactose is not sufficient for binding.

Monoclonal antibodies with specificity to GD3, GM2 and GD2 have been reported [12–15] but to our knowledge this is the first report of a specific monoclonal antibody to any of the major brain gangliotetraosyl gangliosides.

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