Cytotactin, an extracellular matrix protein of neural and non-neural tissues that mediates glia–neuron interaction

(cell–cell adhesion/cell–substrate-adhesion molecule/embryogenesis)

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ABSTRACT An extracellular matrix protein, cytotactin, with widespread tissue distribution has been identified, isolated, and partially characterized. Cytotactin mediates glia–neuron adhesion *in vitro*, but unlike Ng-CAM, the neuron–glia cell-adhesion molecule, it is absent from neurons. Cytotactin was isolated from 14-day embryonic chicken brains as structurally related polypeptides of M_r 220,000, 200,000, and 190,000. These polypeptides were efficiently extracted in the absence of detergent and appeared to be disulfide-linked into higher polymers. Immunofluorescence staining with specific antibodies indicated that cytotactin is found in extracellular spaces and in basement membranes of a variety of non-neural tissues including smooth muscle, lung, and kidney. In the cerebellum, it appears on glial end-feet, on Bergmann glial fibers, and in extracellular spaces. The molecule is synthesized by glia and cells from smooth muscle, lung, and kidney. It is found at the surface of glia in culture in a cell-associated fibrillar pattern. A survey of the times and sites of its appearance during embryogenesis is consistent with the hypothesis that cytotactin is a cell–substrate adhesion molecule that may mediate cell migration in a site-restricted fashion.

Evidence is accumulating that the modulation of expression of cell–cell adhesion molecules (CAMs) and cell–substrate adhesion molecules (SAMs) plays a key role in pattern formation during embryogenesis and histogenesis (1–3). A number of CAMs of different specificity have been isolated and each has been shown to undergo characteristic modes of developmental expression. For example, the coordinated modulation of expression of neural CAM (N-CAM) and liver CAM (L-CAM) is observed at several sites of embryonic induction (4, 5). In a particularly striking case, the feather (6, 7), three successive rounds of induction that occur are each presaged by the apposition of collectives of cells expressing N-CAM with other collectives expressing L-CAM. Perturbation of the binding functions of CAMs can lead to alterations in morphogenesis: for example, anti-N-CAM antibodies distort the pattern of the retinotectal projections (8). Moreover, the interruption of tissue structure can alter CAM expression, as shown by the observation that denervation leads to an increase in muscle N-CAM concentration (9). This is consonant with the idea that modulation is under local control of interacting tissues.

Although extracellular matrix proteins have not so far been found to have expression sequences that are as locally restricted as those of CAMs, these SAMs play key roles in developmental primary processes such as morphogenetic motion (10). The fact that the migration and subsequent interactions of neural crest cells appear to be controlled by the coordinated expression of a CAM (N-CAM) and a SAM (fibronectin) (11, 12) prompted us to search for SAMs with

restricted patterns of expression in morphogenesis. The strategy used was based on the observations that a monoclonal antibody made against ciliary ganglia that also recognizes migrating neural crest cells, NC-1 (13), recognizes a carbohydrate antigenic determinant carried by a group of proteins (14, 15) present at widespread locations, including basement membranes (11, 16). Further, an antiserum prepared against glial cells blocked glia–neuron adhesion and recognized certain proteins recognized also by NC-1. We therefore used NC-1 and HNK-1 (a monoclonal antibody of similar but not identical specificity) to search for molecules involved in glia–neuron adhesion and then scanned for these proteins at other embryonic sites.

The result of this search was the identification and isolation of a protein, cytotactin, that (i) is found in a wide but nonetheless specialized distribution in both neural and non-neural tissues; (ii) is synthesized and secreted by glial cells and by cells isolated from smooth muscle, kidney, and lung; and (iii) is involved in glia–neuron adhesion in an *in vitro* assay. The results raise the possibility that some extracellular matrix proteins contribute to pattern formation in embryogenesis as a result of their restricted expression at some loci but not at others.

MATERIALS AND METHODS

Preparation of Cytotactin. Cytotactin was purified from $95,000 \times g$ supernatants of homogenates of 14-day embryonic chicken brains extracted in P_i /NaCl (10 mM phosphate, pH 7.4/0.15 M NaCl)/1 mM EDTA/Trasyol (200 units/ml) in the presence or absence of 0.5% Nonidet P-40. The extract from 1000 brains was incubated 1.5 hr at 4°C with 150 mg of monoclonal antibody HNK-1 immobilized on 50 ml of Sepharose CL-2B; the beads then were washed with extraction buffer, and absorbed material was eluted with 100 ml of 50 mM diethylamine, pH 11.5/1 mM EDTA. The eluate was neutralized to pH 8.0 with 10 ml of 1 M potassium phosphate (pH 7.0) and loaded onto a column containing 4 ml of DE-52 (Whatman) in P_i /NaCl. The DE-52 was washed with P_i /NaCl and bound material was eluted with a $1 \times$ to $4 \times$ P_i /NaCl gradient. Highly purified cytotactin was eluted between $1.4 \times$ and $2 \times$ P_i /NaCl (pool 1) and was used in all experiments except where indicated. A fraction (pool 2) that contained both cytotactin and material that did not enter a NaDod-SO₄/6% polyacrylamide gel and stained blue with StainsAll (Eastman) (see *Results*) was eluted between $2 \times$ and $3 \times$ P_i /NaCl. In a typical preparation, ≈ 1 mg of cytotactin was obtained.

Preparation of Antibodies. Rabbits were immunized with proteins bearing the NC-1 antigenic determinant (NC-1 Ag) that were purified by immunoaffinity absorption of Nonidet P-40 extracts of 14-day chicken embryo brain membranes. To

remove any antibodies crossreactive with N-CAM and neuron-glia-CAM (Ng-CAM), we incubated antiserum to NC-1 Ag with N-CAM or Ng-CAM immobilized on Sepharose CL-2B (17); antibodies remaining in the supernatant are referred to hereafter as absorbed anti-NC-1 Ag. Anti-cytotactin antibodies were immunoaffinity-purified from absorbed anti-NC-1 Ag by use of cytotactin immobilized on Sepharose CL-2B. IgG fractions of rabbit antibodies to N-CAM (18) and Ng-CAM (15) and monovalent Fab' fragments (19) were prepared as described. Affinity-purified anti-laminin antibodies were purchased from E-Y Laboratories (San Mateo, CA), and anti-fibronectin antibodies were from Collaborative Research (Waltham, MA).

Adhesion Assays. Heterotypic binding of suspensions of fluorescein-labeled neurons to monolayers of glial cells (20) was measured using cells obtained from 9-day chicken embryo brains (7.5×10^6 neurons per 35-mm dish of confluent glia).

Immunofluorescence Studies. Frozen sections were prepared and stained (5, 17) using anti-cytotactin Ig at 1 μ g/ml. Cultures containing both neurons and glial cells were prepared (15) and extracted at -20°C with 5% acetic acid in ethanol to allow cytoskeletal staining.

Analytical Procedures. NaDodSO₄/polyacrylamide gels were immunoblotted (21) or stained with Coomassie blue (22), StainsAll (23), or silver (24). Peptide mapping of isolated gel bands was performed by the method of Cleveland *et al.* (25).

Biosynthesis of Cytotactin. Cell monolayers were prepared from 13-day chicken embryo organs, after treatment with 0.25% trypsin (GIBCO)/1 mM EDTA/0.5% collagenase (Worthington) for 30 min at 37°C , and were incubated with 0.5 mCi (1 Ci = 37 GBq) of [³H]leucine per 100-mm dish for 12 hr in leucine-free Dulbecco's modified Eagle's medium containing 5% fetal bovine serum. The cells were washed and extracted on ice in P_i/NaCl/0.5% Nonidet P-40/1 mM EDTA/Trasyolol (200 units/ml). Culture supernatants and cell extracts were clarified by centrifugation and labeled cytotactin was isolated by affinity adsorption with 4 μ g of anti-cytotactin Ig bound to protein A-Sepharose.

RESULTS

Identification and Characterization of Cytotactin. As a first step in identifying a molecule recognized by anti-glial antibodies and NC-1, a rabbit antiserum was prepared against a mixture of molecules extracted from embryonic chicken brains and bearing the NC-1 antigenic determinant (anti-NC-1 Ag). Anti-NC-1 Ag antiserum recognized several proteins, including N-CAM and Ng-CAM; antibodies to N-CAM and Ng-CAM, however, were quantitatively removed by absorption with the immobilized CAMs. Fab' fragments prepared from this absorbed antiserum (absorbed anti-NC-1 Ag) inhibited glia-neuron adhesion in an *in vitro* assay (Table 1). Antibodies to Ng-CAM also inhibited adhesion, whereas anti-N-CAM had no effect (20).

To identify the specific molecule(s) mediating the glia-neuron adhesion, we tested purified fractions from Nonidet P-40 extracts of whole embryonic chicken brains for their ability to neutralize the inhibitory effect of the antibodies on the adhesion. Sequential immunoaffinity chromatography, using monoclonal antibody HNK-1 coupled to Sepharose CL-2B, and ion-exchange chromatography (see *Materials and Methods*) yielded a fraction (Fig. 1a, lane 1) containing a polypeptide of M_r 220,000, as well as polypeptides of M_r 200,000 and 190,000 in varying amounts. In the absence of 2-mercaptoethanol, no protein from this fraction entered a 6% gel (Fig. 1a, lane 2), suggesting that the native form of the molecule may be disulfide-linked. This material completely neutralized the inhibition of adhesion by the absorbed anti-

Table 1. Inhibition of glia-neuron adhesion

Fab' fragment added	Bound neurons*	% inhibition
Preimmune (1 mg)	314 $\pm$ 18	0
Anti-N-CAM (1 mg)	321 $\pm$ 20	0
Anti-Ng-CAM (1 mg)	185 $\pm$ 9	41
Absorbed anti-NC-1 Ag [†] (1 mg)	188 $\pm$ 14	40
Absorbed anti-NC-1 Ag [†] (1 mg)	319 $\pm$ 16	0
[+ cytotactin (2 μ g)]		
Anti-cytotactin (2 μ g)	191 $\pm$ 11	39
Anti-cytotactin (6 μ g)	187 $\pm$ 13	40
Anti-cytotactin (2 μ g)	116 $\pm$ 17	63
+ anti-Ng-CAM (1 mg)		

Adhesion was assayed as described in *Materials and Methods* and ref. 20. To reduce aggregation of neurons in suspension during the assay, 0.5 mg of anti-N-CAM Fab' was included except when preimmune and anti-Ng-CAM antisera were tested individually.

*No. of neurons bound to 1 mm² of the monolayer (mean of duplicate samples $\pm$ SEM).

[†]Antibodies to N-CAM and Ng-CAM were removed from anti-NC-1 Ag Fab' by absorption.

NC-1 Ag Fab' at amounts as low as 2 μ g/mg of antibody (Table 1).

As shown below, these proteins are related and are secreted into the extracellular matrix by a variety of neural and non-neural cells. We have therefore named this material cytotactin, in view of its apparent function in cell-cell and cell-substrate adhesion.

Cytotactin coupled to Sepharose CL-2B was used to affinity-purify Ig and Fab' (anti-cytotactin Ig or Fab'). The anti-cytotactin Fab' inhibited glia-neuron adhesion at a protein concentration 1/500th that required for inhibition by the parent antibody; additional antibody gave no further inhibition (Table 1), indicating that this dose is functionally saturating. In contrast, a mixture of the saturating dose of

FIG. 1. Structural characteristics of cytotactin polypeptides. (a) Cytotactin (10 μ g of pool 1) isolated from 14-day chicken embryo brains, was resolved by NaDodSO₄/6% PAGE in the presence (lane 1) or absence (lane 2) of 2-mercaptoethanol and detected by Coomassie blue stain. Adult chicken brain Nonidet P-40 extract (100 μ g; lane 3) was analyzed by electrophoresis followed by immunoblotting with anti-cytotactin Ig. Cytotactin (10 μ g of pool 2) was electrophoresed and stained with Coomassie blue (lane 4) or StainsAll (lane 5). (b) Peptide maps of cytotactin polypeptides and the M_r 180,000 and 160,000 polypeptides isolated from adult brains by HNK-1 immunoaffinity chromatography. Polypeptides (10 μ g) were isolated by NaDodSO₄/6% PAGE, digested with *Staphylococcus aureus* V8 protease (0.5 μ g), resolved in NaDodSO₄/15% polyacrylamide gels, and detected by silver staining: M_r 220,000 cytotactin polypeptide (lane 1), M_r 200,000 plus M_r 190,000 cytotactin polypeptides (lanes 2), adult M_r 180,000 polypeptide (lane 3), and adult M_r 160,000 polypeptide (lane 4). Lane 5: V8 protease alone. Migration and $M_r \times 10^{-3}$ of standards are indicated at left in a and in b.

anti-cytotactin antibodies together with anti-Ng-CAM antibodies inhibited adhesion to a greater extent than either antibody alone (Table 1), suggesting that Ng-CAM and cytotactin are parts of two separate molecular mechanisms involved in glia–neuron interaction.

To determine the structural relationship among the cytotactin polypeptides, peptide maps (25) of the M_r 220,000 polypeptide and a mixture of the M_r 200,000 and 190,000 polypeptides were compared. The two maps were almost identical (Fig. 1*b*, lanes 1 and 2), indicating that the components of cytotactin comprise very similar polypeptide chains. When extracts of adult chicken brains were immunoblotted with anti-cytotactin Ig, polypeptides of M_r 180,000 and 160,000 and a minor component of $M_r \approx 300,000$ were found to crossreact (Fig. 1*a*, lane 3). Peptide-mapping experiments indicated that the M_r 180,000 and 160,000 polypeptides are structurally related to each other but may not be related to the components of authentic cytotactin isolated from embryonic chicken brains (Fig. 1*b*, compare lanes 3 and 4 with lanes 1 and 2). An explanation for the apparent immunological crossreactivity of these molecules will depend on further structural characterization and the preparation of more specific antibodies.

Localization and Cellular Synthesis of Cytotactin. Although an *in vitro* glia–neuron adhesion assay was used to identify cytotactin, the widespread distribution of proteins bearing the NC-1 antigenic determinant suggested that cytotactin might also be found outside the nervous system. To determine the localization of cytotactin, frozen sections from a variety of regions of different stage embryos were stained with anti-cytotactin Ig. Cytotactin was detected in the basement membrane and mesenchyme of the gastrointestinal tract as early as 3 days of embryonic development (Fig. 2*a*); staining was also observed in the basement membranes of the notochord and aorta. By 7 days, strong staining was observed in the lung (Fig. 2*b*) and kidney basement membranes and in extracellular spaces between smooth muscle cells [for example, gizzard (Fig. 2*c*)]; no staining of striated muscle was detected.

In the central nervous system, in contrast to N-CAM and Ng-CAM, low levels of cytotactin staining were not observed

until 7 days of development; significantly higher levels were achieved by 9 days. In the 17-day cerebellum, staining was observed on glial end-feet and along Bergmann glia (Fig. 2*d*); in addition, extensive extracellular staining was observed throughout the cerebellum. Observation at higher magnification (Fig. 2*f*) revealed that the staining pattern was extracellular and fibrillar. All staining was blocked by prior incubation of the antibody with soluble cytotactin (Fig. 2*e*).

Immunofluorescence localization experiments indicated that cytotactin is associated with glia but not neurons. Cultures containing both neurons and glia were double-stained with a monoclonal antibody (R5) specific for cytoskeletal elements in glial cells in the brain (26) and a rabbit antibody (anti-N-CAM, anti-Ng-CAM, or anti-cytotactin Ig). Anti-N-CAM antibodies brightly stained cells with typical neuronal morphology but only weakly stained glial cells (Fig. 3, compare *a* and *b*); in contrast, R5 stained only glial cells (Fig. 3*c*). As shown previously (15), anti-Ng-CAM stained only neurons (Fig. 3, compare *d*, *e*, and *f*). In contrast, anti-cytotactin Ig only stained cells that were R5-positive (Fig. 3, compare *g*, *h*, and *i*) indicating that glia but not neurons are recognized by this antibody. Higher magnification photographs (Fig. 3*j*) indicated that staining occurred both in an extracellular fibrillar pattern and in a punctate, perinuclear pattern typical of proteins awaiting secretion from the Golgi apparatus (10). All staining was blocked when the antibody was preincubated with purified soluble cytotactin.

To confirm that the widespread distribution of anti-cytotactin staining reflected synthesis of the protein, cultured cells were intrinsically labeled with [3 H]leucine. Cytotactin polypeptides of characteristic molecular weight were synthesized at high levels by glial cells and cells from the gizzard, intestine, lung, and kidney (Fig. 4). More than 90% of the radioactive cytotactin was found in the culture supernatants. Neurons and cells from the meninges expressed little cytotactin, consistent with the possibility that the observed synthesis in these primary cultures was due to the presence of small numbers of glial cells. When cells were surface-labeled by lactoperoxidase-catalyzed radioiodination, glia contained the characteristic cytotactin polypeptides, whereas neurons did not (Fig. 4*c*, lanes 1 and 2). These findings are

FIG. 2. Distribution of cytotactin in the developing embryo, as detected by immunofluorescence. (a) Transverse section of a 3-day embryo through the level of the lung buds shows extensive cytotactin staining in the basement membranes surrounding the neural tube (NT), notochord, aorta (A), gastrointestinal/respiratory tract (G), and dermamyotome as well as in cells of the sclerotome, gut mesenchyme, and aortic smooth muscle. Staining is not detected at this stage in the neural tube. ($\times 45$.) (b) Transverse section through a 9-day embryonic lung shows strong basement membrane staining in extending bronchi (B) and some cell-associated mesodermal staining. ($\times 110$.) (c) Transverse section of a 7-day embryonic gizzard shows strong cell-associated staining in the developing smooth muscle layer and low-level basement membrane staining under the epithelium (E). ($\times 110$.) (d and e) Intense cytotactin staining is found in extracellular spaces throughout the 17-day embryonic cerebellum (d) which is completely neutralized (e) by purified soluble cytotactin ($1 \mu\text{g}/\mu\text{g}$ anti-cytotactin). ($\times 45$.) (f) Higher magnification shows staining in the outer layers along radial Bergmann glial fibers (arrow) and on glial end-feet (arrowhead). ($\times 290$.)

FIG. 3. Localization of cytotoxin in cultured brain cells. Cultures containing both neurons and glia (shown in phase-contrast in *a*, *d*, and *g*) were double-stained with R5 (50 μ g/ml), a monoclonal antibody specific for cytoskeletal proteins in glial cells (*c*, *f*, and *i*), and rabbit antibodies to N-CAM (100 μ g/ml; *b*), Ng-CAM (100 μ g/ml; *e*) or cytotoxin (1 μ g/ml; *h*). Antibodies to N-CAM (*b*) brightly stain neurons and faintly stain some non-neuronal cells; antibodies to Ng-CAM (*e*) specifically label neuronal cell surfaces most intensely on neurites. Anti-cytotoxin stains only the cells (*h* and *j*) that are also stained with the glial marker R5 (*i* and *k*), indicating that cytotoxin is associated with glia but not neurons. High-magnification micrograph of a sparse glial culture (*j*, *k*) shows intense cytotoxin staining in a punctate pattern around the nucleus corresponding to the Golgi apparatus (arrowheads) and extracellularly in cell-associated fibrillar material (arrows). (Magnifications: *a*–*i*, $\times 200$; *j* and *k*, $\times 280$.)

consistent with the positive staining of tissue sections by anti-cytotoxin Ig, which appears to be due mainly to the presence of cytotoxin and not to the presence of other proteins that might be recognized by the same antibodies.

Extrinsic Nature of Cytotoxin. The cytotoxin polypeptides do not appear to be intrinsic membrane proteins. More than 50% of these molecules in whole brains can be extracted in

FIG. 4. Cytotoxin synthesis and secretion in primary cultures of various tissues. [3 H]Leucine-labeled cytotoxin from culture supernatants (*a*) and from detergent extracts of cells (*b*) was immunoaffinity-purified using anti-cytotoxin Ig and resolved in NaDodSO₄/6% polyacrylamide gels. Lanes: 1, glia; 2, gizzard; 3, lung; 4, kidney; 5, intestine; 6, meninges. Whereas *b* represents all the cytotoxin purified from the cells, *a* represents only 20% of the cytotoxin from the corresponding culture supernatants. (*c*) Highly enriched cultures (27) of 9-day chicken embryo glia (lane 1) and neurons (lane 2) were labeled by lactoperoxidase-catalyzed radioiodination (18) and cytotoxin was immunoaffinity-purified with anti-cytotoxin Ig. In contrast to glia, little or no cytotoxin could be detected on neurons.

the absence of detergent. Mixing experiments indicate that the molecules purified with or without detergent comigrate precisely on gel electrophoresis, suggesting that the molecules purified without detergent are not cleavage products of the intact molecule. In accord with these results, the localization of cytotoxin in the Golgi apparatus, in extracellular spaces, in basement membranes, and in culture supernatants all suggest that cytotoxin is an extracellular matrix protein secreted by cells. Nevertheless, no antigenic crossreactivity with either fibronectin or laminin was detected by immunoblotting.

A second fraction, which was eluted from DE-52 at higher salt concentrations (Fig. 1*a*, lane 4), contained the cytotoxin polypeptides as well as a component that barely entered a NaDodSO₄/6% polyacrylamide gel and was stained blue with StainsAll [a characteristic of polyanions (23)] (Fig. 1*a*, lane 5). This StainsAll-positive material also was present in the main cytotoxin pool, but at a level only one-tenth as high. The material that was eluted at high salt concentrations contained high levels of sialic acid and uronic acid, consistent with the hypothesis that the StainsAll-positive material is a proteoglycan. Material in this fraction was much less effective (per μ g of protein) than purified cytotoxin at neutralizing the inhibition of adhesion by antibody, indicating that the adhesion-blocking antibodies are directed primarily against the cytotoxin glycopeptides rather than the proteoglycan. Whether the copurification of cytotoxin and the proteoglycan is coincidental or is due to an interaction between the molecules remains to be investigated.

DISCUSSION

We report here the identification, isolation, and partial characterization of a secreted glycoprotein, cytotoxin, that is

widespread in tissue distribution and is involved in glia-neuron interactions. Cytotactin has a number of interesting properties. (i) It appears to be an extracellular matrix component, as judged by its extractability in the absence of detergent, its release into culture media, and its localization *in vitro* and *in vivo*. (ii) Cytotactin is found in both neural and non-neural tissues. In some tissues, it appears in basement membranes; in others (including the brain), it is found between cells. (iii) It is synthesized by a variety of cell types, including glia but not neurons. (iv) The native form of the molecule appears to be disulfide-crosslinked. (v) Although anti-cytotactin antibodies recognize polypeptides found in adult brain tissue, these polypeptides differ in their molecular weights and peptide maps from cytotactin isolated from embryonic tissue.

While this manuscript was in preparation, a murine molecule (J1 glycoprotein) that mediated neuron-glia interactions *in vitro* was reported (30). The molecular weight, antigenic properties, and extrinsic character were similar to those of cytotactin, but J1 was described as a nervous system CAM. Cytotactin is in fact a SAM and is not exclusive to the nervous system. If the two proteins are related evolutionarily, it is likely that J1 will also be found at extraneural sites.

Affinity-purified anti-cytotactin Fab' fragments inhibit the adhesion of neurons to glial monolayers at very low concentrations. In previous studies (15, 17), a molecule specific to neurons, Ng-CAM, was implicated in neuron-glia adhesion. Cytotactin does not appear to be the glial ligand for Ng-CAM, because of the enhanced inhibition of glia-neuron adhesion observed with a mixture of anti-Ng-CAM and anti-cytotactin antibodies at saturating levels, because Ng-CAM is restricted to neuroectodermal derivatives (16), and because Ng-CAM and cytotactin are expressed on different developmental schedules in neural tissue. In any event, the importance of both molecules in neuron-glia interactions *in vivo* and a possible function for cytotactin in supporting cell migration are suggested by the observation that in an *in vitro* assay, antibodies to cytotactin as well as to Ng-CAM (17) can inhibit the migration of external granule cells along Bergmann glial fibers to the internal granular layer (unpublished observations).

A specific receptor for cytotactin on neurons and other cells remains to be identified. One potential candidate is the presumptive proteoglycan is eluted in the high-salt fractions from DE-52. Several other SAMs, including laminin (29), fibronectin (29), and a M_r 170,000 retinal SAM (28), have been reported to bind to proteoglycans. Cytotactin is similar to laminin and fibronectin in that both are disulfide-linked high molecular weight SAMs that are present in both neural and non-neural tissues. Nevertheless, these molecules are distinct in their subunit composition, immunological specificity, and tissue distribution.

Because it is possible that the purified cytotactin contained a small amount of proteoglycan, the anti-cytotactin antibodies may also have contained some anti-proteoglycan specificity, raising the possibility that such antigenic determinants contribute to some extent to the staining patterns observed. This does not seem likely because we have recently isolated monoclonal antibodies that recognize cytotactin polypeptides of M_r 220,000, 200,000, and 190,000 but not the proteoglycan and that show identical staining patterns. Nonetheless, the observations that glia and cells from gizzard, intestine, lung, and kidney all synthesize high levels of cytotactin polypeptides confirm the conclusion that cytotactin is distributed widely in both neural and non-neural tissue.

Cytotactin, although an extracellular matrix protein secreted by glia into extracellular spaces, nonetheless mediates local glia-neuron interactions. In the cerebellum, the secret-

ed cytotactin appears to be closely associated with the Bergmann glial cells and thus this cell-associated SAM may comprise part of the pathway provided by radial glial fibers for migrating neurons. In view of the widespread distribution of cytotactin, this function of supporting cell migration may prove to be general in other tissues. This raises the possibility that cytotactin may be one of a number of extracellular matrix proteins that contribute to morphogenetic patterning by their selectively modulated expression at some tissue sites but not others.

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