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The anti-oligosaccharide antibodies present in sera from patients with motor neuron disease and neuropathy recognize the *N*-glycolylneuraminic acid containing gangliotetrahexosyl oligosaccharide

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We found that serum antibodies present in the serum of patients with motor neuron disease and neuropathy, which were previously shown to react with the oligosaccharide chain of ganglioside GM1(Neu5Ac), can be recognized and titred using the *N*-glycolylneuraminic acid containing monosialo-gangliotetrahexosylceramide, GM1(Neu5Gc), which is not a component of normal human cells. The antibody-antigen reaction was abolished by immunoabsorption with the free oligosaccharide chain. This result, together with the knowledge that these antibodies recognize several glycoconjugates, supports the conviction that these antibodies are non-specific for a gangliosidic structure.

Keywords: membrane glycoconjugates, neuropathy, antibodies

Introduction

It has been shown that antibodies that recognize carbohydrate epitopes shared by glycoconjugates, components of mammal and bacterial cells, are present in human serum. Though these antibodies are normally found in the serum of normal subjects [1–5], high titres are found only in patients with motor neuron disease and neuropathy [2, 4, 6–12], but the relationship between the antibodies and the neurological disease remains unclear.

Gangliosides‡ are widely used in TLC-immunostaining and ELISA titration experiments to recognize serum anti-carbohydrate antibodies. On the other hand more recent results show that these antibodies also recognize neutral glycosphingolipids, polysialogangliosides and glycoproteins from human tissues, and lipopolysaccharides of bacterial membranes [13–18]. The results of this work reinforce this position by showing that the serum from patients with motor neuron disease and chronic inflammatory demyelinating polyneuropathy has similar titres to *N*-acetylneuraminic acid containing GM1 and *N*-glycolylneuraminic acid containing GM1, this latter being not a component of normal human cell membranes [19].

Materials and methods

Ganglioside GM1(Neu5Ac) was extracted from calf brain [20] and purified [21]. GM1 containing *N*-glycolylneuraminic acid, GM1(Neu5Gc), and gangliotetrahexosylceramide were prepared from GM1(Neu5Ac) [22, 23]. The glycosphingolipid free oligosaccharide moieties were released from the corresponding sphingolipids by the ozonolysis procedure [24].

Human sera were from patients with motor neuron disease (MND) [9] and chronic inflammatory demyelinating polyneuropathy (CIDP) [25]. HPTLC-immunostaining was performed as reported [9] at serum dilutions of 1:100, 1:1 000 and 1:10 000.

For immunoabsorption studies 200 μl of the sera were diluted 1:2 000 with PBS-1% albumin containing different amounts of galactose or 400 $\mu g\,ml^{-1}$ of the glycosphingo-

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‡Ganglioside nomenclature is in accordance with Svennerholm (1980) [30].

lipid oligosaccharide moiety. After 12 h incubation the immunoabsorbed sera were tested for reactivity to glycosphingolipids by TLC-immunostaining.

Results and discussion

Yoshino *et al.* [26] reported that sera from patients with neurological diseases reacted with GM1(Neu5Gc). According to this information the reactivity to GM1(Neu5Gc) of two sera from patients with motor neuron disease (MND) and chronic inflammatory demyelinating polyneuropathy (CIDP), was studied and compared with that of GM1(Neu5Ac). These two sera were previously shown [9, 25] to have high IgM titres to GM1(Neu5Ac)

Figure 1 shows the reactivity of the two sera to GM1(Neu5Ac) and GM1(Neu5Gc). The reactivity of MND-serum to GM1(Neu5Ac) and GM1(Neu5Gc) is very similar. In fact, both gangliosides are stained to the same extent even at a serum dilution of 1:10 000. The reactivity of CIDP-serum to both gangliosides is up to 1:1 000 but it seems that the reactivity to GM1(Neu5Gc) is a little higher than to GM1(Neu5Ac). The reactivity of the sera to gangliosides was abolished by immunoabsorption with both the GM1(Neu5Ac) and GM1(Neu5Gc) oligosaccharide moiety.

The discovery that the sera from patients with neurological diseases, recognize GM1(Neu5Gc), a ganglioside which is never found in normal human tissues [19, 27], suggests that they are not very specific. Moreover, to know for a fact that the serum immunoabsorption with the free oligosaccharide abolishes the serum reactivity, indicates that the ceramide portion of gangliosides is not necessary for the antibody-antigen reaction. This confirms the previous results which showed that these sera also recognize membrane glycoproteins and lipopolisaccharides from bacterial membranes [13–18]. On the other hand, our results indicate that the ganglioside mixture used for therapeutic purposes [10], must be devoided of the Neu5Gc containing gangliosides, which due to their immunogenetic properties [27] could elicit the production of antibodies. The sera used in this work were from patients who were never therapeutically treated with gangliosides.

The MND-serum [10], was studied in detail and is now well characterized. This serum shows a similar high reactivity to gangliotetrahexosylceramide, GM1-(Neu5Ac), GM1(Neu5Gc) (the reactivity was abolished by immunoabsorption with every free oligosaccharide chain) and GD1b but no reactivity to GD1a, Fuc-GM1 and GM2. These results indicate that the presence of a terminal Gal is essential. To study this we incubated the MND-serum with galactose, but the reactivity to GM1(Neu5Ac) and to GM1(Neu5Gc) was not abolished even at a Gal concentration of 30 mg ml^{-1}. Therefore the terminal Gal itself does not explain the serum reactivity. Moreover, if the neutral Gal-GalNAc-Gal-Glc oligosaccharide chain shared by all the glycolipids mentioned

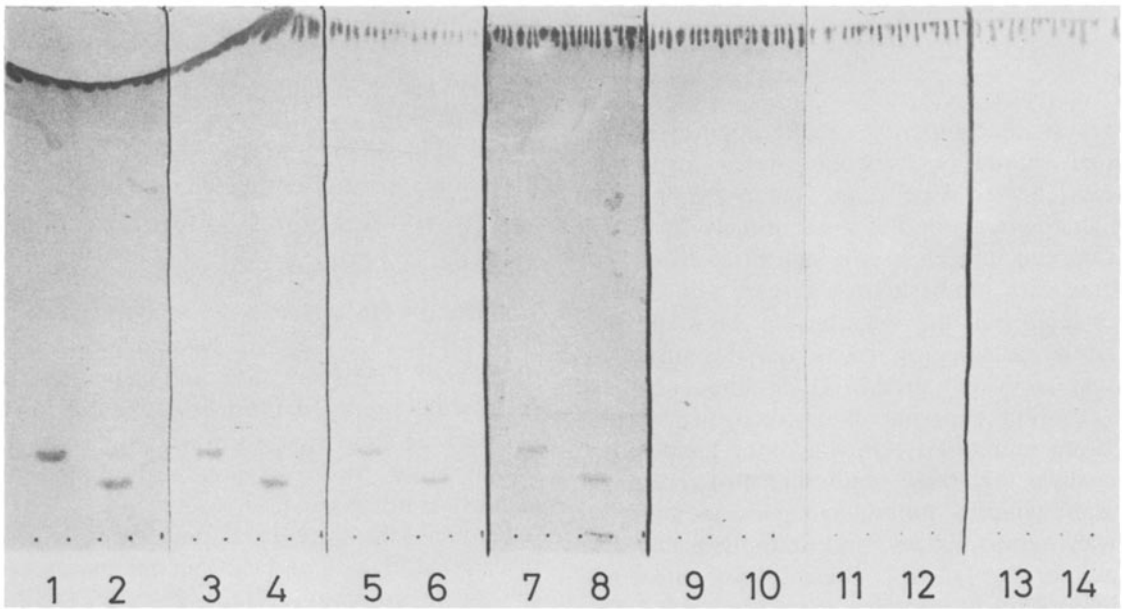

Figure 1. HPTLC of GM1(Neu5Ac) and GM1(Neu5Gc) followed by immunostaining with serum from patients with motor neuron disease (MND) and chronic inflammatory demyelinating polyneuropathy (CIDP). Odd numbers, GM1(Neu5Ac); even numbers, GM1(Neu5Gc). 1 and 2, 1:100 MND; 3 and 4, 1:1000 MND; 5 and 6, 1:10000 MND. 7 and 8, 1:100, CIDP; 9 and 10, 1:1000 CIDP; 11 and 12, 1:10000 CIDP. 13 and 14, staining with the second antibody (αIgM), control. HPTLC solvent system, chloroform: methanol:water:32% NH_4OH, 60:35:7:1.5 by vol.

above is considered, it is clear that the terminal Gal must be free; in fact the addition of a Fuc or a Neu5Ac residue to the external Gal completely abolished the reactivity. On the other hand it has been previously shown [1] that in sera from patients with similar neuropathies, the antibody-antigen reaction can be inhibited by the disaccharide β-Gal-(1-3)-β-GalNAc, confirming that the terminal disaccharide is essential for the antigenity of the complex molecule. Finally the addition of a sialic acid, Neu5Ac or Neu5Gc, or a disialyl chain does not abolish the original reactivity to the tetrahexosylceramide. The sialic acid is linked to the inner Gal but is also close to GalNAc, so that it forms a network of interactions [21, 28–29]. This could suggest that the antibodies recognize the Gal-GalNAc disaccharide side which is opposite that occupied by the sialic acid residue.

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