

Expression of Cytotactin in the Normal and Regenerating Neuromuscular System

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Abstract. Cytotactin is an extracellular glycoprotein found in a highly specialized distribution during embryonic development. In the brain, it is synthesized by glia, not neurons. It is involved in neuron–glia adhesion *in vitro* and affects neuronal migration in the developing cerebellum. In an attempt to extend these observations to the peripheral nervous system, we have examined the distribution and localization of cytotactin in different parts of the normal and regenerating neuromuscular system. In the normal neuromuscular system, cytotactin accumulated at critical sites of cell–cell interactions, specifically at the neuromuscular junction and the myotendinous junction, as well as at the node of Ranvier (Rieger, F., J. K. Daniloff, M. Pinçon-Raymond, K. L. Crossin, M. Grumet, and G. M. Edelman. 1986. *J. Cell Biol.* 103:379–391). At the neuromuscular junction, cytotactin was located in terminal nonmyelinating Schwann cells. Cytotactin was also detected near the insertion points of the muscle fibers to tendinous structures in both the proximal and distal endomysial regions of the myotendinous junctions. This was in striking contrast to staining for the neural cell adhesion molecule, N-CAM, which was accumu-

lated near the extreme ends of the muscle fiber.

Peripheral nerve damage resulted in modulation of expression of cytotactin in both nerve and muscle, particularly among the interacting tissues during regeneration and reinnervation. In denervated muscle, cytotactin accumulated in interstitial spaces and near the previous synaptic sites. Cytotactin levels were elevated and remained high along the endoneurial tubes and in the perineurium as long as muscle remained denervated. Reinnervation led to a return to normal levels of cytotactin both in inner surfaces of the nerve fascicles and in the perineurium. In dorsal root ganglia, the processes surrounding ganglionic neurons became intensely stained by anticytotactin antibodies after the nerve was cut, and returned to normal by 30 d after injury.

These data suggest that local signals between neurons, glia, and supporting cells may regulate cytotactin expression in the neuromuscular system in a fashion coordinate with other cell adhesion molecules. Moreover, innervation may regulate the relative amount and distribution of cytotactin both in muscle and in Schwann cells.

NEURON–NEURON, neuron–glia, and neuron–muscle cell adhesion play a fundamental role in the formation and maturation of the central and peripheral nervous systems (8, 9, 24, 25). It has been proposed that the coordinate spatiotemporal expression of cell adhesion molecules (CAMs)¹ and substrate adhesion molecules regulates various processes involved in morphogenetic patterning (8–11). The peripheral nervous system provides an excellent

opportunity to examine such cell interactions because of its ready accessibility during development and its capacity for regeneration.

A number of studies have demonstrated extensive modulation in the expression of two neuronal CAMs, the neural cell adhesion molecule (N-CAM), and the neuron–glia cell adhesion molecule (Ng-CAM), during development of the PNS (7, 10, 11, 31), particularly in areas of early cell migration and of tract formation. We previously compared normal expression of N-CAM and Ng-CAM with their expression after peripheral nerve injury (7). Changes in CAM expression were correlated with known events of nerve degeneration and regeneration, suggesting that the neuronal CAMs play various roles in degenerative and regenerative processes (5, 7). The data also support the hypotheses that local signals between neurons and glia regulate CAM expression in the spi-

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¹ Abbreviations used in this paper: AChR, acetylcholine receptor; CAM, cell adhesion molecule; DRG, dorsal root ganglion; N-CAM, neural CAM; Ng-CAM, neuron–glia CAM.

nal cord and nerve during regeneration and that activity regulates N-CAM expression in muscle (7).

We have identified an extracellular molecule, cytotactin, that is involved in neuron–glia adhesion *in vitro* (14). The molecule was isolated from embryonic brain tissue as three closely related polypeptides of M_r 220,000, 200,000, and 190,000 under reducing conditions. Similar molecules have been described as myotendinous antigen (2) or tenascin (3). In the central nervous system, cytotactin was shown to be synthesized by glia and not by neurons (14, 18). Cytotactin has been shown to bind to a chondroitin sulfate proteoglycan of neurons as well as to fibronectin (17). In the peripheral nervous system, cytotactin is found on Schwann cells and is highly concentrated at the node of Ranvier (26). The combined observations from previous work suggest that cytotactin appears in embryonic tissues critically involved in histogenesis, particularly in areas of cell movement in the early embryo (6), in neural crest development (30), and in development of the cerebellum (4).

These observations suggested that, like CAMs (7), cytotactin is important in phenomena involving guidance and movement in development and during regenerative events. This hypothesis prompted the present study of cytotactin in the neuromuscular system. In this report, we describe in detail the cellular distribution of cytotactin in the sciatic nerve and muscle of adult chicken and mouse, and emphasize its localization on neuromuscular and myotendinous junctions. We then describe the changes in cytotactin expression in nerve and muscle brought about by crushing or cutting the adult sciatic nerve. These studies taken together with previous analyses (7) suggest that during regeneration, cellular morphology and differentiation are related to the expression of CAMs by a complex signaling system and that there is an identical or similar system acting coordinately for a substrate adhesion molecule such as cytotactin.

Materials and Methods

Animals and Surgical Procedures

White Leghorn chicken embryos and young adult chickens (4 wk old) and C57Bl/6J (8-wk-old) mice were used. Adult chickens were anesthetized with chloral hydrate (8%; 0.33 ml/100 g body weight) for surgery; for sacrifice, chickens were overdosed with this anesthetic while all mice and embryos were subjected to cervical dislocation. Adult chickens were subjected to a unilateral crush or cut of the sciatic nerve equidistant between the greater trochanter and lateral condyle of the femur. Damage to the nerve at this site has previously been shown to result in efficient regeneration (7). The sciatic nerve was crushed for ~3 s with flat, blunt-tipped forceps (2-mm tip) or nerves were transected by removing a 2-mm segment. The site of the injury was located morphologically and with the help of an overlying suture. Animals were sacrificed after 3, 10, 20, or 60 d, and tissues were removed for anatomical analysis. Spinal cords, dorsal root ganglia (DRGs), and nerves were dissected from chickens that had been transcardially perfused with fixative (see below) for 20 min; DRGs from spinal segments 23–26 were examined. Control tissues were taken from the side contralateral to the injury in experimental animals or from animals that were not injured; staining patterns were comparable in both types of control tissue. Sciatic nerves and gastrocnemius muscles of chickens and mice were dissected and processed immediately for further anatomical analysis.

Cell Culture Techniques

Experiments on muscle cells in culture were performed using the C2 cell line (C2C12), a gift from Dr. A. Minty (INSERM; Hôpital Cochin, Paris). Cells were plated at an initial density of 10^5 cells/100-mm culture dish in

a growth medium (DME supplemented with 20% FCS and 1.25% chick embryo extract). When cells were 50–60% confluent, the medium was changed (DME supplemented with 10% horse serum) to induce myoblast fusion. Fibroblast cultures were prepared from the body walls of embryonic day 10 chicken embryos and maintained in DME supplemented with 2% chicken serum and 2% tryptose phosphate broth (Gibco Laboratories, Grand Island, NY). The culture supernatants were analyzed biochemically on 6 or 7.5% polyacrylamide gels after intrinsic labeling of the cells using 0.1 μ Ci/ml of culture medium of 3 H-Leu, followed by immunoprecipitation and autoradiography (7, 25, 26).

For microscopy, cells were fixed for 10 min in 2.5% paraformaldehyde/0.02% glutaraldehyde/0.1 M phosphate buffer (pH 7.4), washed in PBS, and stained as described below for histological sections.

Immunocytochemical Techniques

Polyclonal and monoclonal antibodies against cytotactin were prepared as previously described (6). Two cell type-specific antibodies were used to identify Schwann cells; polyclonal rabbit IgG fraction of antisera against S100 protein (7) or mouse mAb 224–58 (a generous gift of Dr. B. Zalc and co-workers INSERM/U134, Paris) which was generated after immunization of mice with human central nervous system myelin and which recognizes an epitope characteristic of Schwann cells in the mammalian peripheral nervous system (13, 15). Polyclonal and monoclonal antibodies prepared against chicken N-CAM and polyclonal antibodies prepared against mouse N-CAM were also used (7). To localize the acetylcholine receptor (AChR) in the motor endplate region, we used its specific irreversible ligand α -bungarotoxin labeled with rhodamine (Sigma Chemical Co., St. Louis, MO) on teased muscle preparations as described previously (25). Fresh pieces of sciatic nerves or hatchling anterior latissimus dorsi muscle were placed on filter paper and fixed for 1 h in 2.5% paraformaldehyde/0.02% glutaraldehyde/0.1 M phosphate buffer (pH 7.4). Adult tissues from perfused animals were postfixed for 20 min. After fixation, all tissues were placed in 30% sucrose in PBS overnight. After equilibrating in embedding compound, the tissues were frozen. Sections (10 μ m thick) were cut and dried overnight at room temperature before incubation with primary antibody. Muscle tissue from perfused animals was immersed in embedding compound (Tissue-Tek; Miles Scientific Div., Miles Laboratories Inc., Naperville, IL), frozen in cold isopentane, sectioned on a cryostat (8 μ m), collected on gelatin-coated slides, and dried. After storage overnight at 4°C, slides were postfixed for 15 min in the fixative described above. Primary antibodies were used at 10–40 μ g/ml in PBS with 4% goat serum. Second antibodies were either fluorescein- or rhodamine-conjugated goat anti-rabbit IgGs (Cappel Laboratories, Malvern, PA). All tissues were viewed and photographed in a Zeiss epifluorescence photomicroscope equipped with the appropriate barrier filters to restrict fluorescence to the appropriate wavelengths. Comparable staining was always observed in single-labeled sections and no staining was evident in the absence of primary antibody. In double-labeled sections, primes after the letter indicate the alternate stain and double primes, the corresponding phase micrographs.

Results

Cytotactin in the Terminal Schwann Cell in Adult Nerve

The distribution of cytotactin at the neuromuscular junction was examined in intramuscular sciatic nerve bundles in adult mouse gastrocnemius muscle using teased muscle fiber preparations. In addition to low levels in the endomysium, bright staining for cytotactin was found in muscle regions that were recognized as the motor endplate regions by double-staining with rhodamine-labeled α -bungarotoxin (which specifically reacts with the AChR) (Fig. 1, *a*, *a'*, *b*, and *b'*). The patterns of staining for AChR and cytotactin were not exactly coincident; cytotactin was more extensive and diffuse than the AChR. Terminal axons were not labeled with anticytotactin antibodies, as evidenced by the fact that immunofluorescent staining by antineurofilament protein did not overlap with the cytotactin pattern (not shown) (25). The pattern of cytotactin

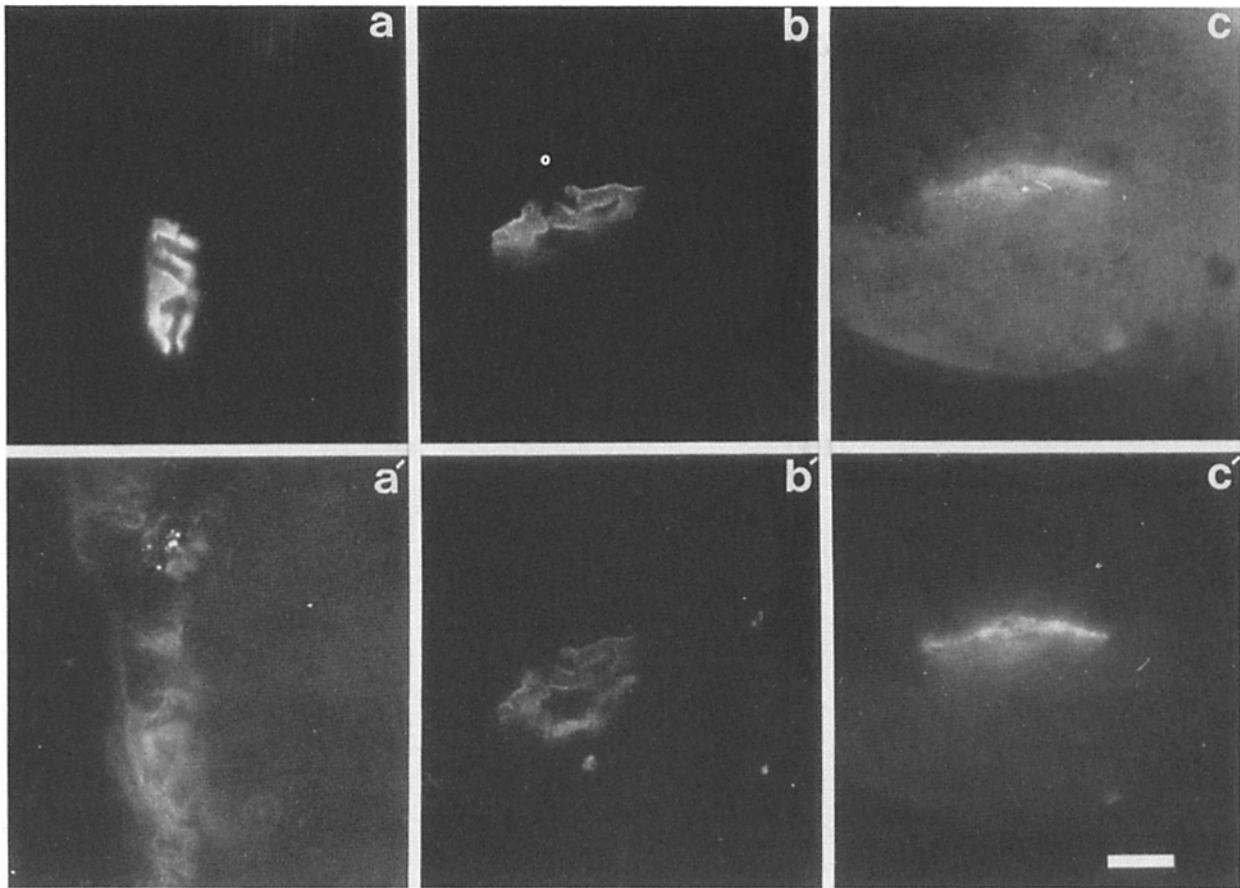


Figure 1. Immunocytochemical staining of terminal Schwann cells in adult mouse skeletal muscle. Two types of double-labeling experiments were performed to determine cytotactin localization in the motor endplate region in teased muscle fiber preparations. First, rhodamine-labeled α -bungarotoxin was used to visualize the AChR in its postsynaptic location (*a* and *b*). Cytotactin staining (*a'* and *b'*) was found in the same region but was more extensive and not exactly coincident with the AChR staining. Second, mAb 224-58 was used to label the Schwann cells (*c*) and was found to stain the same structures as the anticytotactin rabbit polyclonal antibody (*c'*). Bar, 50 μ m.

staining was found to be coincident with that of the specific mouse Schwann cell marker, mAb 224-58 (13, 15) in double-labeled sections (Fig. 1, *c* and *c'*). Thus, it appeared that the cytotactin observed in the motor endplate region was associated with the terminal Schwann cell.

Cytotactin Labeling of the Myotendinous Junction in Striated Muscle

The distribution and biochemical characterization of cytotactin reported elsewhere (6, 14, 18) suggested that it is similar to the myotendinous antigen isolated from fibroblast and

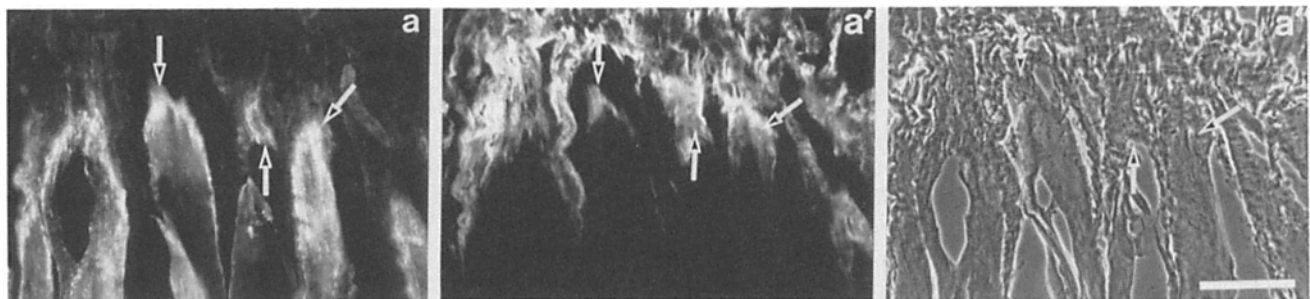


Figure 2. Directional distribution of cytotactin and N-CAM at the myotendinous junction. Double-staining experiments were performed on longitudinal sections of hatchling chicken anterior latissimus dorsi muscle sections using monoclonal anti-chicken N-CAM antibody (*a*) and rabbit polyclonal anticytotactin antibody (*a'*). *a''* shows the corresponding phase-contrast micrograph. N-CAM staining, which is very low or negative on adult muscle fibers except at synaptic sites (5, 25), was restricted to the very extremities of the muscle fibers (*a*) as indicated by phase-contrast observations (*a''*). Cytotactin staining (*a'*) was found extending from the tips of the muscle fibers at the myotendinous junction and in association with tendinous structures that extend away from the muscle fibers into the periosteal region. Arrows point at corresponding points (*a*, *a'*) demonstrating the region of overlap between cytotactin and N-CAM staining. Bar, 50 μ m.

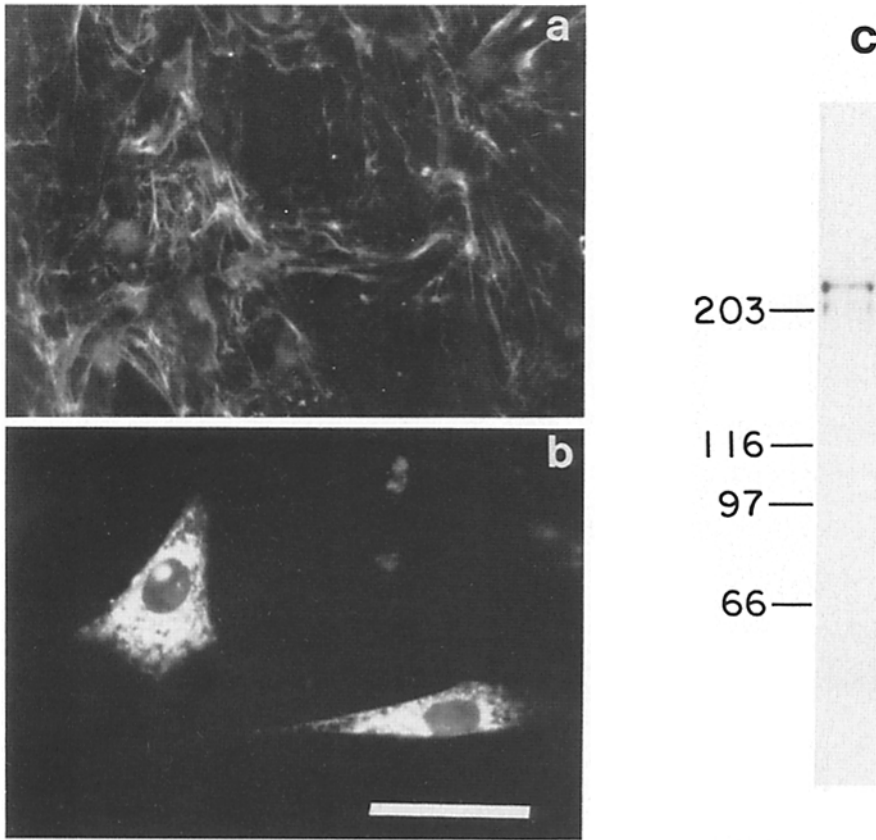


Figure 3. Cytotactin in primary fibroblast cultures. (a) In confluent cultures, cytotactin appeared in an extracellular, fibrillar pattern. (b) Sparse cultures permeabilized with detergent showed intense perinuclear staining, characteristic of secreted proteins. (c) Secretion of cytotactin from confluent fibroblast cultures was confirmed by intrinsic labeling of confluent cells. Immunoprecipitates were resolved on a 6% polyacrylamide gel. Characteristic cytotactin polypeptides of M_r 220,000 and 200,000 were observed. $M_r \times 10^{-3}$ are shown to the left in c. Bar, 50 μm .

muscle cultures (2), which is highly localized to the myotendinous junction in developing and adult muscles. The identity of cytotactin and myotendinous antigen has recently been demonstrated (18). We therefore examined the distribution of cytotactin at the myotendinous junction and compared it with the staining for N-CAM on muscle in this specialized region. In both adult chicken anterior latissimus dorsi (Fig. 2) and mouse diaphragm muscle (not shown) a localized distribution of cytotactin was observed, with heavy staining extending from the tip of the muscle fibers towards the bone insertion, in sharp contrast to other extracellular matrix proteins such as fibronectin, which are widespread in both muscle and tendon and have no such directionality of distribution (2). Cytotactin accumulated in the myotendinous junction region mostly within tendinous structures and possibly overlapping into muscle (Fig. 2 a'). By contrast, N-CAM (Fig. 2 a) was restricted to the muscle components of the junction, with sharper contours that seemed to correspond to the extremities of the muscle fibers themselves. N-CAM was present more on the muscle fiber than on the myotendinous junction itself. The region where cytotactin and N-CAM overlap may include the muscle basal lamina, where N-CAM has been observed in frog muscle (27). Thus, intense cytotactin staining was observed where structural elements involved in anchoring muscle fibers to tendons and tendons to bones are located. Moreover, N-CAM remained high at such sites in muscle, in striking contrast to its otherwise specialized localization at the motor endplates throughout the muscle fiber.

Cellular Synthesis of Cytotactin

This morphological study could not answer the question of the cellular origin of cytotactin at the myotendinous junction. The molecule may originate from fibroblasts involved in the maintenance of tendinous structure, from the muscle cells themselves, or from both. We therefore examined the expression of cytotactin in cultured fibroblasts and muscle cells. In primary cultures of chicken embryo fibroblasts, cytotactin was strongly expressed in cells cultured at either high or low density (Fig. 3, a and b). At high density (Fig. 3 a), cells produced a thick fibrillar matrix of cytotactin. When cells were plated at low density to allow examination of single cells and were permeabilized to expose cytoplasmic structures, cytotactin was observed in a perinuclear pattern characteristic of secreted proteins. When the culture supernatant from these cells was analyzed by intrinsic labeling, high levels of two characteristic cytotactin polypeptides (M_r 200,000 and 220,000) were found (Fig. 3 c).

To obtain some indication on the biosynthetic capabilities of muscle cells to produce cytotactin, we studied C2C12 muscle cell cultures at the myoblast and the myotube stages. Cytotactin staining was detectable on myoblasts and became more intense and dispersed on differentiated myotubes with superimposed bright spots and streaks (Fig. 4, a and b). Moreover, high levels of cytotactin were secreted into the culture medium (Fig. 4 c). These high levels of synthesis may not be characteristic of terminally differentiated muscle cells *in vivo*, however, because primary chicken muscle cell cul-

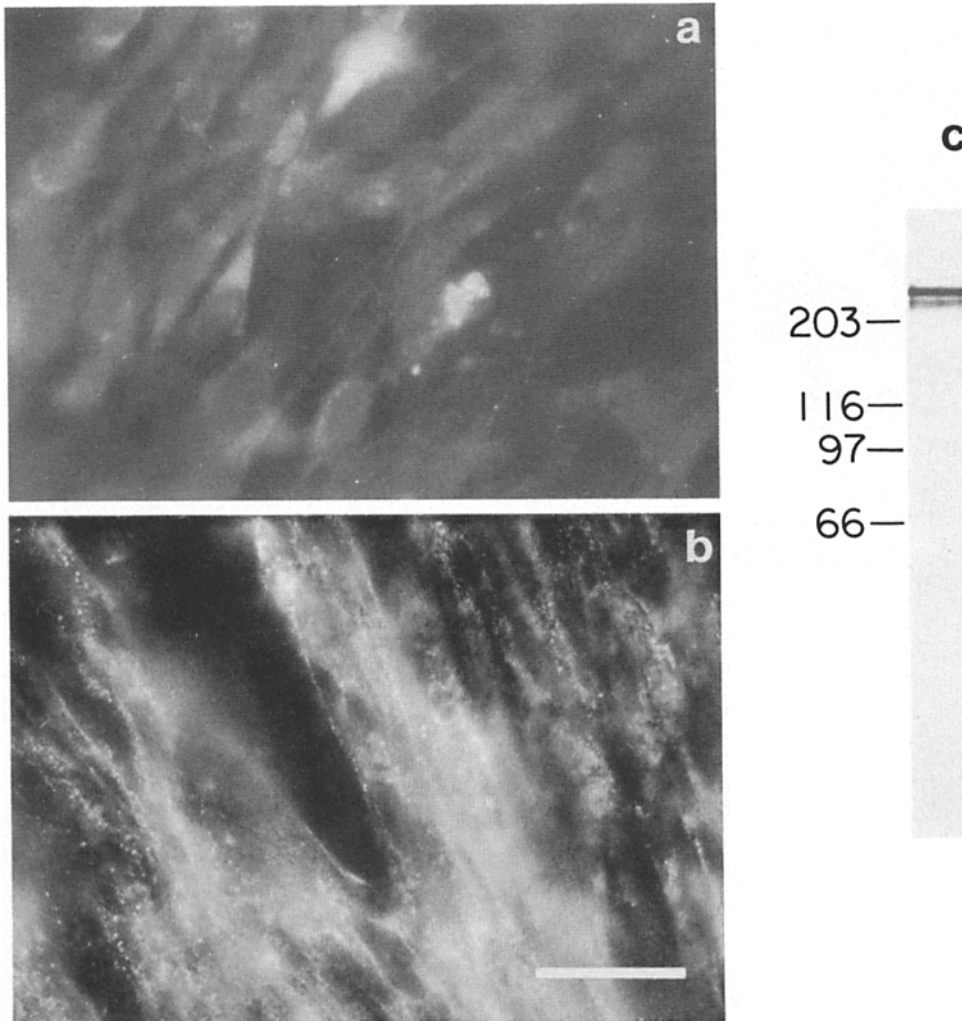


Figure 4. Cytotactin in the mouse C2C12 muscle cell line in culture. Cytotactin staining was detected at low levels on young dividing myoblasts under cell growth conditions (*a*). Intense cytotactin staining was observed on the surface of nonpermeabilized contractile myotubes (*b*), disposed in small bright spots and streaks with no apparent special topographical arrangement relative to the substratum and cell-substratum surfaces. Some large, bright accumulations of cytotactin were observed, such as those at the center of the micrograph, which may correspond to sites of cell-cell contact or fusion. Bar; 50 μm . (*c*) Culture medium from intrinsically labeled contracting myotubes was analyzed on a 7.5% polyacrylamide gel. Very high levels of cytotactin were released into the medium. The two immunoreactive bands at slightly higher molecular mass (M_r 220,000 and 240,000) than those found in fibroblast culture supernatants may result from reduced mobility in higher percentage gels or may reflect alternative forms such as those seen in smooth muscle (20). $M_r \times 10^{-3}$ are shown to the left in *c*.

tures were observed to synthesize cytotactin only after ~ 3 wk in culture (data not shown).

Appearance of Cytotactin during Nerve Regeneration after Nerve Crush or Transection

It was postulated that cytotactin may play a role in regeneration because of its widespread distribution in the neuromuscular system, and its association with glial and connective tissue elements. We therefore examined the expression of cytotactin in adult chicken muscle, nerve, spinal cord, and dorsal root ganglia during the regeneration that follows nerve compression or nerve transection (7, 16, 23). Longitudinal sections of normal, crushed, and transected sciatic nerves, 3, 10 and 20 d after compression (Fig. 5 *A*) and 3, 10, 20, and 60 d after transection (Fig. 5 *B*) were compared with normal nerve (Fig. 5 *A*, panel *d*). At 3 and 10 d after injury, cytotactin staining was strongly positive along the endoneurial tubes and in the perineurium. Near the site of injury, cellular structures were stained proximal and distal to the injury in both crushed (Fig. 5 *A*, panels *a* and *b*) and transected (Fig. 5 *B*, panels *a* and *b*) nerves. There was a peak of intensity of the cytotactin staining inside the damaged nerve (Fig.

5 *A*, panel *b*; Fig. 5 *B*, panel *b*) about 10 d after injury, the time when the beginning of regeneration has been observed (22). Increased staining in the perineurium remained elevated until 10–20 d after injury. Levels of cytotactin returned to near normal (Fig. 5 *A*, panel *d*) by 20 d after nerve crush (Fig. 5 *A*, panel *c*) or 60 d after nerve transection (Fig. 5 *B*, panel *d*). Observations at higher magnification showed that, in the distal stump of a nerve transected 10 d earlier, cytotactin was mainly localized in the cytoplasm of Schwann cell bodies (not shown). Cytotactin was also found in the endoneurium (Fig. 6 *a*) around regenerating axons, which were identified in double staining experiments by their intense staining for Ng-CAM (Fig. 6 *a*).

To assess the changes in cytotactin distribution in the muscle target tissues after nerve injury, we examined the gastrocnemius muscle in the adult mouse after nerve crush. In longitudinal sections of normal muscle, there was a slight interstitial, endomysial staining (Fig. 7 *a*). The intensity of cytotactin staining increased after the nerve was crushed, but was observed in the same locations as in control muscle (i.e., in the endomysial spaces of denervated muscles [Fig. 7 *b*]). Deposits of cytotactin were observed in interstitial spaces between denervated muscle cells, but were most intense near

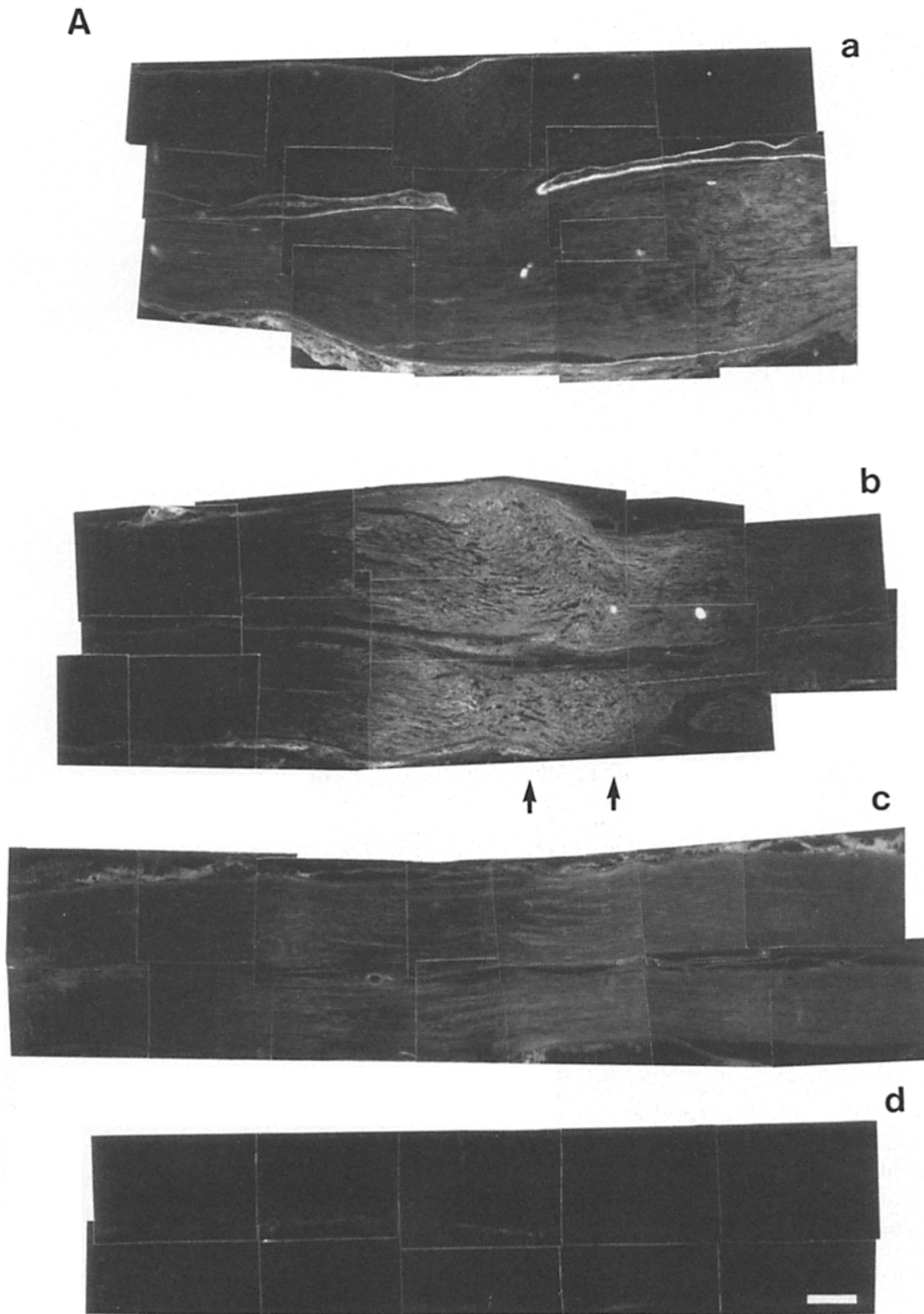
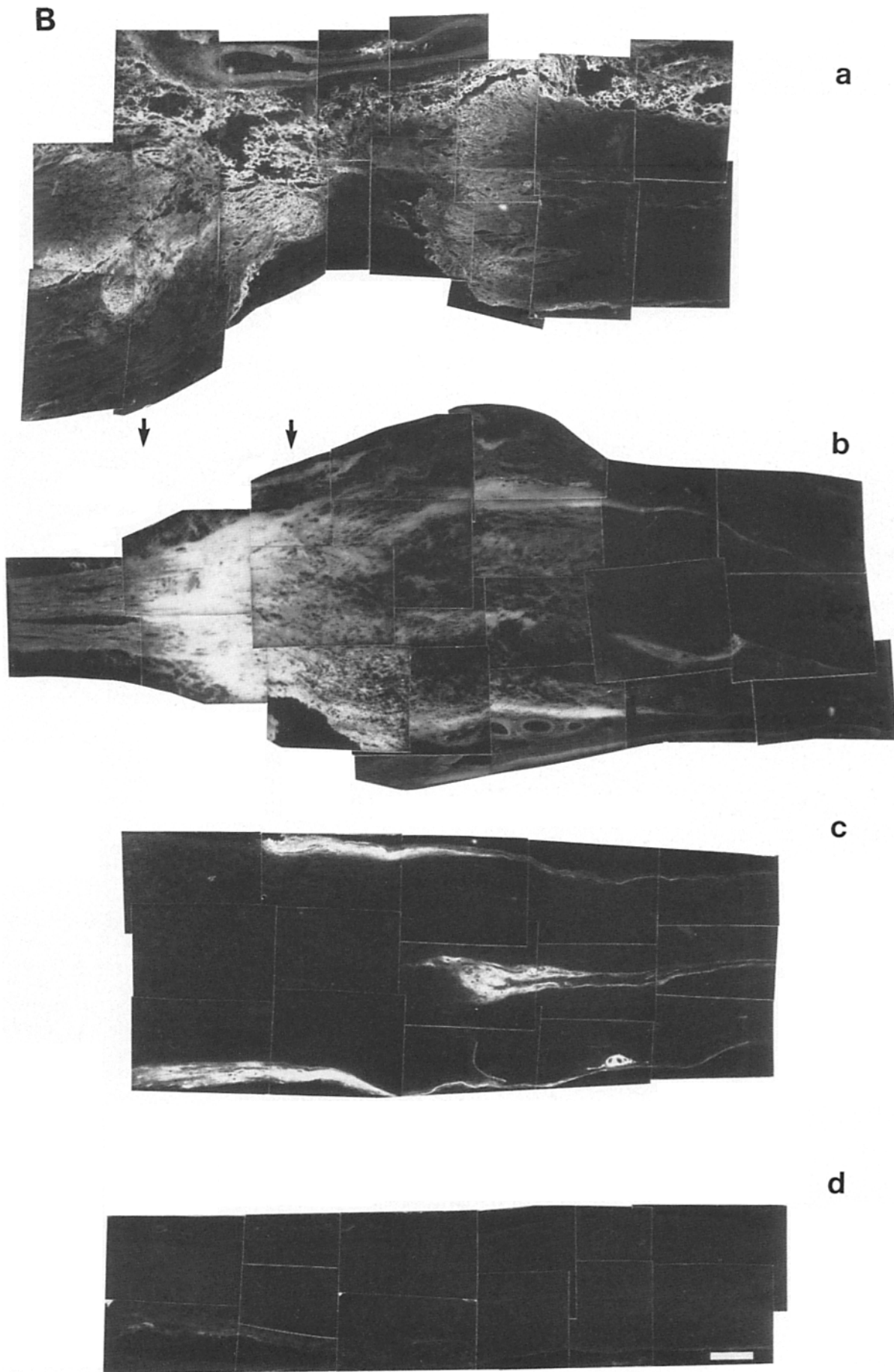


Figure 5. Low magnification views of cytotactin staining in frozen longitudinal sections of normal, crushed, and transected adult chicken nerve. Crushed nerve was examined at 3, 10, and 20 d after injury and transected nerve 3, 10, 20, and 60 d after injury. The site proximal to injury is on the right in all panels. Arrows indicate the crush or cut site, on each plate. (A) *Crush*: in normal nerve, a very slight endoneurial and perineurial staining for cytotactin was observed (d). In crushed nerve, staining became intense in the crushed area, 3 d after nerve injury (a), with a maximal intensity 10 d after injury (b) with prominent perineurial and endoneurial staining near the site of injury and staining reminiscent of the bands of Büngner within the nerve. 20 d after injury (c), the overall staining started decreasing and was greatly decreased to near normal levels (compare with [d]). (B) *Cut*: in cut nerve, the cytotactin staining changed in parallel (a-d); the main difference was that the scar region remained swollen until 20 d after injury (c). There is a peak of intensity of the cytotactin staining about 10 d after injury inside the nerve and also on the perineurium. By 60 d after cut (d), staining returned to normal levels. Bar, 500 μ m.



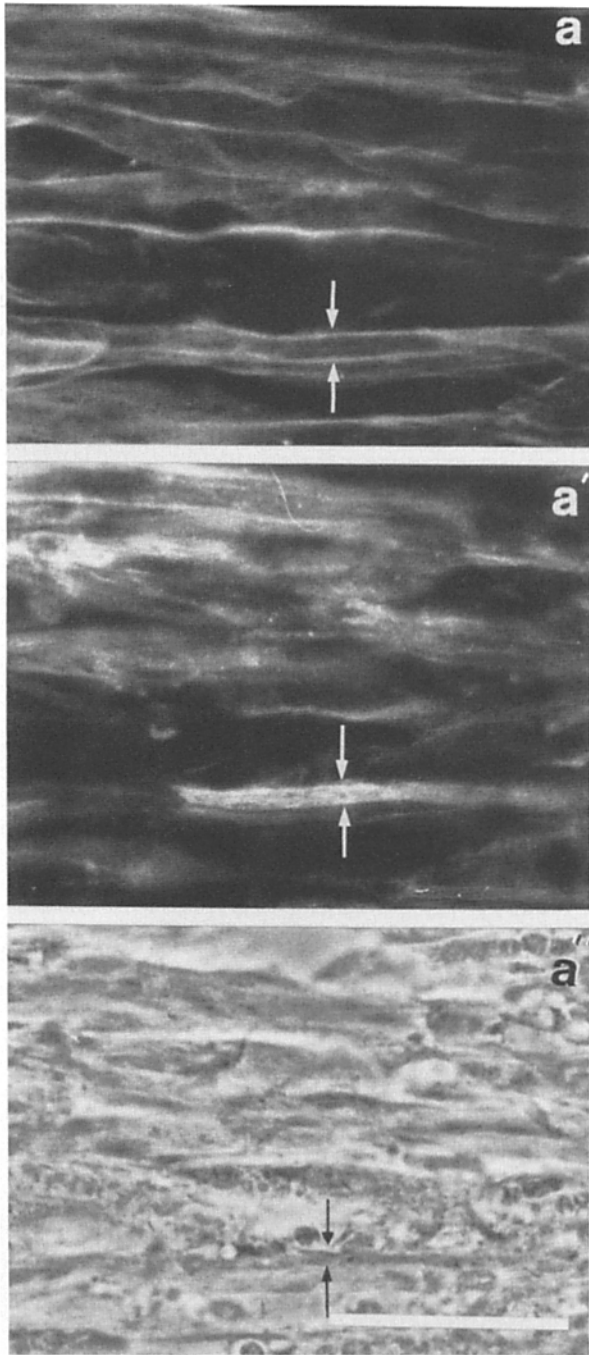


Figure 6. High magnification view of cytotactin staining in adult chicken sciatic nerve 10 d after transection. On the proximal side of the lesion, cytotactin staining was found to surround regenerating axons ([*a*] a single axon is shown enclosed in arrows; [*a'*] double-stained with an anti-Ng-CAM antibody). *a''* is the corresponding phase micrograph. There was intense and continuous cytotactin staining all along the regenerated nerve fibers up to 20 d after nerve transection. Bar, 50 μm .

denervated synaptic sites labeled with α -bungarotoxin (not shown). During the first days after injury the staining was more diffuse and less concentrated in the motor endplate region than at later times. Cytotactin accumulated near the old synaptic sites 1–3 wk after injury, in both compressed or transected nerves (not shown).

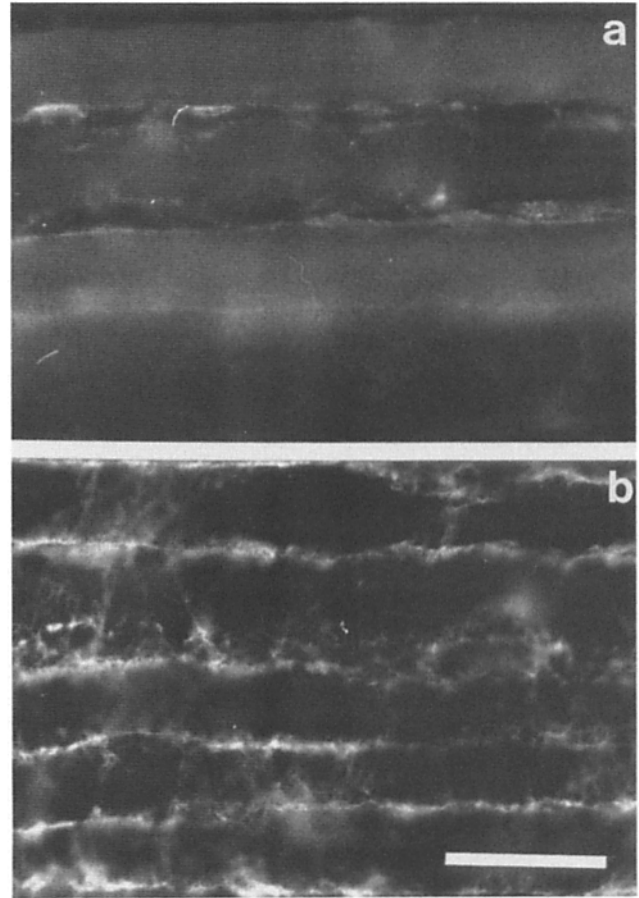


Figure 7. Consequences of nerve injury on the distribution of cytotactin in skeletal muscle. Cytotactin staining in longitudinal sections of normal adult mouse gastrocnemius muscle (*a*) was low and was distributed along the muscle fiber in interstitial spaces. In denervated muscle (*b*), cytotactin staining had increased dramatically 5 d after nerve compression in corresponding locations. Bar, 50 μm .

Patterns of cytotactin staining in the DRG and spinal cord on the side contralateral to a nerve cut (Fig. 8, *a*, *a'*, and *a''*) were compared with the experimental side (Fig. 8, *b*, and *b'*) 10 d after transection. Staining of the nonlesioned side was the same as normal at all times after injury. No change in staining was seen in the spinal cord (not shown). In either normal DRGs or DRGs on the nonlesioned side, there was a slight but significant staining all around the neurons (Fig. 8 *a*). These areas were also labeled by the anti-S100 antibodies (Fig. 8 *a'*) and thus may include glial processes. On the experimental side, the cytotactin staining was greatly increased in these same areas surrounding the Ng-CAM-positive neurons (Fig. 8, *b* and *b'*) showing that DRG cells react to nerve injury by cytotactin accumulation. The cytotactin increase in DRG cells was a transient phenomenon, which returned to normal 20–30 d after injury.

Discussion

In the present study, we have examined the cellular localization of cytotactin in the neuromuscular system and found local changes and modulation in its expression during regenera-

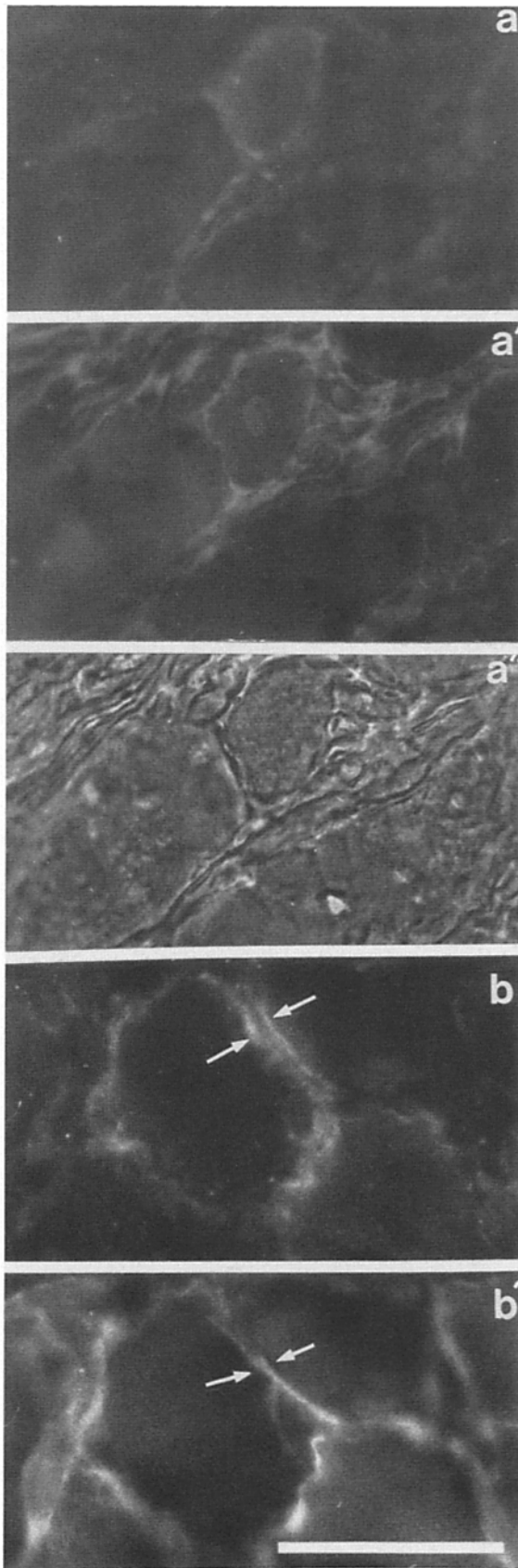


Figure 8. Cytotactin staining in DRGs. In normal DRGs, slight but significantly over background staining for cytotactin was observed in areas surrounding the neurons (*a*), which were also labeled by anti-S100 protein antibodies in double-staining experiments (*a'*). Phase-contrast micrograph (*a''*). The area outside the neurons, the

tion. Cytotactin accumulated at three critical sites of cell-cell interactions in the normal neuromuscular system: between Schwann cell and axon, at the neuromuscular junction, and at the myotendinous junction. Cytotactin has been localized to the region of the node of Ranvier (26). At the neuromuscular junction, cytotactin was found in the terminal Schwann cell. At the myotendinous junction, cytotactin accumulated on the tendon side of the junction, while N-CAM was prominent at the adjoining tips of the muscle fibers. During the regeneration that follows peripheral nerve injury, cytotactin increased in cells within the nerve both at the site of injury and at the neuromuscular junction. The distribution at these sites returned to normal after reinnervation was completed. These observations are consistent with the hypothesis that local signals between neurons and glia regulate the expression of cytotactin during regeneration of the neuromuscular system and possibly during its development as well, and that cytotactin plays a role in cell migration and cell interaction involved in the formation and stabilization of contacts among neurons, glia, and muscle. The regulatory pattern (but not the distribution) greatly resembled that already seen for two neuronal CAMs (7).

The pattern of cytotactin staining found in the motor endplate region was similar but not exactly coincident with that of the postsynaptic AChR. The cytotactin staining was, however, remarkably similar to that of a Schwann cell-specific antigen, which suggests its presence on the terminal nonmyelinating Schwann cell (13, 15). This result is consistent with the idea that cytotactin is synthesized and secreted by Schwann cells and may be an important component of their extracellular matrix, particularly at the node of Ranvier (26). Preliminary evidence suggests that Schwann cells in culture secrete cytotactin (our unpublished observations). It will be of interest to examine the components of cytotactin produced by various cells in the neuromuscular system in light of recent evidence indicating that the multiple cytotactin polypeptides arise from differential splicing of a single gene (20).

Another prevalent site of localization of cytotactin in muscle was the myotendinous junction. Intense cytotactin staining was restricted to proximal and distal portions of muscle fibers near their insertion points to tendon. Staining was very low or absent from medial portions of the muscle. The tendinous side of the myotendinous junctions and tendon fascicles were brightly labeled for cytotactin. Chiquet and Fambrough (1, 2) have described and characterized a molecule, the myotendinous antigen (also called tenascin [3]), which is very similar to cytotactin in its distribution at the myotendinous junction. During development both molecules are similarly located in the perichondria of vertebrae, in limb cartilage elements, and in smooth muscles including the gizzard and aorta (1, 6). Moreover, recent biochemical data have established the identity of cytotactin and the myotendinous antigen (18). It has been hypothesized that the mechanical coupling between skeletal muscle fibers and their

surfaces (*arrows*) of which label with anti-Ng-CAM (*b*), showed increased cytotactin staining 10 d after nerve injury (*b'*) in a pattern suggestive of the glial satellite cells enveloping the neurons. Bar, 50 μ m.

tendons at the myotendinous junction involves components of the extracellular matrix originating from both muscle and fibroblasts (12, 21). Consistent with the current findings, the extracellular nature of cytotactin and its involvement in cell adhesion suggest a possible role in the establishment and maintenance of connections between muscle fibers and tendon fascicles. Cytotactin may not only modulate cell movement (30) but also represent an extracellular matrix protein involved in the supramolecular associations responsible for the mechanical transduction of tension between muscle and bone.

In the present investigation, we found marked local changes in the distribution and amounts of cytotactin after peripheral nerve injury. We previously reported that the amount of two neuronal CAMs, N-CAM and Ng-CAM, increased after denervation both in neurons and in Schwann cells in the area surrounding the lesion (7). In a similar manner, the present study has focused on cytotactin, the expression of which is restricted to Schwann cells and certain connective tissue elements, but not neurons (18, 26). After nerve injury, high levels of cytotactin staining were observed both proximal and distal to the injury, as found for N-CAM and Ng-CAM (7). Cytotactin was highly concentrated along the endoneurial tubes and on the perineurium. Cytotactin also accumulated on the surface of the Schwann cells present at or near the site of injury in crushed or cut nerves. Another reaction to nerve injury, specifically associated with transection of nerve, was the increased cytotactin staining of cells that surround neurons in the DRG.

In muscle, cytotactin levels and distribution increased after denervation and thus, like N-CAM, they may potentially be regulated by synaptic function (5, 7, 25, 28). Cytotactin levels remained high as long as muscle remained denervated, with increased interstitial staining, especially near the motor endplate region. Light microscopic observations of normal muscle indicated that this increase may be due to staining of terminal Schwann cells in the motor endplate region and perhaps of connective tissue elements. Thus, cytotactin may play a role in axon guidance by contributing (along with other adhesion molecules) to the orientation and growth of regenerating axons along basal lamina components (19) within the nerve trunks and bands of Büngner (22). Such a role has also been suggested for the J1 glycoprotein (29), which consists of a number of components recognized by a polyclonal antibody, at least some of which appear to be related to tenascin (12). The extent of reinnervation measured by choline acetyltransferase activity correlated well with a down regulation of cytotactin, similar to that observed for N-CAM (7). It thus appears that increased synthesis or accumulation of cytotactin characterizes part of the response to nerve injury. This was seen in various cells at the site of injury, in the DRGs and the target muscle. During the nerve regeneration process, complex signals are exchanged among neurons, glia, muscle, and connective tissue cells (24), possibly by means of soluble factors. It is tempting to speculate that cytotactin constitutes part of the signal path between cells particularly with regard to cell migration events after injury, as well as during development. Two recent studies also support a role for cytotactin in cell migration. Both cytotactin and its proteoglycan ligand have been shown to be involved in the migration of neural crest cells on adhesive substrates such as fibronectin (30). Furthermore, antibodies to cytotactin in-

hibit neuronal migration on glia within the molecular layer of the developing cerebellum (4). Thus the decrease in cytotactin during embryonic development (6, 18) and its increase upon nerve injury may be correlated with cell migration events known to occur at these times (22) and perhaps with other regenerative and developmental processes as well.

Further experiments are required to evaluate precisely the coordination of the respective roles of the cell adhesion molecules and cytotactin in nerve development and regeneration. It is of particular significance that the expression of cytotactin is high in cells participating in the regenerative events, but is relatively low in normal adult cells of the neuromuscular system and that the molecule occurs in adult tissue with very specialized distribution, localized at sites of heterotypic cell interactions (26). In particular, both embryonic development (26) and regeneration lead to the preferential localization of cytotactin at the node of Ranvier in myelinated nerve fibers and cytotactin synthesis or accumulation is down-regulated at other sites, suggesting that cytotactin may play a decisive role in Schwann cell positioning and stabilization along developing and regenerating axons.

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