APPLICATIONS OF MONOCLONAL ANTIBODIES TO GANGLIOSIDES IN NEUROSCIENCE

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ABSTRACT

We established an improved method for the generation of mouse monoclonal antibodies (MAbs) to gangliosides by immunizing gangliosides. These MAbs enabled us to examine the distribution of ganglioside in the brain. Immunohisto- and immunocyto-chemical studies suggested that there is a cell type-specific expression of gangliosides in the central nervous system.

Introduction

Gangliosides, sialic acid-containing glycosphingolipids, are normal membrane constituents and are highly expressed in the vertebrate central nervous system (1). Owing to their topological localization on the outer surface of neural plasma membranes and their unique chemical structure, gangliosides have been implicated in a variety of phenomena involving cell-cell recognition, neurite outgrowth, synaptogenesis, transmembrane signaling, and cell growth and differentiation (2-5). An understanding of the cellular localization of gangliosides in the brain could provide insight into the possible function of these molecules. In the past decade, cholera and tetanus toxins, and several polyclonal and monoclonal antibodies (MAbs) reacting with gangliosides have been used as probes for detecting gangliosides in neurons and glia (6, 7). It was, however, difficult to generate MAbs specific for individual gangliosides. We recently established an improved method for the generation of mouse MAbs to gangliosides by immunizing mice with purified gangliosides (8-15). These MAbs enabled us to examine the distribution of ganglioside in the central nervous system. These studies revealed the differential distribution patterns of gangliosides in the brain regions (16-19).

Materials and Methods

MAbs to gangliosides

The production and characterization of MAbs has been described previously (9-15). Briefly, all of the MAbs were generated by immunizing C3H/HeN mice with purified gangliosides adsorbed to *Salmonella minnesota* mutant R595. The binding specificity of these MAbs was determined by an enzyme-linked immunosorbent assay and an immunostaining on thin-layer chromatogram. Most of these MAbs show highly restricted binding specificity, reacting only with the immunizing ganglioside. None of other various authentic gangliosides or neutral glycolipids were recognized. Although most of the MAbs were of IgM, some MAbs belonged to IgG.

Immunohistochemistry

The expression of gangliosides in frozen sections of rat brain was determined by the indirect immunofluorescence technique with specific MAbs as previously described (16).

Immunocytochemistry

Cells were fixed with paraformaldehyde and stained in an immunofluorescence procedure as previously described (18).

Conclusions

Immunohistochemical studies of gangliosides in the rat brain

At first, we attempted to investigate the localization of major gangliosides in the adult rat brain by an immunofluorescence technique with mouse MAbs. Five MAbs that specifically recognize gangliosides GM1, GD1a, GD1b, GT1b and GQ1b, respectively, were used. We have found that there is a cell type-specific expression of the gangliosides in the rat central nervous system (16). As a next step, we studied the distribution of minor gangliosides in the adult rat brain by an immunofluorescence technique with mouse MAbs. Ten MAbs that specifically recognize GM3, GM2, GT1a, GD3, O-Ac-disialoganglioside, GD2, GM1b, GM4, IV^{3}NeuAcα-nLc$_4$Cer, and IV^{6}NeuAcα-nLc$_4$Cer, respectively, were used. Our study revealed that there is a cell type-specific expression of minor gangliosides as well as major gangliosides in the rat brain. (17) Subsequently, we studied the distribution of gangliosides during the development of postnatal rat cerebellum by an immunofluorescence technique with mouse MAbs. Eleven MAbs that specifically recognize each ganglioside changed dramatically during the development (19).

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Immunocytochemical study of gangliosides in primary cultured neuronal cells

Then, we studied the expression of ganglioside antigens in primary cultures of rat cerebellum using an immunocytochemical technique with mouse MAbs specific for various gangliosides. Twelve MAbs that specifically recognize each ganglioside were used. Our study revealed that there is a cell type-specific expression of ganglioside antigens in the primary cultures (19). Some caution must be used in interpreting the expression of ganglioside antigens based on immunocytochemistry, since a lack of immunorecognition of ganglioside epitope on cells does not necessarily mean that a ganglioside is absent. There are indications that a number of factors are involved in influencing the reactivity of MAbs with specific cells: (i) the density of ganglioside on cells is involved in the reactivity of antibodies, (ii) other components of the cell surface may influence antibody reactivity; and (iii) the ceramide portion of gangliosides may be involved in the reactivity (20-22). Further study will be needed for elucidating the precise mechanisms of immunoreactivity, particularly in normal cells, since previous reports were based mainly on the studies of cancer cells. An immunoelectron microscopy study will be necessary to further evaluate the localization of the gangliosides in cells in the rat brain.

Acknowledgments

The author wishes to thank Drs. I. Kawashima, H. Ozawa, M. Kotani, and K. Ogura (Tokyo Metropolitan Institute of Medical Science) for their collaborations. He also thanks Drs. T. Terashima and Y. Nagata (Tokyo Metropolitan Institute of Neuroscience) for their valuable suggestions.

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DIFFERENCES IN IMMUNOLOGICAL BEHAVIOR BETWEEN NAcGM3 AND NGcGM3 GANGLIOSIDES

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Gangliosides expression in breast tumors which were histopathologically diagnosed as invasive ductal carcinoma were examined. Total gangliosides level measured as lipid-bound sialic acids were found to be significatly higher than those in normal tissues (19.7 +/- 13.0 microgram sialic acid/g of wet tissue *versus* 8.8 +/- 5.3 microgram/g, $p < 0.005$).

Eighteen of 37 tumor cases showed levels of gangliosides higher than 2 SD the mean in normal tissues. Major gangliosides were GM3 and GD3 and they accounted for 85-90 % of the lipid-bound sialic acid in both normal tissues and tumor tissues. The levels of GM3 and GD3 in tumor tissues were 2.8 fold and 1.7 fold greater than those in normal tissues, respectively.

O-acetyl gangliosides were characterized by TLC-immunostaining using anti-O-acetyl ganglioside monoclonal antibodies, GMR2 and 493D. N-glycolylneuraminic acids were detected in breast cancer gangliosides by positive reactions with H-D antibody and monoclonal antibody P3. These minor gangliosides were also characterized using FAB/MS. Unusual gangliosides such as O-acetyl GD3, O-acetyl GT3 (present in fetal brain) and N-glycolyl GM3 (not present in human and chicken tissues) were found to be expressed in most tumor samples.

In order to evaluate if NGcGM3 is more immunogenic in chickens than NAcGM3, different strains of these animals were immunized with both gangliosides adsorbed in human very low density lipoproteins (VLDL) in the presence of adjuvants and the titers of anti-ganglioside specific IgG antibodies were measured. All chickens inoculated with NGcGM3/VLDL raised IgG antibodies whereas in those inoculated with NAcGM3/VLDL specific antibodies were absent. Interestingly, IgG antibodies induced by immunization with NGcGM3/VLDL also recognized other N-glycolylated gangliosides and did not react with others N-acetylated gangliosides. These results suggest that the "non self" character of the N-glycolylated sialic acid moiety could be critical for the different immunological behavior of these gangliosides.

The finding that NGcGM3 ganglioside is present in human breast tumor has provided the rationale for the design of a cancer vaccine project currently ongoing.

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