Cell Assembly Patterns of Embryonic Mouse Cerebellar Cells on Carbohydrate-derivatized Polylysine Culture Substrata

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ABSTRACT

Four carbohydrate derivatives of poly-D-lysine have been synthesized and assayed as substrates for the tissue culture of embryonic mouse cerebellar cells. On poly-β-(D-glucopyranosyl)-poly-D-lysine and on poly-β-(N-acetyl-D-glucosaminyl)-poly-D-lysine, dissociated cerebellar cells formed a monolayer. On poly-β-(D-galactopyranosyl)-poly-D-lysine, cellular aggregates were formed and cables of processes were extended between the aggregates. On poly-β-(L-fucosyl)-poly-D-lysine, cerebellar cells failed to attach and died within 24 h. Onpoly-[(N-acetyl)-poly-D-lysine, cell attachment was identical to that on poly-D-lysine. At low concentrations of underivatized poly-D-lysine (0.5–2.0 μg/ml) dissociated embryonic cerebellar cells formed cellular aggregates, whereas at higher concentrations of poly-D-lysine monolayering was extensive.

The presence of particular complex carbohydrates at the cell surface may provide a mechanism for cell positioning in the developing mammalian brain (1, 2, 20). As specific probes of carbohydrate sequences, lectins have been used to catalogue changes in carbohydrate-containing moieties that are developmentally regulated (13, 16, 21). In the embryonic mouse cerebellum, studies with lectins (11, 12) have revealed binding sites for concanavalin A and Lens culinaris, wheat-germ, and Ricinus communis agglutinins, but not for peanut, Dolichos florisbundis, wysteria, soybean, or Ulex europaeus agglutinins.

Changes in the agglutination with and binding of the former lectins occur in the mouse cerebellum between embryonic days 13 and 17 (9–12).—a period when specific cell contacts are shaping the basic form of the organ (13–17)—which raises the question of the importance of these changes to the complex cell behavior required for histogenesis. The present study seeks to determinewhetherthe carbohydrates recognized by concanavalin A (D-glucose, methyl-α-D-mannose), wheat germ agglutinin (N-acetyl-D-glucosamine), Ricinus communis agglutinin (D-galactose), and Ulex europaeus agglutinin (L-fucose), presented as insoluble analogues attached to tissue culture dishes, influence cell attachment and cellular assembly in vitro.

Carbohydrates have been immobilized on Sephadex beads and nylon fibers to study adhesive properties of fibroblasts (5) and to fractionate some cell types (6, 19). The present study extends the use of insoluble carbohydrate analogues to tissue culture surfaces in order to study cell behavior important to histogenesis: cell assembly, fiber outgrowth, and cell migration. We report the derivatization of polylysine, a promoter of cell attachment in vitro (14), with D-glucose, N-acetyl-D-glucosamine, D-galactose, and L-fucose. These insoluble synthetic "glycoproteins" have been used in three microculture systems to directly evaluate the efficacy of specific carbohydrates as substrates for in vitro cellular assembly and fiber outgrowth.

MATERIALS AND METHODS

Synthesis of Carbohydrate-derivatized Poly-D-lysine

Poly-D-lysine was used to minimize metabolic conversion by the cells (11). Synthesis of poly-β-(D-glucopyranosyl)-poly-D-lysine (Glc-Plys), poly-β-(D-galactopyranosyl)-poly-D-lysine (Gal-Plys), poly-β-(N-acetyl-D-glucosaminyl)-poly-D-lysine (GlcNAc-Plys), and poly-β-(L-fucosyl)-poly-D-lysine (Fuc-Plys) were carried out as follows: poly-D-lysine (Plys) was dissolved in CHCl:pyridine (3:1), reacted with aceiochloropyranose (Koch-Light; Sigma Chemical Co., St. Louis, Mo.) and the reaction products were separated by silica gel chromatography. The carbohydrate-derivatized Plys product was deacetylated in LiOH and purified by Ambulite CG 50 ion-exchange chromatography, and identified by infrared spectroscopy. The amount of neutral or aminosugar coupled to polylysine was measured by automated ion-exchange chromatography and was found to be 22% of the total molecular weight for Glc-Plys, 54% for Gal-Plys, 26% for GlcNAc-Plys, and 18% for Fuc-Plys (expressed as molar ratio of carbohydrate to lysine, these values were 0.23 for Glc-Plys, 0.94 for Gal-Plys, 0.28 for GlcNAc-Plys, and 0.20 for Fuc-Plys). As a control for destruction of polylysine, the described procedure was carried out in the absence of acetolchloropyranose. The described procedure was carried out in the absence of acetolchloropyranose. A more detailed report of the synthesis and characterization of carbohydrate derivatives of polylysine will follow elsewhere.1

Poly-[(N-acetyl)-poly-D-lysine (NAc-Plys) was prepared as follows: 200 mg of polylysine was dissolved in 10 ml H2O. The pH was raised to 10 with NaOH (2 M), acetic anhydride (1.5 M, 0.153 ml; Aldrich Chemical Co., Inc., Milwaukee, Wisc.) was added and the mixture was stirred vigorously for 1 h. Unreacted acetic

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Density was either 1-5 x 10^5 cells/ml (sparse cell cultures) or 1-4 x 10^6 cells/ml to correlate cellular assembly patterns with the presence of covalently bound CHO-tensions necessary for the hydrolysis of bound sugar destroyed the plastic dishes. To treat substrata, first cells were plated in microwells (10 µl/well; Falcon Labware, catalogue no. 1006). The cover slip was secured with a mixture of petroleum jelly and paraffin (Vaseline/Tissue Tek [Fisher Scientific Co., Springfield, N. J., 1:3] applied at 60°C and hardened at 20°C. The plates were sterilized by exposure to ultraviolet irradiation (short wave) for 1 h at 20°C, rinsed once with distilled water, and air-dried, and the cover slip was added to the well (30 µl). This method avoided technical difficulties encountered in removing the 5-mm cover slips from the microtest plate II wells and facilitated light microscopy at high magnification. The method also reduced the amount of culture fluid required per well, thereby increasing the number of replicates possible per experiment. In all, 62 cover-slip cultures were carried out with Glc-Plys, Glc-NAc-Plys, and Gal-Plys, and 33 cover-slip cultures were carried out with Fuc-Plys. In all cases, cover slips were treated with 5 mg/ml of CHO-Plys (8). The amount of CHO-Plys per unit area of the treated cover slips used in microcultures was assumed to approximate that of the analyzed 22 x 22 mm cover slips.

For each experiment, five controls (12 microwell or three cover-slip cultures each) were carried out. Cells were plated on: untreated control surfaces, surfaces treated with Plys (10 µg/ml), surfaces treated with the coupling agent (carbodiimide for microwells and γ-aminopropyl-trietoxysilane for glass cover slips) and washed three times with H2O, surfaces treated with the coupling agents and washed three times with glycine (50 mM) and three times with H2O, and finally surfaces treated with the coupling agent and underivatized Plys (10 µg/ml).

Three parameters were measured in each experiment. First, plating efficiency was assayed 24 h after plating by counting the total number of cells per microculture, either by phase-contrast microscopy or by removing the cells and counting with a hemocytometer, and expressing that as a percentage of the total number of cells plated. Second, cell counts were repeated 72 h after plating and, in some cases, 2-3 wk after plating. Third, the pattern of cell assembly was assayed by phase-contrast microscopy.

Specific inhibition of cell attachment to derivatized substrata was measured by two methods. First, cells were plated in microtest plate I microwells as described but with constant gentle agitation in the presence of free monosaccharide (0.01-0.10 M). After 4 h (35.5°C), the number of cells remaining in the supernate was expressed as a percentage of the total number of cells plated. Second, cells were plated on Glc-NAc-Plys as described in the presence of free monosaccharide (5-100 mM). The number of cells per square millimeter of surface area was counted by phase-contrast microscopy.

RESULTS

Embryonic cerebellar cells formed dramatically different patterns on different carbohydrate-derivatized polylysyl substrates. On Glc-NAc-Plys, extensive monolayering of the cells was observed at both low and high cell densities (Figs. 1a and 2e). Numerous microcolonies were projected by the cells and plating efficiency was >95%. On Glc-Plys, the patterning of embryonic cerebellar cells was similar to that on Glc-NAc-Plys. Extensive monolayering and some aggregation were observed over the range of cell densities tested (Figs. 1b and 2f). On Gal-Plys, the cells reaggregated into aggregates of 2,000-10,000 cells each (Fig. 1c) when plated at high cell density and 10-500 cells each when plated at low cell density (Fig. 2c). Little monolayering was observed on Gal-Plys, even at low cell density. On Fuc-Plys, no cell attachment or survival was observed (Figs. 1d and 2d).

The different responses of embryonic cerebellar cells to four carbohydrate derivatives of polysylne depended on the method by which the derivatives were applied to the substratum. In contrast to underivatized Plys, which could be used to coat tissue culture plastic or glass by simple application of aqueous solution, best results with derivatized Plys were obtained with a cross-linking agent. For glass, γ-aminopropyl-trietoxysilane treatment followed by glutaraldehyde-mediated linkage of the polysylne derivative was effective. For tissue culture plastic, carbodiimide improved the treatment of the substratum. A higher concentration of carbohydrate-derivatized Plys (0.5-10 µg/ml) was effective.

Embryonic Cerebellar Cell Cultures

All studies were performed with cerebellar tissue harvested from C57Bl/6J mouse embryos at the 13th d of gestation, the day of impregnation being designated embryonic day zero. Single cell suspensions of embryonic cerebellum were prepared as described (11). Washed cells were resuspended in Eagle’s basal medium (Earle’s Salts; Grand Island Biological Co., Grand Island, N. Y.), supplemented with glucose (5 mM), glutamine (4 mM), penicillin-streptomycin (100 µl/ml), and horse serum (10%; Microbiological Associates, Walkersville, Md.), heat-treated horse serum (50°C, 1 h; 10%), or fetal calf serum (10%; Microbiological Associates). Cells were introduced into microwell cultures with a 0.250-ml syringe fitted with a repeatering dispenser (Hamilton Co., Reno, Nev.) and a sterile tip. The final plating density was either 1-5 x 10^3 cells/ml (sparse cell cultures) or 1-4 x 10^6 cells/ml (high-density cell cultures). For all experiments, the viability of the plating cell suspensions, as measured by exclusion of the dye trypan blue, was >85%. Cultures were maintained at 35.5°C with 5% CO2 and 100% humidity.

Poly-D-lysine (150,000 mol wt, hydrobromide), α-glucose, α-galactose, N-acetyl-D-glucosamine and chitobiose were the generous gifts of Dr. Roger W. Jeanloz, Boston, Mass.

Carbohydrate Derivatization of Polylysine (CHO-Plys, NAc-Plys, or Plys) was used to coat tissue culture dishes by three methods. First, an aqueous solution of CHO-Plys (1-10 mg/ml), NAc-Plys (1-50 µg/ml), or Plys (1-50 µg/ml) was added to the microwell (10 µl/well) or culture dish (0.5 ml/well) for 2 h (35.5°C). The microwells or cover slips were individually rinsed with distilled water three times, air-dried, and used immediately. Second, CHO-Plys, NAc-Plys, or Plys was coupled to tissue culture plastic with carbodiimide as a linking agent (1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide-metho-p-toluenesulfonate [6]; Aldrich Chemical Co.). The polylysine compound was mixed with carbodiimide (5 µg/ml), and the tissue culture dish was treated as described above. Treated culture dishes were washed three times with distilled water and incubated at 35.5°C to remove unreacted carbodiimide. Third, the covers were covalently coupled to glass cover slips by the method of Gottlieb and Glaser (8). Treated culture dishes were washed three times with glycine (50 mM) to remove unreacted glutaraldehyde and three times with distilled water, and then were air-dried. Derivatized culture substrata were sterilized by exposure to ultraviolet irradiation (short wave) for 1 h at 20°C.

The amount of CHO-Plys bound to glass cover slip culture surfaces was determined enzymatically. Although it was not possible to assay individual cover slips, the amount of CHO-Plys bound to 10 x 22 mm glass cover slips. The cover slips were covalently derivatized with Glc-Plys, Gal-Plys, or Fuc-Plys (5 mg/ml) (6), hydrolyzed with HCl (3 N, 6 h, 100°C) and evaporated to dryness, and the amount of bound sugar released was measured enzymatically (7). Expressed as micrograms of carbohydrate per cover slip, the amounts bound were 1.1 for Glc-Plys, 5.4 for Gal-Plys, and 0.9 for Fuc-Plys. The amount of carbohydrate bound per cover slip was not changed significantly after incubation of the treated cover slips in supplemented Eagle’s basal medium for periods of 24 or 72 h (35.5°C, 5% CO2, 100% humidity).

Poly-D-lysine (150,000 mol wt, hydrobromide), α-glucose, α-galactose, N-acetyl-D-glucosamine and chitobiose were the generous gifts of Dr. Roger W. Jeanloz, Boston, Mass.

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mg/ml) than underivatized Plys (5–50 μg/ml) was required for cell attachment. Presumably carbohydrate derivatives of Plys, being less charged than the parent compound, did not readily adhere to the highly charged tissue culture plastic or glass surfaces.

The development of the cellular pattern on GlcNAc-Plys is shown for sparse cultures in Fig. 3. At plating, the cells were “rounded up” and monodisperse (Fig. 3 a). After 4 h in culture, most of the cells had attached to the culture substratum and had started to flatten out (Fig. 3 c). 12 h after plating, the cells had elaborated processes and distributed as a monolayer (Fig. 3 e). On Glc-Plys, the cell population appeared to be quite homogenous. An identical time-course of cellular pattern development was observed for high cell density cultures.

The cellular pattern depended on the method used to treat the culture substratum with GlcNAc-Plys. Equivalent plating efficiencies were obtained when the compound was linked to the substratum with carbodiimide in the case of tissue culture plastic or with γ-amino-propyl-triethoxysilane in the case of glass coverslips. When the substratum was simply coated with an aqueous solution, cell attachment was markedly less. When the growth medium was changed every 3 d, cell survival was 2–6 wk without any change in cell pattern or cell number. After that time, in some cultures, the cells retracted from the culture dish as a sheet and formed one or two large aggregates. This was followed by detachment from the culture substratum and a rapid decline in cell viability. The release of the cells was presumably caused by the leakage of the GlcNAc-Plys from the culture dish.

Identical results were obtained when the cells were grown in the presence of heat-treated horse serum (10%) or fetal calf serum (10%). Because the latter treatment inactivated serum glycosidases (4), it is unlikely that the binding of cells to GlcNAc-Plys was affected by the action of serum glycosidases. This result was not surprising, as the GlcNAc was coupled to polylysine via an N-glycosidic and not an O-glycosidic linkage.

The time-course of the development of the cellular pattern on Glc-Plys was identical to that for GlcNAc-Plys. Equivalent plating efficiencies were observed when the polylysine derivative was attached via carbodiimide (tissue culture plastic) or γ-amino-propyl-triethoxysilane (glass coverslips). Growth on Glc-Plys was similar for cells grown in the presence of horse serum, heat-treated horse serum, or fetal calf serum. When the medium was changed at 3-d intervals, cultures were viable for 1–3 wk without any change in cellular pattern or cell number. In general, cells grown in Glc-Plys extended fewer processes and subjectively appeared to be slightly less healthy than those grown on GlcNAc-Plys.

The development of the cellular pattern for cells plated at sparse density on Gal-Plys is shown in Fig. 3. At plating, the cells were rounded up and monodisperse (Fig. 3 b). During the first 4 h in vitro, small aggregates of several dozen cells each formed (Fig. 3 d). By 12 h in vitro, almost all of the cells were located in aggregates (Fig. 3 f). The details of the formation of
the cellular pattern were identical for cells plated at high cell density (1–2 × 10⁶ cells/ml).

On Gal-Plys the cultures survived for long periods, up to 6 wk, without any change in cellular pattern or cell number when the medium was changed at 3-d intervals. When the cells were removed from the microwell by gentle treatment with trypsin (0.1%, 5 min, 20°C) and replated in a microwell treated with Plys (10 μg/ml), the cells formed a monolayer with 70% plating efficiency. No difference was observed when the cells were grown in medium supplemented with heat-treated horse serum (10%) or fetal bovine serum (10%).

No significant cell division was observed on either CHO-Plys or Plys-treated culture substrata. Cell counts at 24-h intervals for 0–120 h in vitro did not reveal any increase in cell number with time in culture. Very few mitotic figures were present in the cultures, <1% of the total cell population, and these were present only during the first 1 or 2 d in vitro. Presumably, mitotic cells were already in mitosis at the time of cell dissociation, or they represented nonneuronal populations present in the cultures.

A number of controls were used to evaluate the carbohydrate specificity of cell patterning on carbohydrate-derivatized Plys. First, cell attachment to CHO-Plys was partially blocked by the addition of excess amounts of free specific carbohydrates.
but not by other carbohydrates. When cells were plated in the presence of soluble sugar, cell attachment to GlcNAc-Plys, measured after 24 h in vitro, was inhibited 40–50% by 0.1 M N-acetyl-D-glucosamine (D-GlcNAc), 0.1 M sucrose, or 0.01 M chitobiose. The addition of 0.1 M D-galactose (D-Gal) or 0.1 M L-fucose (L-Fuc) had no effect. For Glc-Plys, attachment was inhibited 30–50% by 0.1 M D-glucose (D-Glc) or 0.1 M sucrose. No effect was observed after the addition of 0.1 M D-Gal or 0.1 M L-Fuc. For Gal-Plys, attachment of cellular aggregates was inhibited 30–50% by 0.1 M D-Gal or 0.1 M lactose, but not by 0.1 M D-Glc, 0.1 M sucrose, 0.1 M D-GlcNAc, 0.1 M L-Fuc, or 0.01 M chitobiose.

Second, soluble carbohydrate was added to the growth medium and cell density was assayed over a period of 84 h in vitro (Fig. 4). No effect on cell attachment or survival on Plys was observed when free D-Glc, D-GlcNAc, D-Gal, or L-Fuc was added (1.0–60 mM) to the growth medium (Fig. 4). Similarly no effect resulted from the addition of methyl-α-D-glucose to the growth medium (1.0–60 mM). However, when the cells were plated on GlcNAc-Plys in the presence of free D-GlcNAc (5–100 mM) cell density was markedly reduced as the concentration of free sugar was increased (Fig. 4).

Third, the masking of charged groups on Plys by acetylation rather than coupling of carbohydrate had no effect on cell patterning. Cell attachment to and survival on substrata treated with NAc-Plys or with Plys subjected to the conditions of
carbohydrate derivatization, but not to carbohydrate derivatization were identical in cell patterning and concentration dependence to Plys (Fig. 5). These observations suggest that the results did not relate exclusively to alterations in the charge distribution or structure of polylysine.

Fourth, it is possible that the distinct cellular responses on the four carbohydrate analogues pertained to the degree of derivatization of Plys rather than to the particular carbohydrate derivative or to the cell density used in the experiment. To evaluate this, a wide range of concentrations of Plys was tested as a culture substratum, and a graded cellular response was observed. In the absence of Plys, embryonic cellular cells aggregated, failed to attach to the substratum, and died within 24 h. At low concentrations of Plys (0.5–2.0 μg/ml), cellular aggregation occurred; at intermediate concentrations (3–5 μg/ml), cellular aggregation and limited monolayering occurred; at higher concentrations (10–50 μg/ml), monolayering occurred. In contrast, cell attachment to and patterning on carbohydrate-derivatized Plys was not strikingly substrate concentration dependent. Below a critical concentration of CHO-Plys (500 μg/ml), no cellular attachment to the substratum was observed. Above that concentration, the cells formed the described assembly patterns.

Above the critical concentration of Glc-Plys and GlcNAc-Plys, it can be argued that cell attachment was caused by the presence of excess amounts of free amino groups on Glc-Plys or GlcNAc-Plys. However, below the critical concentration of Glc-Plys and GlcNAc-Plys, in spite of the presence of free polylysyl amino groups in excess of the concentration of Plys required for cell attachment, monolayering was not observed. On Fuc-Plys, approximately the same concentration of free amino groups should have been available as was the case for GlcNAc-Plys or Glc-Plys, but the cells failed to attach or survive even at a very high concentrations of Fuc-Plys (10 mg/ml). On Gal-Plys, derivatization of free amino groups was relatively complete and the cells aggregated. These results suggest that cell assembly on CHO-Plys was more dependent on the particular carbohydrate residue present than on the degree to which the lysyl amino groups were derivatized with any carbohydrate.

No significant cell survival or process extension was observed when cells were grown on untreated surfaces or on surfaces treated with the coupling agent but not with Plys or CHO-Plys.

DISCUSSION

The specific attachment of embryonic cerebellar cells to GlcNAc-Plys and Glc-Plys suggests the presence of cell surface proteins or glycoproteins that bind D-Glc- and D-GlcNAc-containing residues. The failure of the cells to attach to or survive on Fuc-Plys suggests the absence of a fucose-binding protein or glycoprotein. The response of the cells to Gal-Plys is more difficult to interpret. It is possible that galactose-binding moieties are present in lower total numbers or in altered conformations than is the case for D-Glc and D-GlcNAc binding sites. Alternatively the binding of cells to Gal-Plys may trigger cell assembly conducive to aggregation.

These studies do not address the many complex features of the cell surface that might control the exposure, conformation, or arrangement of specific binding sites. Factors such as the heterogeneity of binding sites for carbohydrate ligands and the possible control of the density, mobility, and interactions between such sites by the cytoskeleton could generate a very complex set of specific cell-carbohydrate interactions. What is clear from these studies is that the cells easily distinguished among four different carbohydrate residues, suggesting that glucose-, N-acetylglucosamine-, and galactose-binding moieties, but not fucose-binding moieties are relevant to cell-cell interactions in the developing cerebellum.

The results reported here are in agreement with previous lectin agglutination and binding studies for embryonic mouse cerebellum (10–12, 21). Whereas lectins revealed exposed carbohydrate sequences, the present studies suggest the availability of carbohydrate binding sites on the cell surface. In spite of the fact that cells are normally exposed to glycoproteins with carbohydrate sequences that are larger than the monosaccharide studies here, the cells bound to monosaccharides linked to a polylysyl backbone via a carbohydrate-specific mechanism. It is of special interest that the carbohydrate specificity of lectins demonstrated to agglutinate or bind to embryonic cerebellar cells in our previous work (10–12) correlated with the carbohydrates that were suitable substrates for in vitro cell attachment and fiber outgrowth in the present study. This may reflect the presence of complementary sets of carbohydrates and carbohydrate-binding proteins on cerebellar cell surfaces.

Biochemical studies of glycoprotein binding sites for lectins have revealed the presence of a heterogeneous group of glycoproteins that bind concanavalin A and wheat-germ, Lens culinaris, and Ricinus communis agglutinins in the embryonic cerebellum (my unpublished observation). It is likely that carbohydrate ligands also bind to a heterogeneous group of sites. The particular sets of binding sites for carbohydrates and carbohydrate-binding proteins and their arrangement on the cell surface may provide one mechanism for specific cell contacts in the developing brain (19).

Cell adhesion to the culture substratum was a critical requirement for cerebellar cell attachment and subsequent survival and process extension. Cerebellar cells failed to attach and died within a short period on untreated tissue culture...
Monolayering of embryonic mouse cerebellar cells plated at $2 \times 10^6$ cells/ml on microwells treated with (A) Plys (10 µg/ml) and (B) NAc-Plys (10 µg/ml). After 4 d in culture; phase-contrast microscopy. × 280.

plastic or on untreated glass coverslips. Cerebellar cells also did not attach to or survive on collagen-treated plastic or glass surfaces (my unpublished observation). On adhesive substrata such as Plys, GlcNAc-Plys, Glc-Plys, or NAc-Plys, the behavior of the cells was qualitatively similar. At the resolution of the light microscope, there were no major differences in cell morphology, extension of processes, or viability of the cells. The distinction between Plys and CHO-Plys as culture substrata was that appropriate hapten sugars inhibited cell attachment and patterning on CHO-Plys, but did not affect these parameters on Plys surfaces.

It is possible that different cell populations survived on GlcNAc-Plys, Glc-Plys, Gal-Plys, and Plys. On GlcNAc-Plys, the cell population appeared to be more homogeneous than was the case for the other substrates studied. A rigorous analysis of the distribution of specific cell types could not be made with light microscopy. We are currently addressing this issue with electron microscopy and specific cell markers.

These studies suggest the relevance of cell-carbohydrate interactions to cell behavior required for embryonic cerebellar cell assembly in vitro. CHO-Plys substrata offer the possibility of mimicking some carbohydrate properties of the cell surface and evaluating their impact on cell behavior important to cerebellar histogenesis. These surfaces should provide novel substrates for studies of in vitro cell migration.

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REFERENCES

1. Aminoff, D., W. F. V. Bruegge, W. C. Bell, K. Sarpolis, and R. Williams. 1977. Role of sialic acid in survival of erythrocytes in the circulation: interaction of neuraminidase-treated and untreated erythrocytes with spleen and liver at the cellular level. Proc. Natl. Acad. Sci. U. S. A. 74:1521-1525.

2. Ashwell, G., and A. G. Morell. 1974. The role of surface carbohydrates in the hepatic recognition and transport of circulating glycoproteins. Adv. Enzymol. 41:99-128.

3. Banker, G., and W. M. Cowan. 1977. Rat hippocampal neurons in dispersed cell culture. Brain Res. 126:397-425.

4. Bischof, R., B. Benesch, and M. M. Burger. 1977. Cell culture in serum depleted of glycosidases by heating. Exp. Cell Res. 104:143-152.

5. Chipowsky, S., Y. C. Lee, and S. Roseman. 1973. Adhesion of cultured fibroblasts to insoluble analogues of cell-surface carbohydrates. Proc. Natl. Acad. Sci. U. S. A. 70:2309-2312.

6. Edelman, G. M., and V. Rutishauser. 1974. Specific fractionation and manipulation of cells with chemically derived fibrous and surfaces. Methods Enzymol. 34:195-225.

7. Finch, P. R., R. Yuen, H. Schachter, and M. A. Moscarello. 1969. Enzyme methods for the microassay of D-mannose, D-glucose, D-galactose and L-fucose from acid hydrolyzates of glycoproteins. Anal. Biochem. 31:296-305.

8. Gottlieb, D. I., and L. Glaser. 1975. A novel assay of neuronal cell adhesion. Biochem. Biophys. Res. Commun. 63:815-831.

9. Hatten, M. E., and A. Messer. 1978. Postnatal cerebellar cells from staggerer mutant mice express embryonic cell surface characteristic. Nature ( Lond.). 276:504-506.

10. Hatten, M. E., M. Schachner, and R. L. Sidman. 1979. Histochemical characterization of lectin binding in mouse cerebellum. Neuroscience 4:921-935.

11. Hatten, M. E., and R. L. Sidman. 1978. Cell reassociation behavior and lectin-induced agglutination of embryonic mouse cells from different brain regions. Exp. Cell Res. 113:111-125.

12. Hutt, S., and M. E. Hatten. 1980. Histochemical characterization of lectin binding to embryonic and early postnatal mouse cerebellum in microcultures. Eur. J. Cell Biol. 22:260 (Abstr.)

13. Kelly, P., C. W. Cotman, C. Gesier, and G. L. Nicolson. 1976. Distribution and mobility of lectin receptors in synaptic membranes of purified neurons in the CNS. J. Cell Biol. 63:541-549.

14. Letiembre, P. C. 1975. Possible roles for cell-to-substratum adhesion in neuronal morphogenesis. Dev. Biol. 48:77-91.

15. Palay, S., and V. Chan-Palay. 1974. Cerebellar Cortex: Cytology and Organization Springer-Verlag, Berlin. 348 pp.

16. Pfennienger, K. H., and M. F. Mayle-Pfennienger. 1975. Distribution of and fate of lectin-binding sites on the surface of growing neuronal processes. J. Cell Biol. 67:332 a (Abstr.)

17. Sidman, R. L. 1974. Cell interactions in the developing mammalian central nervous system. In Cell Interactions: Proceedings of the Third Lepetit Colloquium L.G. Silvestri, editor. North Holland Biomedical Press, Amsterdam. 1-13.

18. Trenthner, E., and R. L. Sidman. 1975. Histogenesis of mouse cerebellum in microwell cultures. J. Cell Biol. 75:915-940.

19. Weigel, P. H., E. Schnett, Y. C. Lee, and S. Roseman. 1973. Specific adhesion of rat hepatocytes to D-galactosides linked to polyacrylamide gels. J. Biol. Chem. 253:330-333.

20. Yen, P. H., and C. E. Ballou. 1974. Partial characterization of the sexual agglutination factor from Hansenula wingei Y-2404 type 5 cells. Biochemistry. 13:2428-2437.

21. Zanetta, J. P., G. Roussel, M. S. Ghouldeur, G. Vincendon, and G. Gombos. 1978. Postnatal development of rat cerebellum: massive and transient accumulation of concanavalin A binding glycoproteins in parallel fiber axolemma. Brain Res. 142:301-319.

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