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GANGLIOSIDE GD2 SPECIFICITY OF MONOCLONAL ANTIBODIES TO HUMAN NEUROBLASTOMA CELL

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Four monoclonal antibodies (3F8, 3A7, 3G6, and 2F7) against human neuroblastoma cells have been suggested to react with surface glycolipids of these cells. In this report these monoclonal antibodies were shown to be specific to the disialoganglioside GD2 using a thin-layer chromatography (TLC)-immunostaining method. When mixed human brain gangliosides were developed by TLC in two different solvent systems and incubated with each of the monoclonal antibodies, only GD2 was stained. These antibodies also reacted with highly purified GD2 on the plate. These findings suggest that GD2 provides an antigenic site on the surface of human neuroblastoma cells.

Several antigenic determinants have recently been demonstrated on the surface of neuroblastoma cell (1-4). Binding studies on human tissues revealed that each of these antigenic determinants is not restricted to neuroblastoma cells, but commonly found in cells of the same lineage, e.g., cells of neuroectodermal origin (4). Although one of these antigens has been shown to be the human "Thy-1" molecule (3), others have not been fully characterized. Gangliosides are a family of sialic acid-containing sphingoglycolipids located primarily on the outer surface of cell membranes. The oligosaccharide moieties of gangliosides as well as glycoproteins are known to provide antigenic sites on the cell surface (5,6). While they are found in essentially all vertebrate

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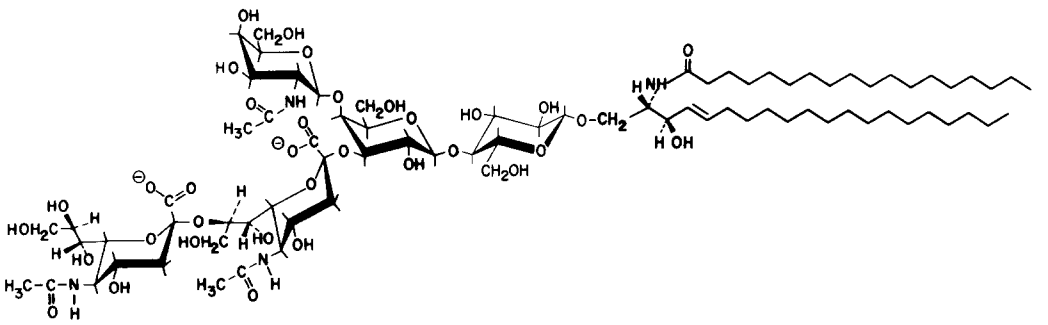

Figure 1. Structure of GD2 ganglioside.

tissues and body fluids, they are relatively enriched in the nervous system and tumor cells of neural origin, such as neuroblastoma cells (5,7).

Cheung et al. (8) have recently prepared four monoclonal antibodies (3F8, 3A7, 3G6 and 2F7) against human neuroblastoma cells by a standard hybridization method, and provided evidence that these antibodies reacted with surface glycolipid antigens. In this report we show that the disialoganglioside GD2(Fig.1), a major ganglioside of human neuroblastoma cells, is specific for all these monoclonal antibodies.

Materials and Methods

Monoclonal antibodies - Murine monoclonal hybridomas against human neuroblastoma were established by fusing SP-2 mouse myeloma cells with spleen cells of BALB/c mice hyperimmunized intraperitoneally with human neuroblastoma cells as previously described (8). Four antibody-secreting hybridomas were subcloned and maintained as stable cell lines. One monoclonal antibody belongs to the IgG3 class (3F8) and others are of the IgM class (3A7, 3G6 and 2F7).

Gangliosides - Gangliosides were purified from adult human brains as previously described (9).

Preparation of ^{125}I-Staphylococcal protein A (SPA) -The iodination of SPA (Pharmacia Fine Chemicals, Sweden) was carried out with the ^{125}I-Bolton-Hunter reagent (10,11).

Thin-layer chromatography (TLC) - A human brain ganglioside mixture or purified GD2 was developed on a high-performance TLC plate (nanoplate, 10 x 20 cm, E. Merck, West Germany) with the solvent system of chloroform/methanol/water (50:45:10) containing 0.02% $CaCl_2$ or chloroform/methanol/5N NH_4OH/0.4% $CaCl_2$ (60:40:4:5).

Immunostaining on the chromatogram - The binding of monoclonal antibodies to gangliosides on the TLC plate was carried out according to the method of Kasai et al. (6) with some modifications. The TLC plate was dried completely in vacuo and immersed in a 0.4% polyisobutylmethacrylate solution (high molecular weight, Aldrich Chemicals, USA) for one min. The polymer solution was prepared by diluting a 2.5% chloroform solution of the polymer with n-hexane. After the plate was dried in air, the chromatogram was incubated with a monoclonal antibody solution at room temperature for 1.5 hours. The antibody solution was prepared by diluting the antibody- containing culture medium or a control medium with 0.3% gelatin- phosphate-buffered saline (gelatin-PBS pH 7.3). For monoclonal antibodies of the IgM type (3A7, 3G6 and 2F7), each lane was incubated with a rabbit anti-mouse immunoglobulin serum (Calbiochem-Behring Corp., USA, diluted to 1/32 with gelatin-PBS) at room temperature for 1.5 hours. It was then incubated with a ^{125}I-SPA (5 x 10^5 cpm/ml of 0.3% gelatin-PBS) under the same conditions as described above. Finally, the chromatogram was thoroughly washed with PBS containing 0.1% Triton X-100 and exposed to an X-ray film at -70°. After the exposure, each band of gangliosides were visualized by spraying with resorcinol/HCl reagent (12). For the monoclonal antibody of

the IgG type (3F8), the incubation step with the rabbit anti-mouse immunoglobulins serum was omitted.

Results and Discussion

The ganglioside composition of human neuroblastoma cells has been reported by several authors (7,13). The major ganglioside is GD2 which constitutes one-fourth to one-fifth of the total; other gangliosides such as GM3, GM2, GM1, GD3, GD1a, GD1b, GT1b and GQ1c, are also present in considerable amounts (13). Thus, any of the gangliosides can be a potential candidate for providing the antigenic determinant on the surface of neuroblastoma cells. The TLC-immunostaining method is particularly useful in such a situation because several gangliosides can be screened simultaneously by using a ganglioside mixture.

When brain gangliosides were separated on TLC with the neutral solvent system and were incubated with each of the antibodies, only one positive band was observed which had the same migratory rate as GD2 in all cases; the control culture medium did not stain any band (Fig. 2). The position of the positive band stained with the 3F8

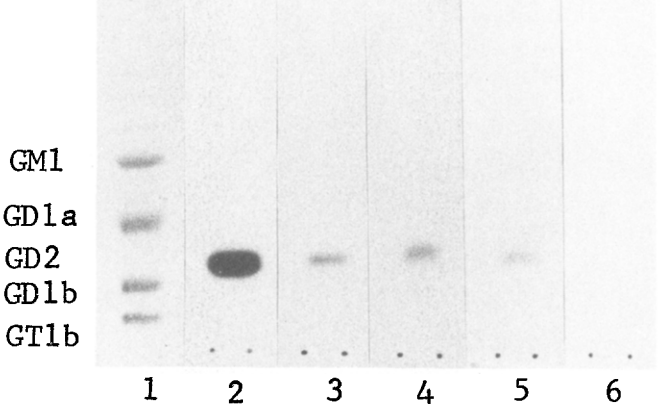

Figure 2. A total ganglioside mixture prepared from human brain grey matter (1.5ug as sialic acid) was developed on a TLC plate with the solvent system of chloroform/methanol/water (50:45:10) containing 0.02% $CaCl_2$. Lanes 2,3,4,5 and 6 were incubated with the 3F8, 3A7, 3G6, 2F7 antibody and the control medium, respectively. Lane 1 was a ganglioside reference visualized with the resorcinol/HCl reagent.

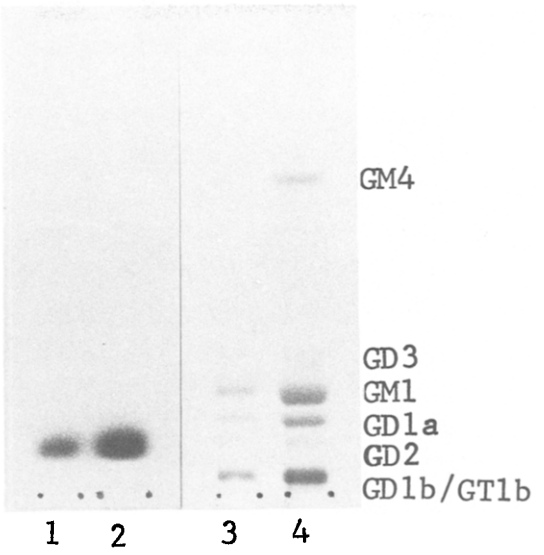

Figure 3. Two different amounts of a total ganglioside mixture were developed with the solvent system of chloroform/methanol/5N NH_4OH/0.4% $CaCl_2$ (60:40:4:5), and incubated with the 3F8 monoclonal antibody: lane 1 (1.5ug as sialic acid) and lane 2 (6ug as sialic acid). Lanes 3 and 4 were ganglioside references for lanes 1 and 2, respectively.

antibody was still identical with that of GD2 even when the gangliosides were separated by developing with the alkaline solvent system (Fig. 3). These results strongly suggest that GD2 is the antigen for these monoclonal antibodies. This conclusion is substantiated by the observation that all these antibodies reacted with highly purified brain GD2 (Fig. 4, data for the 3A7, 3G6, and 2F7 antibodies not shown). Since the monoclonal antibodies failed to interact with GM3, GD3 and GM2, the terminal sialic acid and N-acetylgalactosamine residues are probably involved in the epitope for these antibodies.

In conclusion, we have shown that GD2 ganglioside reacts specifically with several monoclonal antibodies prepared using human neuroblastoma cells as the immunogen. These monoclonal antibodies appear to have the same antigenic specificity as the murine monoclonal antibody (Mab 126), which is of the IgM type, produced independently by Schultz et al. (14). The availability of

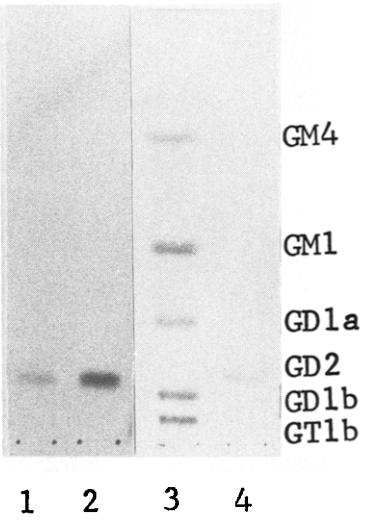

Figure 4. A ganglioside mixture prepared from human brain white matter (1.5ug as sialic acid, lanes 1 and 3) or purified GD2 (0.5ug as lipid, lanes 2 and 4) was developed with the same solvent system as described in Fig. 1. Lanes 1 and 2 were incubated with the 3F8 monoclonal antibody. Lanes 3 and 4 were ganglioside references for lanes 1 and 2, respectively.

these antibodies thus provides us with a suitable probe for the immunocytochemical examination of the distribution and localization of GD2 ganglioside on tumor cells. They also should facilitate the detection of GD2 in sera of patients with neuroblastoma (14,15). Additionally, the possibility of employing these highly specific antibodies for the immunosuppression of neuroblasma, as seems possible for the inhibition of melanoma cell growth by monoclonal anti-GD3 antibody (16), should be seriously contemplated.

Acknowledgments

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