Glycoconjugate Journal (1994) **11**:345–352

Determination of structural elements of the L2/HNK-1 carbohydrate epitope required for its function

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Received 7 April 1994, revised 22 June 1994

The L2/HNK-1 carbohydrate epitope has been shown to carry an unusual 3′-sulfoglucuronic acid linked *O*-glycosidically through a neolactosyl-type backbone to a ceramide residue. Using monoclonal antibodies, the same or a closely related epitope has also been detected *N*-glycosidically linked to glycoproteins, amongst them several neural cell adhesion molecules. We used synthetic glycolipids carrying sulfated or non-sulfated glucuronic acid attached to ceramide through glycans of different length to show that not only the sulfated glucuronic acid but also the neolactosyl-type backbone is essential for the recognition of the L2/HNK-1 carbohydrate by a monoclonal antibody, its binding to laminin and its role in neural cell migration and outgrowth of processes from neurons and astrocytes.

Keywords: L2/HNK-1 carbohydrate; antibody recognition; laminin binding; neural migration and process outgrowth

Abbreviations: mab, monoclonal antibody; TLC, thin layer chromatography; HRP, horseradish peroxidase; glcA, glucuronic acid; gal, galactose; glcNAc, *N*-acetyl-glucosamine; man, mannose.

Introduction

The L2/HNK-1 carbohydrate (abbreviated to L2) is expressed on cells of the immune and nervous system [1, 2]. Except for its binding to P- and L-selectins and laminin [3–5], little is known of its function outside the nervous system. In the nervous system, the L2 epitope has been shown to be implicated in migration of neural crest cells [6], the adhesion of astrocytes and neurons to laminin [7], the outgrowth of astrocytic and neuritic processes [8], the preferential outgrowth of neurites from motor neurons [9], and finally the homophilic binding of the neural cell adhesion molecule P0 [10]. The structure of L2 has been identified as having 3′-sulfated glucuronic acid at the non-reducing end of glycolipids with a neolactosyl-type backbone (Fig. 1) [11, 12]. Whether the L2 epitope detected by monoclonal antibodies on neural cell adhesion molecules [1, 13] has the same structure when linked *N*-glycosidically to glycoproteins is still an undecided issue [14–16].

The monoclonal antibodies recognizing the L2 carbohydrate were either experimentally produced against enriched or purified antigen preparations [2, 17–19], or were IgM-proteins detected in the serum of patients with neuropathy and plasma cell dyscrasia [2, 12, 19–21]. The antibodies seemed to differ in their reactivity towards the 3′-sulfoglucuronic acid containing glycolipids and several modified structures related to it. Some of them, for example the HNK-1 antibody, showed a strong requirement for sulfate, but not for glucuronic acid; another group of antibodies required the free carboxylic group but not the sulfate; other antibodies needed only one or other of these negatively charged groups [5, 20, 22].

The fact that these antibodies, which have also been used for functional studies, seem to recognize different aspects of what has been designated as the L2 carbohydrate epitope, raised the question as to what structural features are required for its function. We studied binding of different synthetic glycolipids to a monoclonal antibody and to laminin as well as the influence of these glycolipids on neural cell migration and outgrowth of processes from neurons and astrocytes. Our results indicate that the L2 carbohydrate epitope comprises not only the sulfated glycuronic acid, but also part of the neolactosyl-type carbohydrate it is attached to.

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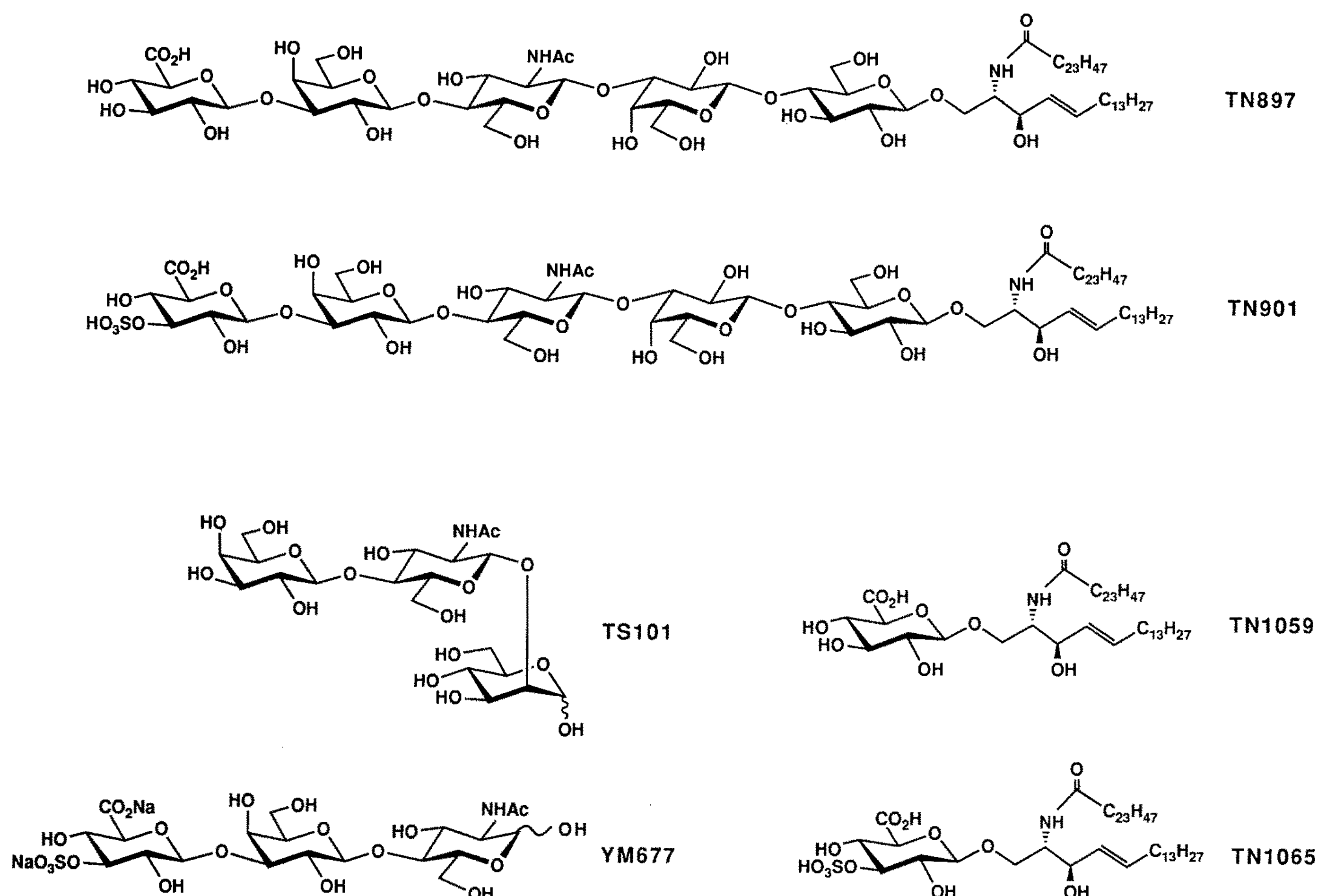

Figure 1. Structures of TN 901, 897, 1059, 1065, YM 677 and TS 101. YM 677 and TS 101 were converted to neoglycolipids. For structural details of neoglycolipids see Pohlentz *et al.* [29].

Materials and methods

Antibodies

Monoclonal L2 antibody 412 (mab 412) and polyclonal antibodies to laminin and fibronectin were obtained as described previously [17, 23, 24]. Polyclonal antibodies to glial fibrillary acid protein (GFAP) were purchased from Dakopatts; horseradish peroxidase (HRP)-conjugated secondary antibodies directed against rat IgG and IgM or rabbit IgG, and fluorescein isothiocyanate-conjugated secondary antibodies were obtained from Dianova, Hamburg, Germany.

Glycolipids

A) Synthesis of oligosaccharides and glycolipids (*Fig. 1*)

TS 101 was synthesized according to the method described by Arnarp and Lönngren [25]. YM 677 was synthesized according to the procedure of Nakano *et al.* [26, 27] and the details will be published separately. Glycolipids TN 897, TN 901, TN 1059 and TN 1065 were synthesized according to the procedure of Nakano [28].

B) Synthesis of neoglycolipids

Neoglycolipids [29] were synthesized from TS 101 and YM 677 according to the procedure outlined by Stoll *et al.* [30]. In brief, 500 μg oligosaccharides in 20 μl methanol:water (1:1, v/v) were mixed with 4 mg dipalmitoylphosphatidylethanolamine (Sigma) dissolved in 800 μl chloroform:methanol (1:1, v/v). After incubation for 2 h at 60 °C, 1 mg sodium cyanoborohydride in 100 μl methanol was added and the reaction continued for up to 5 days. The progress of the reaction was monitored by TLC. The chromatogram was first developed with chloroform:methanol:water (105:100:28, v/v) until the solvent front had reached about 2/3 of the height of the plate and, after drying, with chloroform:methanol:water (75:25:4, v/v) until the solvent front had reached the top of the thin layer plate. Glycolipids were visualized by staining with orcinol.

The neoglycolipids were purified on Sephadex LH 20 (column size: 140 × 18 mm; Pharmacia) with chloroform:methanol:water (5:5:1, v/v) as eluent and then on Iatrobeads (column size: 80 × 18 mm; Macherey & Nagel, Düren, Germany) first with chloroform:methanol:water

(75:25:4, v/v) until the underivatized phosphatidylethanolamine eluted and finally with chloroform:methanol:water (105:100:28, v/v). The fractions from each column were analysed by TLC and the neoglycolipid-containing fractions pooled.

C) Isolation of L2 glycolipids

The L2 carbohydrate carrying glycolipids (3′-sulfoglucuronylneolactotetraosyl- and -hexaosyl-ceramide) were isolated from human sciatic nerve according to Chou *et al.* [11] with the only difference being that the digestion of gangliosides with neuraminidase from *Arthrobacter ureafaciens* (Boehringer Mannheim) was carried out prior to ion exchange chromatography on DEAE-Sephadex A-25 (Sigma). The L2 glycolipids were also kindly provided by Dr Robert K. Yu.

Enzyme-linked immunosorbent assays (ELISA)

A) Determination of antibody binding to substrate-coated glycolipids

Antibody binding to glycolipids coated on to the plastic of 96-well microtitre plates was carried out as described previously [7]. The amount of glycolipids bound to the plastic of the microtitre plates was determined as described [31] and found to be approximately the same for all glycolipids used.

B) Binding of glycolipids to laminin and fibronectin

Coating of glycolipids on to microtitre plates and blocking was carried out as above. Incubation of substrate-coated glycolipids for 1.5 h with laminin from Engelbreth-Holm Swarm sarcoma (Boehringer Mannheim; in 0.1 M $NaHCO_3$ containing 2 mM EDTA) by serial doubling dilution starting with 1.2 μM, or with fibronectin from human serum (Sigma; 10 μg ml^{-1} PBS), was followed by incubation with polyclonal laminin or fibronectin antibodies for 2 h and HRP-conjugated secondary antibodies for 1 h. All steps were carried out at room temperature.

Cell culture and immunocytological procedures

The influence of glycolipids on cell migration and outgrowth of neurites and astrocytic processes from cerebellar microexplants (see below) on laminin- or fibronectin-coated glass coverslips was investigated as described [8]. Cerebella were taken from 5- to 7-day-old ICR mice and freed from meninges, choroid plexus and deep cerebellar nuclei. The remaining tissue was then forced through a Nitrex nylon mesh, pore size 300 μm, and the tissue particles thus obtained (= microexplants) washed three times in serum-free hormone-supplemented medium [32]. Microexplants were plated on to glass coverslips in the same culture medium (50 μl per 16 mm diameter coverslip). The glass coverslips had been previously coated with 20 μg per ml laminin or 25 μg per ml fibronectin in basal medium Eagle's (BME). Glycolipids were dried under a stream of nitrogen, resuspended at a concentration of 20 μg ml^{-1} in BME by vortexing and added 4 h after plating on laminin and 16 h after plating on fibronectin. Microexplants on laminin as substrate were examined 1 day later by phase contrast microscopy and by indirect immunofluorescence staining of glial fibrillary acidic protein according to Fischer *et al.* (33). Microexplants on fibronectin as substrate were examined by phase contrast microscopy 2 days later.

Results

The sulfated glucuronic acid of the L2 carbohydrate is not sufficient for recognition by monoclonal antibody 412

Monoclonal antibody 412 (mab 412) has been shown to detect the L2/HNK-1 carbohydrate epitope on several neural cell adhesion molecules and also on glycolipids isolated from peripheral nerve [1]. We here show that mab 412 recognizes the synthetic L2 glycolipid TN 901 (Fig. 2) which is identical to the 3′-sulfoglucuronyl-neolactotetraosyl-containing L2 glycolipid from peripheral nerve (see Fig. 1 for the structures of all glycolipids used). The

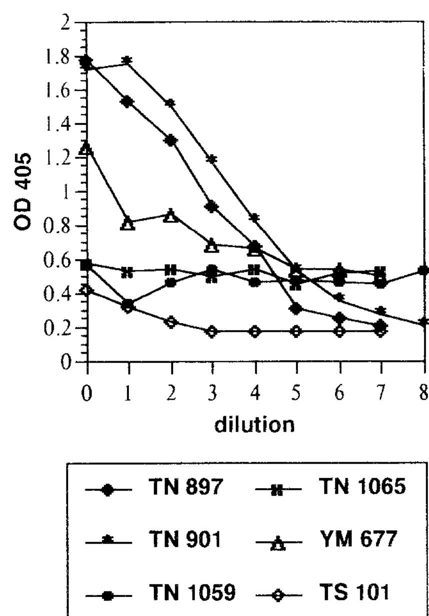

Figure 2. Determination of the reactivity of mab 412 with the glycolipids TN 897, TN 901, TN 1059, TN 1065 and the neoglycolipids YM 677 and TS 101 as quantified by ELISA (for structures of the glycolipids see Fig. 1). Glycolipids were coated in serial doubling dilution starting with 1.5 μM for TN 901 and TN 897 and from 3 μM for TN 1059, TN 1065 and neoglycolipid YM 677. After incubation with mab 412 (5 μg ml^{-1}) and secondary anti-rat antibodies, the optical density at 405 nm (OD 405) of reaction products with HRP-conjugated secondary antibodies was measured. One representative out of three experiments is shown. Standard deviations were always less than 10%.

reactivity of mab 412 with the non-sulfated glycolipid TN 897 was slightly less than with TN 901 at all concentrations determined (Fig. 2). Reactivity of mab 412 with YM 677-neoglycolipid, which contains only the trisaccharide 3-sulfo-glcA-gal-glcNAc, was significantly lower than with TN 901 (Fig. 2). The two other compounds tested which contain sulfated (TN 1065) or non-sulfated (TN 1059) glucuronic acid directly linked to ceramide, were not recognized by the antibody (Fig. 2). A control neoglycolipid containing the trisaccharide gal-glcNAc-man (TS 101) was also not recognized by mab 412.

The sulfation of glucuronic acid and the neolactosyl-type structure of the L2 carbohydrate are required for the binding to laminin

It has been shown that L2 glycolipids bind to laminin [5, 7].

In order to investigate whether binding to laminin would also require part of the oligosaccharide backbone in addition to the sulfated glucuronic acid, glycolipids were coated on to microtitre plates and incubated with laminin. Laminin bound to substrate-coated TN 901 as expected in a concentration-dependent saturable manner (Fig. 3). No significant binding of laminin to any of the other glycolipids tested was observed (Fig. 3).

Our results indicate that both the sulfation of glucuronic acid and part of the oligosaccharide to which the sulfo-glucuronic acid is attached, are needed for the binding of laminin.

The sulfation of glucuronic acid and the neolactosyl-type structure of L2 glycolipids are required for inhibition of cell migration and outgrowth of processes from neurons and astrocytes

The L2 carbohydrate has been implicated in cell migration and outgrowth of processes from neurons and astrocytes [8]. In order to investigate the structural elements of the L2 carbohydrate required for the previously observed inhibitory effects on cell migration and outgrowth of processes from neurons and astrocytes [8], we studied the influence of TN 901, 897, 1059 and 1065 on these functions in microexplant cultures from the cerebellum of early postnatal mice. When microexplants were maintained on laminin in the presence of TN 901 added to the culture medium, the same effects were observed as in the presence of the L2 glycolipids from sciatic nerve (Figs 4E, F and 5E, F; see also [8]), i.e. outgrowth of neurites and astrocytic processes and migration of neuronal and astrocytic cell bodies were strongly reduced. In contrast, TN 897, 1059 and 1065 did not change outgrowth or migration patterns (Figs 4C, D and 5A, B, C, D).

Since, of all glycolipids tested, only TN 901 or the L2 glycolipids bind to laminin, one possible mechanism for the inhibition of cell migration and outgrowth of processes was that the binding of L2 positive cells to laminin was competitively inhibited by the added glycolipids, thus

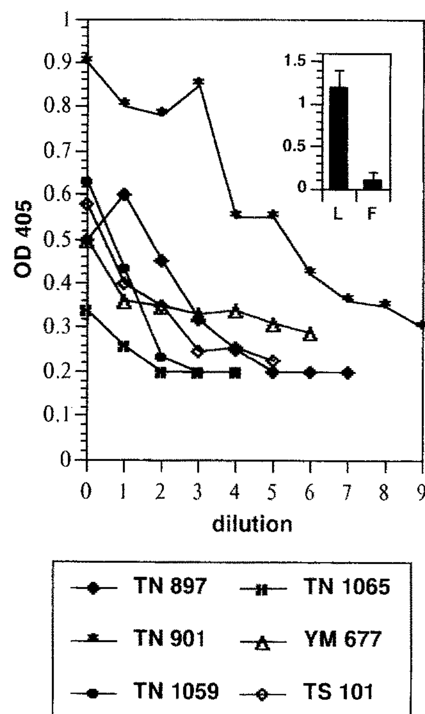

Figure 3. Determination of the binding of laminin and fibronectin to glycolipids. TN 897, TN 901, TN 1059, TN 1065 and neoglycolipids of TS 101 and YM 677 were coated at a concentration of 1 μg ml^{-1} in wells of microtitre plates and incubated with laminin by serial doubling dilution starting with 0.6 μM. The insert shows binding of fibronectin (F, 10 μg ml^{-1}) to L2 glycolipids from sciatic nerve in comparison to laminin (L, 10 μg ml^{-1}). Bound laminin and fibronectin were detected with their respective polyclonal antibodies. The optical density at 405 nm (OD 405) of reaction products with HRP-conjugated secondary antibodies is shown. One representative out of five experiments for all glycolipids except for TS 101 (three experiments) is shown. Mean values ±SD of one representative out of three experiments for laminin binding in comparison to fibronectin binding (insert) carried out in triplicate are shown.

affecting cellular functions. The inability of TN 897, 1059 and 1065 to bind to laminin could, then, be the reason for the lack of any effect of these glycolipids on migration and outgrowth. To investigate whether soluble or substrate bound L2 glycolipids interfere with outgrowth and cell migration, microexplants were plated on fibronectin as substrate, which does not bind the L2 glycolipids (insert of Fig. 3).

On fibronectin, microexplants showed a morphology which was different from that observed on laminin. Outgrowth of processes was much slower and neurites were more strongly fasciculated than on laminin (Fig. 6). Also, many less cells migrated out of the microexplants than on laminin. When microexplants were maintained on fibronectin in the presence of L2 glycolipids, similar inhibitory effects were observed as on laminin: virtually no cells

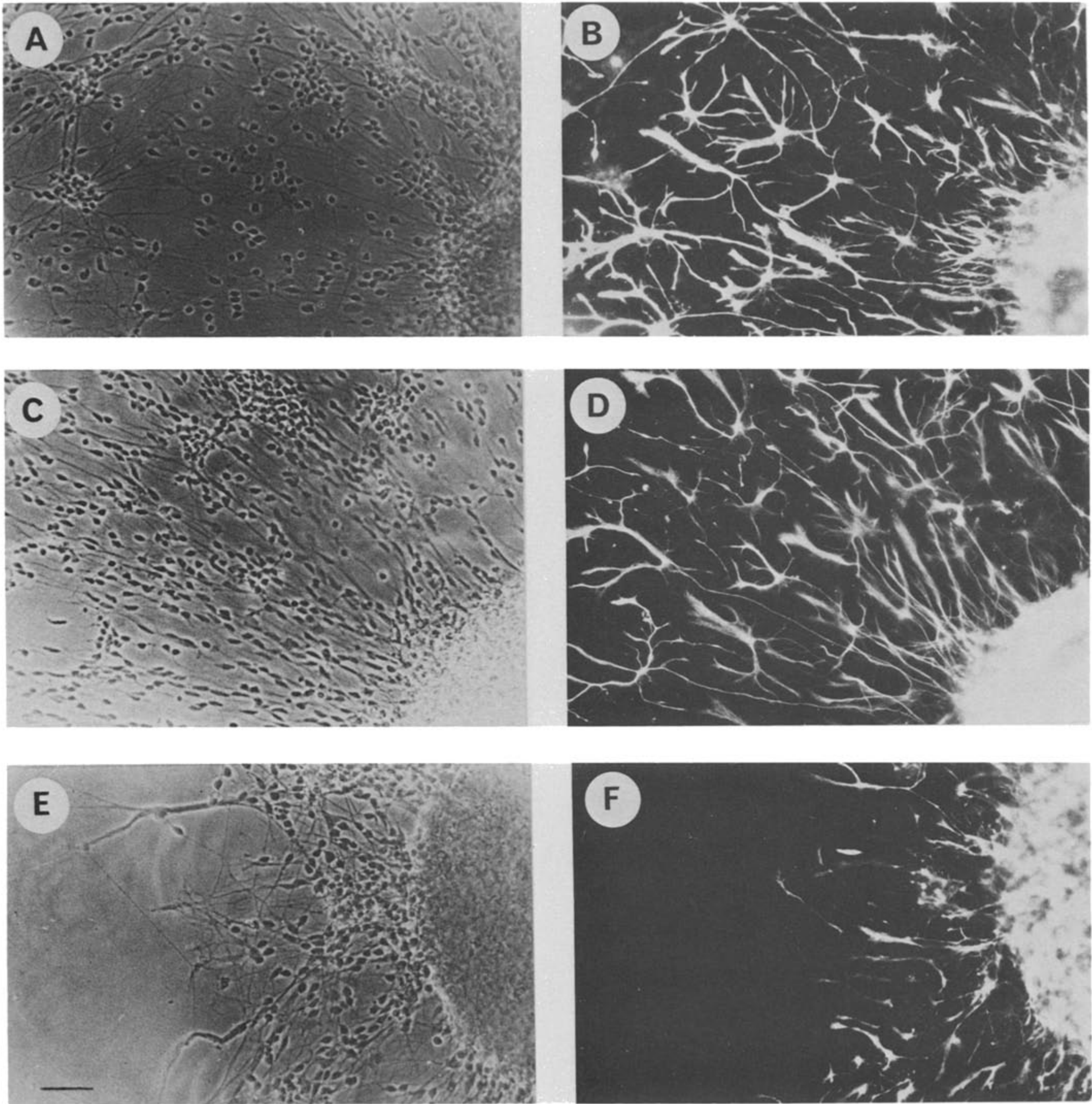

Figure 4. Influence of TN 901 and TN 897 on outgrowth patterns in microexplant cultures on laminin. Cerebellar microexplants were maintained for 2 days in culture. TN 897 (C, D) and TN 901 (E, F) were added to the culture medium 4 h after plating. (A, B) shows the control experiment in the absence of additives. The phase contrast micrographs in A, C, and E correspond to the indirect immunofluorescence staining images for glial fibrillary acidic protein in B, D, and F. The bar in E represents 50 μm for A–F. Note the reduced cell migration and outgrowth of neuritic and astrocytic processes in the presence of TN 901 (E, F).

migrated out of the microexplants and neurites were only very short and often extremely fasciculated (Fig. 6). Maintenance of microexplants in the presence of the ganglioside GD1b did not alter outgrowth and migration patterns on laminin or on fibronectin (not shown). These observations suggest that L2 glycolipids may exert their effects by binding to a cell surface receptor and thus trigger a process resulting in inhibition of neurite outgrowth.

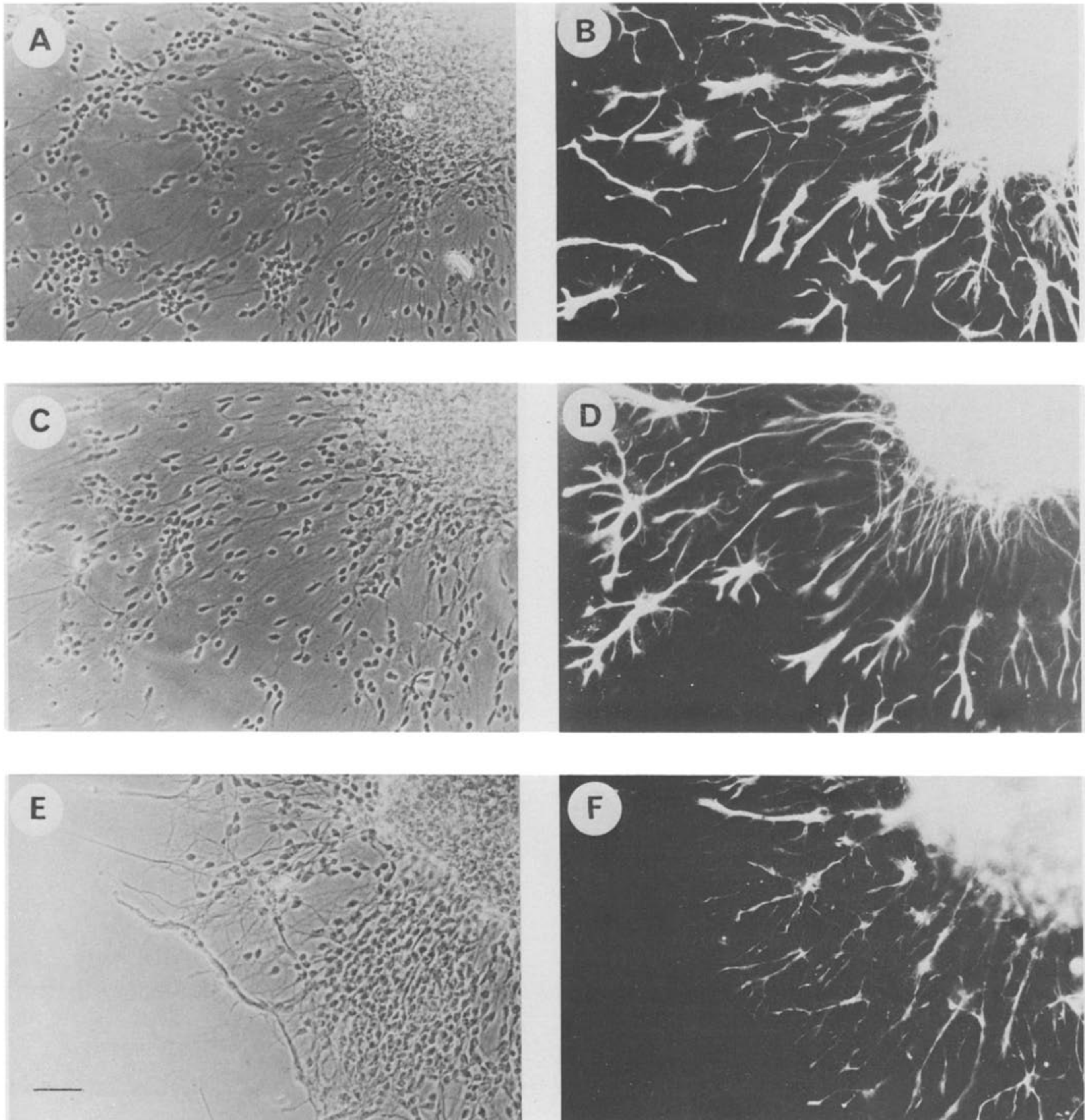

Figure 5. Influence of L2 glycolipids, TN 1059 and TN 1065 on outgrowth patterns of microexplant cultures on laminin. Cerebellar microexplants were maintained for 2 days in culture. TN 1059 (A, B), TN 1065 (C, D) and L2 glycolipids (E, F) were added to the medium 4 h after plating. The phase contrast micrographs in A, C, and E correspond to the indirect immunofluorescence staining images for glial fibrillary acidic protein in B, D, and F. The bar in E represents 50 μm for A–F. Note the reduced cell migration and outgrowth of neuritic and astrocytic processes in the presence of L2 glycolipids.

Discussion

In this study we used synthetic glycolipids containing sulfated or non-sulfated glycans of different length to investigate the structural features of the 3′-sulfoglucuronyl-neolactotetraose as part of the L2 glycolipid that are required for recognition by mab 412 and binding to laminin, as well as its involvement in cell migration and the

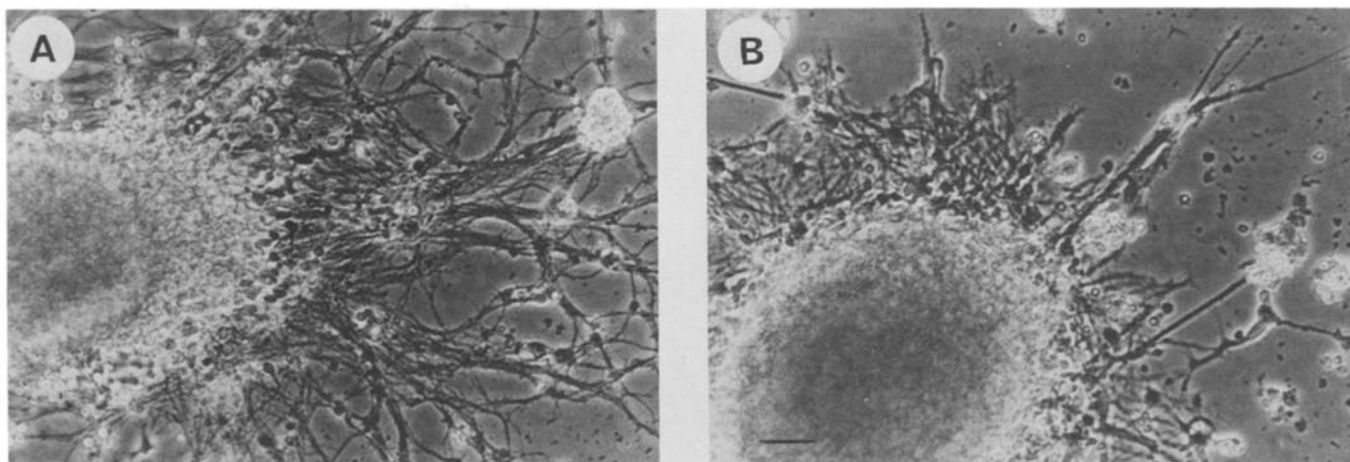

Figure 6. Influence of L2 glycolipids on neurite outgrowth in microexplant cultures on fibronectin. Cerebellar microexplants were maintained for 3 d in culture. (A) shows microexplants in the absence and (B) in the presence of L2 glycolipids added to the medium 16 h after plating. The bar in B represents 25 μm for A and B. Note the strong fasciculation and inhibition of neurite outgrowth in (B).

outgrowth of processes from cerebellar explant cultures.

Binding studies with mab 412 reveal that in addition to the sulfated or non-sulfated glucuronic acid at the non-reducing end of the glycolipid, the tetrasaccharide to which the sulfated glucuronic acid is attached is also required for antibody binding. This is demonstrated by the observation that mab 412 showed reduced binding to the glycolipid YM 677 containing a trisaccharide and no binding to the glycolipid that only contained sulfated glucuronic acid. The non-sulfated glycolipid TN 897 was bound only slightly less than the sulfated glycolipid TN 901 by mab 412; in contrast, mab HNK-1 and mab 336, a monoclonal antibody raised against membrane glycoproteins from mouse brain [17], bind only weakly to non-sulfated L2 glycolipids ([5]; Schmitz, unpublished observations).

We also investigated the structural elements that are important for the binding of the L2 carbohydrate to laminin, implicated in the adhesion of neural cells to laminin [7], as well as its function for the migration of cells and the outgrowth of processes from neurons and astrocytes [8]. Binding studies of glycolipids to laminin indicate that both sulfation and the neolactosyl-type structure are important, because none of the glycolipids other than the L2 glycolipid TN 901 showed a significant binding to laminin. In agreement with observations by Mohan *et al.* [5] the non-sulfated glycolipid TN 897 did not bind to laminin, showing that the sulfate is required. On the other hand, the glycolipids containing sulfated glucuronic acid linked either through a trisaccharide (YM 677-neoglycolipid) or directly to ceramide also did not bind to laminin, showing that the neolacto-tetraose structure is equally necessary. It is unlikely that the direct linkage of the sulfated monosaccharide of TN 1065 to the ceramide sterically hinders binding to laminin, since sulfatide, which contains a sulfated galactose linked to ceramide, binds well to laminin [5, 34]. When microexplants from the cerebellum of early postnatal mice were maintained in the presence of TN 901, migration of cells and outgrowth of processes from neurons and astrocytes were strongly reduced as has been described previously for the L2 glycolipids and the L2 tetrasaccharide [8]. Again, no inhibition was observed in the presence of the other compounds tested, supporting the notion that the sulfation of glucuronic acid as well as the tetrasaccharide to which it is linked, are required for function.

Taken together, our results show that the functionally active epitope designated as the L2/HNK-1 carbohydrate, does not solely comprise the sulfated glucuronic acid but also the neolactosyl-type structure to which it is attached. It remains to be seen whether the activity of the L2 carbohydrate depends, for example, on the monosaccharide composition or sequence or the type of anomeric linkages, or whether the neolactosyl-type structure merely serves as a spacer necessary for the efficient binding to the antibodies, to laminin or to a cell surface receptor, which has to be postulated to be involved in the inhibitory effects on migration and outgrowth patterns from microexplants (see below for discussion). Also we do not know whether the pentasaccharide of TN 901 and of the major compound of the naturally occurring L2 glycolipids, represents the optimal length for its interaction with antibodies and receptors, or whether the heptasaccharide structure of the minor component of L2 glycolipids detected in sciatic nerve [11, 12] is even more active. Sulfatide which binds to a different binding site on laminin than the L2 epitope [7], does not inhibit cell migration and neurite outgrowth (unpublished observations).

Since TN 897, 1059 and 1065 do not bind to laminin it could be argued that these glycolipids were ineffective as inhibitors in the cell culture experiments because binding

to the substrate-coated laminin would be a prerequisite for their influence on cellular functions. To investigate this possibility, we plated microexplants on fibronectin, which in contrast to laminin, does not bind L2 glycolipids. An inhibition of neurite outgrowth and cell migration from microexplants of similar potency was observed in the presence of L2 glycolipids on fibronectin and laminin, indicating that binding of L2 glycolipids to laminin does not play a role in altering neurite outgrowth. This observation is of interest in the context of previous results which showed that only the L2 glycolipids or the free L2 tetrasaccharide, but not the Fab fragments of mab 412, inhibited neurite outgrowth from microexplant cultures [8]. In contrast, outgrowth of astrocytic processes was reduced when microexplants were maintained in the presence of both types of inhibitors, i.e. either the L2 carbohydrate or the L2 antibody. One possible explanation for these differing results could be that different ligand-receptor interactions are involved. For the outgrowth of astrocytic processes the interaction between the cell surface-expressed L2 and laminin may be essential, which can be inhibited either by the binding of the antibody to the epitope at the cell surface, or by competition of the added L2 glycolipids with the cell surface expressed L2 for binding to the binding site of laminin [5, 7]. In contrast, binding of the cell surface expressed L2 to laminin seems not to play a role in neurite outgrowth and neuronal cell migration because otherwise these cellular events should also be inhibited by the antibody. One possible explanation is that the binding of L2 glycolipids to a putative cell surface receptor triggers a signal transduction mechanism resulting in the inhibitory effects observed. It remains to be seen which molecular mechanisms underly the L2-mediated effects leading ultimately to the inhibition of neurite outgrowth and cell migration.

Acknowledgements

The authors thank A. Marxer for preparing antibodies, A. Schwarze for her expert technical assistance, Dr P. Pesheva for antibodies to laminin and fibronectin, Dr R. K. Yu for L2 glycolipids and Dr R. A. Klein for critical reading of the manuscript.

References

1. Schachner M (1989) In *Carbohydrate Recognition in Cellular Function, Ciba Foundation, Symposium 145* (Bock G, Harnett S eds) pp. 156–72 Chichester: John Wiley and Sons.
2. Abo T, Balch CM (1981) *J Immunol* **127**:1024–9.
3. Asa D, Gant T, Oda Y, Brandley BK (1992) *Glycobiol* **2**:395–400.
4. Green PJ, Tamatani T, Watanabe T, Miyasaka M, Hasegawa A, Kiso M, Yuen CT, Stoll MS, Feizi T (1992) *Biochem Biophys Res Comm* **188**:244–51.
5. Mohan PS, Chou DKH, Jungalwala FB (1990) *J Neurochem* **54**:2024–31.
6. Bronner-Fraser M (1987) *Dev Biol* **123**:321–31.
7. Hall L, Liu L, Schachner M, Schmitz B (1993) *Eur J Neurosci* **5**:34–42.
8. Künemund V, Jungalwala FB, Fischer G, Chou DKH, Keilhauer G, Schachner M (1988) *J Cell Biol* **106**:213–23.
9. Martini R, Xin Y, Schmitz B, Schachner M (1991) *Eur J Neurosci* **4**:628–39.
10. Griffith SL, Schmitz B, Schachner M (1992) *J Neurosci Res* **33**:639–48.
11. Chou DKH, Ilyas AA, Evans JE, Costello C, Quarles RH, Jungalwala FB (1986) *J Biol Chem* **261**:11717–25.
12. Ariga T, Kohriyama T, Freddo L, Latov N, Saito M, Kon K, Ando S, Suzuki M (1987) *J Biol Chem* **262**:848–53.
13. Jessell TM, Hynes MA, Dodd J (1990) *Annu Rev Neurosci* **13**:227–55.
14. Field MC, Wing DR, Dwek RA, Rademacher TW, Schmitz B, Bollensen E, Schachner M (1992) *J Neurochem* **58**:993–1000.
15. Uyemura K, Kitamura K (1991) *Comp Biochem Physiol* **93C**:63–72.
16. Oka S, Terayama K, Kawashima C, Kawasaki T (1992) *J Biol Chem* **267**:22711–14.
17. Kruse J, Mailhammer R, Werneke H, Faissner A, Sommer I, Goridis C, Schachner M (1984) *Nature* **311**:153–5.
18. Noronha AB, Ilyas A, Antonicek H, Schachner M, Quarles RH (1986) *Brain Res* **385**:237–44.
19. Ilyas AA, Dobersen MJ, Willson HJ, Quarles RH (1986) *J Neuroimmunol* **12**:99–106.
20. Kohriyama T, Ariga T, Yu RK (1988) *J Neurochem* **51**:869–77.
21. O'Shannessy DJ, Ilyas AA, Dalakas MC, Mendell JR, Quarles RH (1986) *J Neuroimmunol* **11**:131–6.
22. Ilyas AA (1990) *J Neurochem* **55**:594–601.
23. Martini R, Schachner M (1986) *J Cell Biol* **103**:2439–48.
24. Pesheva P, Horwitz AF, Schachner M (1987) *Neurosci Lett* **83**:303–6.
25. Arnarp J, Lönngren J (1981) *J Chem Soc* Perkin Trans. **1**:2070–4.
26. Nakano T, Yukishige I, Ogawa T (1990) *Tetrahedron Lett* **31**:1597–600.
27. Nakano T, Yukishige I, Ogawa T (1991) *Tetrahedron Lett* **32**:1569–72.
28. Nakano T (1992) Dissertation (University Tokyo).
29. Pohlentz G, Schlemm S, Egge H (1992) *Eur J Biochem* **203**:387–92.
30. Stoll MS, Mizuochi T, Childs RA, Feizi T (1988) *Biochem J* **256**:661–4.
31. Schmitz B, Peter-Katalinic J, Egge H, Schachner M (1993) *Glycobiology* **3**:609–17.
32. Fischer G (1982) *Neurosci Lett* **28**:325–9.
33. Fischer G, Keilhauer G, Schachner M (1986) *Dev Brain Res* **28**:247–57.
34. Roberts DD, Weber UM, Liotta LA, Ginsburg V (1988) *Cancer Res* **48**:3367–73.