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Molecular identity, distribution and heterogeneity of glial fibrillary acidic protein: an immunoblotting and immunohistochemical study of Schwann cells, satellite cells, enteric glia and astrocytes

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Summary

Glial fibrillary acidic protein has been firmly established as the predominant component of astrocyte intermediate filaments. It has also been detected immunohistochemically in the glial cells of the enteric nervous system and some Schwann cells in the P.N.S. The molecular identity of this GFAP immunoreactivity in the P.N.S. has so far not been investigated. This study compares GFAP in the C.N.S. and P.N.S. of adult rats both immunochemically and immunohistochemically.

Using SDS polyacrylamide gel electrophoresis combined with immunoblotting, and a polyclonal antiserum to brain GFAP, we show that the peripheral GFAP immunoreactivity resides in a polypeptide with a molecular weight of 49 kd, which is identical to that of rat brain GFAP. Furthermore, we find that this GFAP reactivity can be detected immunohistochemically in Schwann cells in a wide variety of nerves in the P.N.S. and in some satellite cells in both sensory and sympathetic ganglia, in addition to enteric glia. The pattern of distribution of GFAP filaments in Schwann cells suggests that, in the nerves surveyed, they may be expressed by most or all non-myelin forming Schwann cells but not by myelin-forming Schwann cells. We also show, using a monoclonal antibody to GFAP (anti-GFAP-3) in both immunohistochemical and immunoblotting studies, that the GFAP found in most peripheral glia is not identical to that of astrocytes since it lacks an antigenic determinant, defined by this monoclonal antibody, which is present in astrocytes. An exception to this finding is seen in the myenteric plexuses where immunohistochemically detectable GFAP is found in some, but not all, of the enteric glia, using the monoclonal antibody.

Thus, the results suggest that GFA polypeptides may be a heterogeneous group, that share some common determinants and a common molecular weight, and show a widespread and complex distribution in the glia of both the C.N.S. and P.N.S.

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Introduction

Glial fibrillary acidic protein (GFAP) has been firmly established as a predominant component of the 10 nm intermediate filaments of astrocytes (Bignami *et al.*, 1972; Schachner *et al.*, 1977; Lazarides, 1980; Anderton, 1981). More recently, it has become clear that GFAP, when detected immunohistochemically, is not restricted to glia of the C.N.S. We have reported that GFAP-like immunoreactivity can be seen in the glial cells of the enteric nervous system in tissue sections, whole mount preparations and tissue culture (Jessen & Mirsky, 1980, 1983), and it has since been detected in Schwann cells in both the sciatic nerve (Yen & Fields, 1981; Dahl *et al.*, 1982) and olfactory nerve (Barber & Lindsay, 1982). So far no information has been available on the molecular basis of the GFAP immunoreactivity, and the nature of the GFAP-like material in the P.N.S. has become controversial (Davison & Jones, 1981; Dahl *et al.*, 1982). Furthermore, it has not been determined how widespread the expression of this antigen is within the P.N.S.

The present work, using immunoblotting with polyclonal antisera, shows that the peripheral GFAP immunoreactivity resides in a protein of molecular weight apparently identical (49 kd) to that of astrocyte GFAP. Nonetheless, we find that the GFAP of most peripheral glia appears to differ from the GFAP of astrocytes in lacking an antigenic determinant defined by monoclonal antibody to GFAP (anti-GFAP-3) (Albrechtsen *et al.*, 1983). This provides the first evidence for molecular heterogeneity among GFAP filaments. The results also show that, rather than being confined to a few specific locations, GFAP-positive Schwann and satellite cells are in fact widely scattered throughout peripheral nerves and ganglia. The pattern of GFAP distribution in Schwann cells suggests that this filament type may be expressed by most or all non-myelin forming Schwann cells while being absent from those that form myelin.

Materials and Methods

Frozen sections

Dorsal root ganglia (DRG), superior cervical ganglia (SCG), dorsal and ventral roots, cervical sympathetic trunk, cerebellum and proximal colon were removed from Wistar-Furth (W/Fu) rats of various ages. Frozen sections of 3–7 μm were cut and thawed on to dry or polylysine-coated microscope slides. In some experiments sections were briefly rinsed with 0.15% Triton in phosphate buffered saline (PBS) prior to application of antibodies.

Antibodies

For routine studies a rabbit antiserum to human GFAP, a gift from Dr R. Pruss, was employed. The specificity of this serum has been described previously (Jessen & Mirsky, 1980). It was used at dilutions of 1:500 or 1:2000 in incubations for 1 h at room temperature or 18 h at 4° C respectively, in immunofluorescence experiments. In immunoblotting experiments it was used at a dilution of 1:30 000. Results obtained with this antiserum in immunofluorescence studies were confirmed using four additional rabbit antisera raised against GFAP (given by Drs A. Bignami, E. Bock, R. Pruss and P. Woodhams). Mouse monoclonal antibody, anti-GFAP-3, was supplied and charac-

terized by Dr E. Bock (Albrechtsen *et al.*, 1983). Hybridoma supernatant was used at a dilution of 1:2 in incubations for 18 h at 4° C in immunofluorescence experiments and 1:3 in immunoblotting experiments. Tetramethyl rhodamine conjugated to goat anti-rabbit Ig (G anti-RIg-Rd) and fluorescein conjugated goat anti-rabbit Ig (G anti-RIg-Fl) adsorbed with mouse Ig to remove cross-reacting antibodies, and fluorescein conjugated goat and anti-mouse Ig (G anti-MIg-Fl) (Nordic Labs Ltd), adsorbed with rabbit Ig to remove cross-reacting antibodies, were used at dilutions of 1:50 or 1:100 in immunofluorescence experiments. Affinity purified sheep anti-rabbit Ig labelled with ^{125}I, and affinity purified rabbit anti-mouse F(ab')$_2$ labelled with ^{125}I by the chloramine T method were used in immunoblotting experiments.

Immunofluorescence

Cryostat sections were incubated for 18 h at 4° C, with GFAP antisera or monoclonal anti-GFAP-3 diluted in PBS containing 10% foetal calf serum (FCS) and 0.1 M lysine. After the incubation, sections were allowed to stand for 1 h at room temperature and then given three 5-min washes in PBS. The sections were then incubated with G anti-RIg-Fl or G anti-MIg-Fl for 30 min at room temperature and again washed three times in PBS before mounting in 50% glycerol/PBS mountant (Chemistry Department, City University). In double label immunofluorescence experiments sections were incubated sequentially with mouse anti-GFAP-3 (18 h), G anti-MIg-Fl (1 h), rabbit anti-GFAP (1 h) and G anti-RIg-Rd (1 h).

Immunoblotting

Brain, sciatic nerve, DRG, SCG and cervical sympathetic trunk were removed from adult W/Fu rats. Myenteric plexus from proximal colon of 14-day-old rats was dissected free from smooth muscle as described previously (Jessen *et al.*, 1983). Tissue extracts were prepared by homogenization for 5 min in 2% SDS, and 2% mercaptoethanol (ME). The homogenate was then boiled for 5 min, spun and the supernatant retained. An aliquot was removed for protein estimation. Samples were subjected to SDS–polyacrylamide gel electrophoresis using 7.5 or 8% total acrylamide slab gels and the buffer system described by Laemmli and Favre (1973). The separated proteins were then electrophoretically transferred to 0.12 μm pore size nitrocellulose sheets essentially as described by Towbin *et al.* (1979). A transfer time of 2.5 h was used. After transfer, excess protein binding sites on the sheets were blocked by overnight incubation in PBS containing 5% bovine haemoglobin. The blots were then incubated for 2 h in 1 μl of rabbit anti-GFAP diluted in 30 ml of PBS containing 3% haemoglobin (PBS–3Hb) or overnight in a 1:3 dilution of mouse anti-GFAP-3 supernatant in PBS–3Hb. After incubation blots were washed for 1 h with five changes of PBS–3Hb and incubated for 1 h 15 min with ^{125}I-labelled sheep anti-rabbit Ig (5×10^6 cpm/blot diluted in 30 ml of PBS–3Hb) in the case of rabbit anti-GFAP, or ^{125}I-labelled rabbit anti-mouse F(ab')$_2$ (15×10^6 cpm/blot diluted in 30 ml of PBS–3Hb) in the case of mouse monoclonal anti-GFAP-3. After washing with five changes of PBS, blots were dried and exposed to pre-flashed X-ray film for 2–21 days in a cassette equipped with an intensifying screen.

Results

GFAP POLYPEPTIDES FROM THE P.N.S. AND C.N.S. HAVE THE SAME MOLECULAR WEIGHT

To investigate the molecular identity of the GFAP-like immunoreactivity in the P.N.S., we combined SDS–polyacrylamide gel electrophoresis of tissue extracts with immunoblotting, using polyclonal antiserum to brain GFAP. Homogenates of myenteric plexus, sciatic nerve, DRG, SCG, cervical sympathetic trunk and brain all showed a single

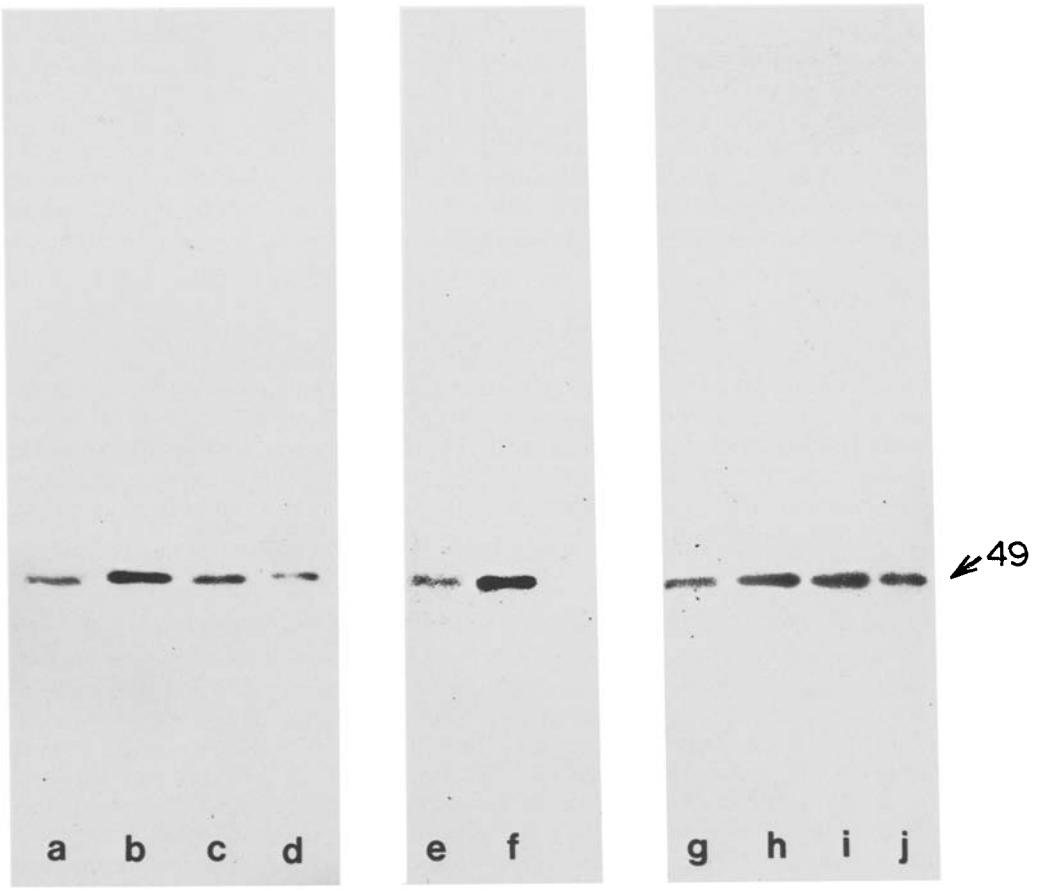

Fig. 1. Immunoblots using rabbit polyclonal antiserum to GFAP. Procedure as described in the Methods. (a) Sciatic nerve extract, 80 μg/track; (b) extract of superior cervical ganglia, 80 μg; (c) extract of dorsal root ganglia, 80 μg; (d) brain extract, 4 μg; (e) extract of cervical sympathetic trunk, 16 μg; (f) brain extract, 16 μg; (g) myenteric plexus extract, 30 μg; (h–j) brain extract, 34, 17 and 8 μg, respectively.

immunoreactive band at 49 kd, the expected molecular weight of GFAP in rat brain (Thorpe *et al.*, 1979) (Fig. 1). In occasional experiments a faintly reactive band could be seen in the vimentin position. Smooth muscle, a possible contaminant in the plexus preparations, showed no band in the 49 kd position. A comparison was made of the quantity of protein from different tissues required to give comparable autoradiographs. Brain extract of 2 μg was equivalent to 8 μg of myenteric plexus extract and 40 μg of sciatic nerve extract. This indicates that on the basis of equivalent quantities of total

protein, brain extracts contained approximately eight times as much immunochemically detectable GFAP as myenteric plexus and twenty times as much as sciatic nerve.

GFAP is present in non-myelin forming Schwann cells and some satellite cells

The immunofluorescence studies described here were carried out on 6–8 month old rats, since other experiments indicated that GFAP distribution changes with age in younger animals (see below). Five different polyclonal antisera showed a similar staining pattern in immunofluorescence studies using frozen sections of various peripheral nerves, including the sciatic nerve, nerves from the brachial plexus, the vagus, dorsal and ventral spinal roots, and cervical sympathetic trunk (Figs. 2–4). Unambiguous and discretely localized filamentous immunoreactivity was seen in all the nerves, apparently restricted to non-myelin forming Schwann cells. This was ascertained by observing the same field alternately with fluorescence and phase contrast optics, and by immunostaining teased nerve preparations in which individual myelinated axons and non-myelinated nerve fascicles can be clearly observed (data not shown). Similar experiments using anti-vimentin antiserum revealed filamentous staining in both the myelin-forming and non-myelin forming Schwann cells of the sciatic nerve (unpublished) indicating that technical difficulties do not preclude the visualization of intermediate filaments in these cells in frozen sections. The size of the GFAP-positive Schwann cell population varied markedly between the various nerves, as would be expected if the antigen was confined to non-myelin forming cells (Figs. 2–4). Thus, as far as could be judged, GFAP immunoreactivity was present in essentially all the Schwann cells of the cervical sympathetic trunk, where 99% of axons are surrounded by non-myelin forming cells (Dyck & Hopkins, 1972). Immunopositive Schwann cells were still frequent in sections of dorsal roots, where about 66% of the axons are accompanied by non-myelin forming cells (Langford & Coggeshall, 1981), while they were much rarer in ventral roots, in which non-myelinated fibres only account for 26 to 29% of the axons (Coggeshall *et al.*, 1975).

In sections from the SCG and ganglia of the sympathetic trunk we found that the satellite cells enveloping the neuronal cell bodies frequently showed GFAP immunoreactivity (Figs. 5, 6). Preliminary counts indicate that 40 to 60% of the neurons in the SCG are associated with GFAP-positive satellite cells. GFAP was also seen in satellite cells in DRG, but such cells were only frequent in ganglia corresponding to cervical and lumbar levels of the spinal cord. In ganglia from mid-thoracic spinal levels, fewer GFAP-immunoreactive satellite cells were seen, and some sections through such ganglia contained only GFAP-negative cells. In both sympathetic and sensory ganglia GFAP immunoreactivity was predominantly associated with cells that enveloped large neurons, rather than with those associated with small or medium-sized neurons. In most GFAP-positive satellite cells, the immunoreactivity appeared weaker than that seen in Schwann cells in nerves, and could only be clearly visualized using very thin frozen sections (3–4 μm). Furthermore, the size of the GFAP-positive satellite cell popu-

lation increased markedly with age, highest numbers being seen at 6 months or older, while GFAP-positive cells were often hard to find in younger animals (unpublished).

In experiments carried out in parallel with those above, intense and characteristic immunostaining was seen in brain astrocytes. No significant staining was seen in smooth muscle, connective tissue or neurons. This indicates that, in the rat, under the

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Fig. 2. GFAP immunofluorescence with polyclonal antibodies to GFAP in 3–4 μm frozen sections of cervical sympathetic trunk stained with rabbit anti-GFAP followed by G anti-RIg-Fl. Sections viewed with (a) fluorescein optics, (b) phase contrast. × 84. Note staining throughout the width of the nerve, and apparent absence of myelinated fibres in b, when compared with dorsal and ventral root (Figs. 3, 4).

Fig. 3. GFAP immunofluorescence with polyclonal antibodies to GFAP in 3–4 μm frozen sections of dorsal root treated as described in Fig. 2. Viewed with (a) fluorescein optics, (b) phase contrast. × 84. Note staining of some Schwann cells, one of which is arrowed, lying among many unlabelled myelinated fibres visualized in b.

Fig. 4. GFAP immunofluorescence with polyclonal antibodies to GFAP in 3–4 μm frozen sections of ventral root treated as described in Fig. 2. Viewed with (a) fluorescein optics, (b) phase contrast. × 84. Notice relatively sparse staining of Schwann cells compared with dorsal root. Many unlabelled myelinated fibres can be visualized in b.

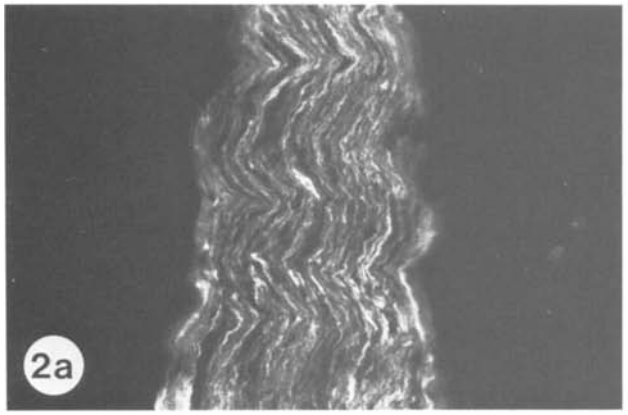
Fig. 5. GFAP immunofluorescence with polyclonal antibodies to GFAP. 3–4 μm frozen sections of DRG treated as described in Fig. 2. Viewed with (a) fluorescein optics, (b) phase contrast. × 250. Note that one neuronal cell body (asterisk) is surrounded by satellite cells containing GFAP, whereas two other neuronal cell bodies are surrounded by satellite cells which do not express GFAP. Myelin sheaths visualized in b are unstained in a, while non-myelinated fibres, poorly visualized in b, are intensely stained. An example is arrowed.

Fig. 6. GFAP immunofluorescence with polyclonal antibodies to GFAP. 3–4 μm frozen sections of SCG treated as described in Fig. 2. Fluorescein optics. × 250. Note staining of satellite cells round neuronal cell bodies, examples of which are labelled with asterisks. Schwann cells within the ganglion are also stained.

Fig. 7. Double-label GFAP immunofluorescence with monoclonal and polyclonal antibodies to GFAP. 4 μm frozen sections of DRG were incubated with mouse anti-GFAP-3 followed by G anti-MIg-Fl, then rabbit anti-GFAP serum followed by G anti-RIg-Rd. Sections viewed with (a) fluorescein, (b) rhodamine optics. × 250. In the DRG, satellite cells around two neuronal cell bodies (asterisks) are labelled with the polyclonal antiserum in b, while satellite cells around other neuronal cell bodies and all the neurons are unlabelled. No labelling of the same satellite cells is seen with the mouse monoclonal antibody.

Fig. 8 Double-label GFAP immunofluorescence with monoclonal and polyclonal antibodies to GFAP in 7 μm frozen sections of sciatic nerve treated as described in Fig. 7. Sections were viewed with (a) fluorescein, (b) rhodamine optics. × 84. Selected fibres are labelled with the polyclonal antiserum in b, while no labelling of the same fibres is seen with the mouse monoclonal antibody in a.

Fig. 9. Double-label GFAP immunofluorescence with monoclonal and polyclonal antibodies to GFAP in 7 μm frozen sections of cerebellum treated as described in Fig. 7. Sections were viewed with (a) fluorescein, (b) rhodamine optics. × 84. In the cerebellum, Bergmann glia are strongly labelled with both the polyclonal antiserum in b and the monoclonal antibody in a.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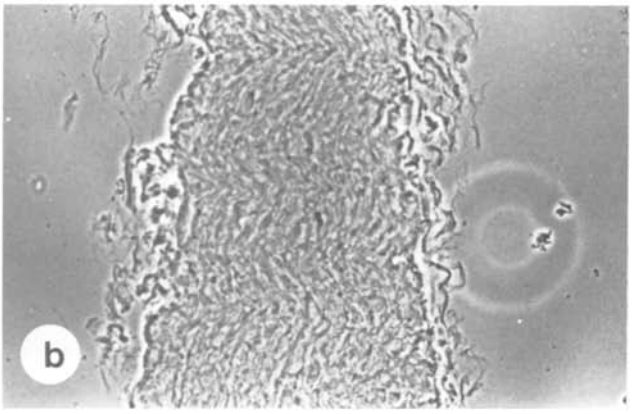
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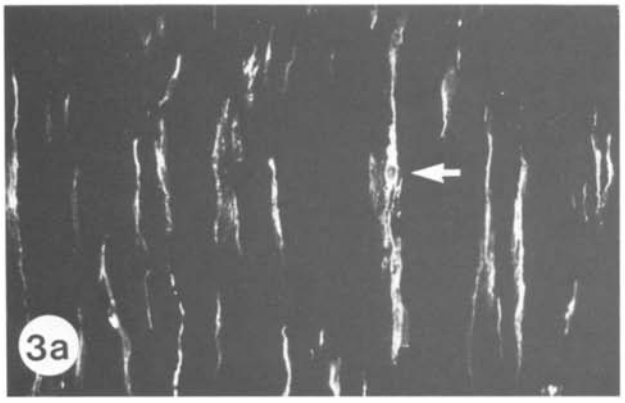
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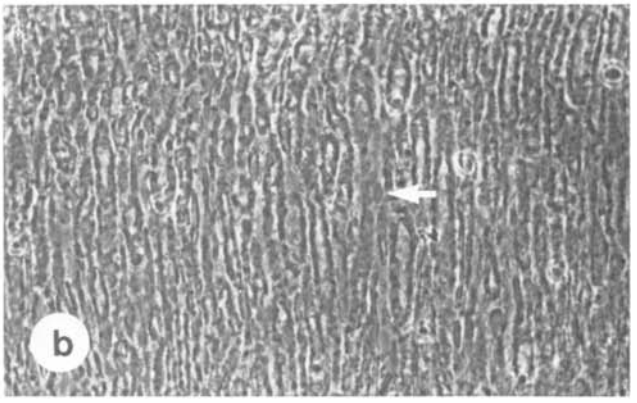
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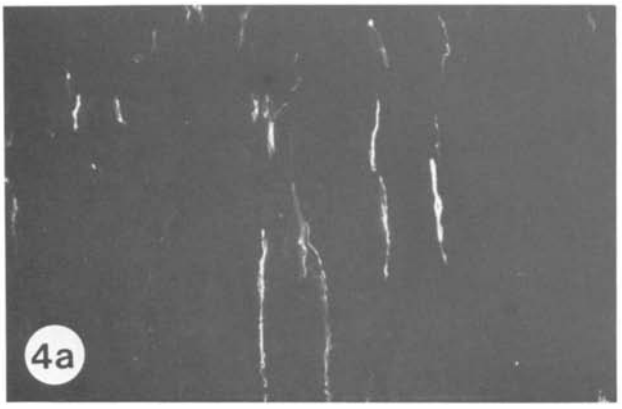
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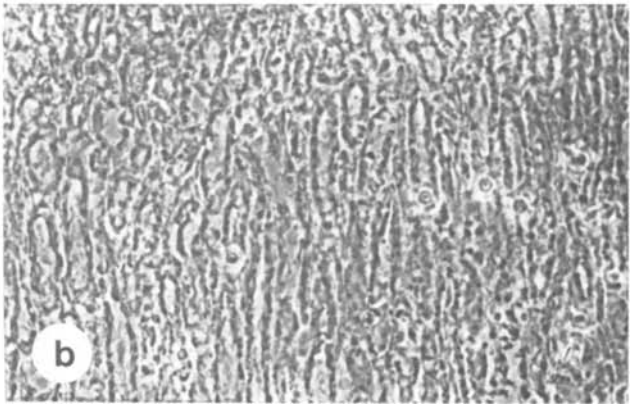
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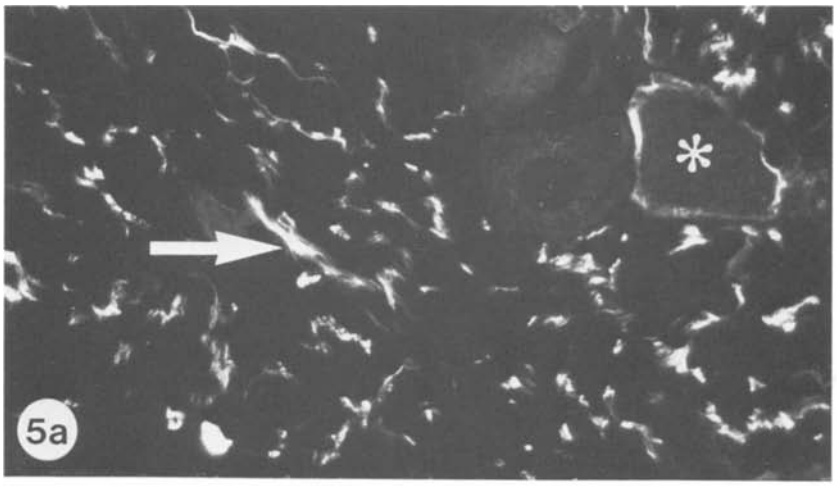
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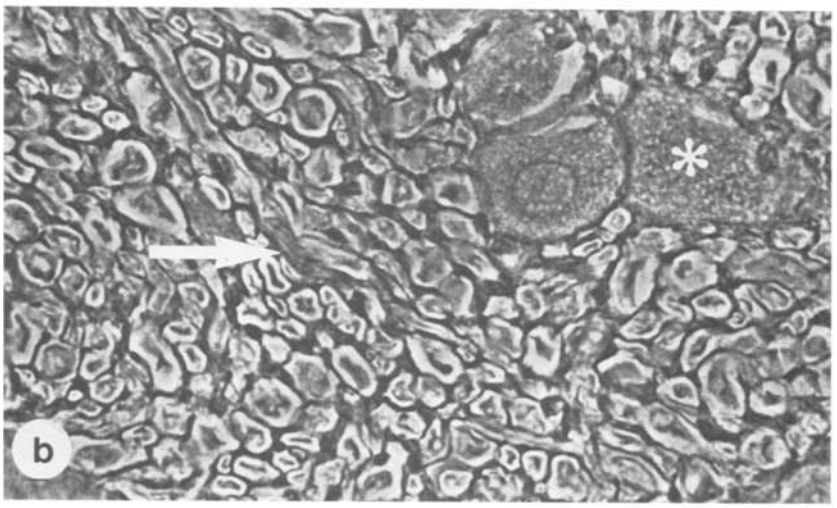
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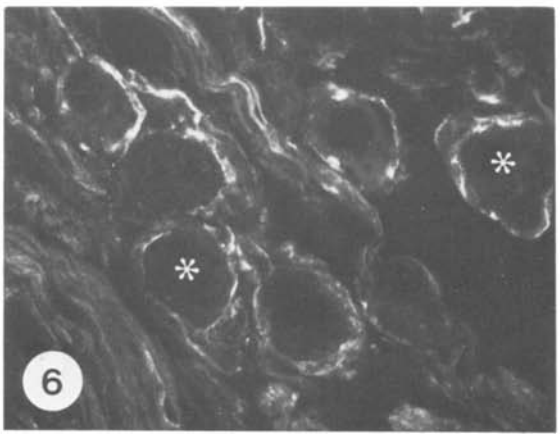
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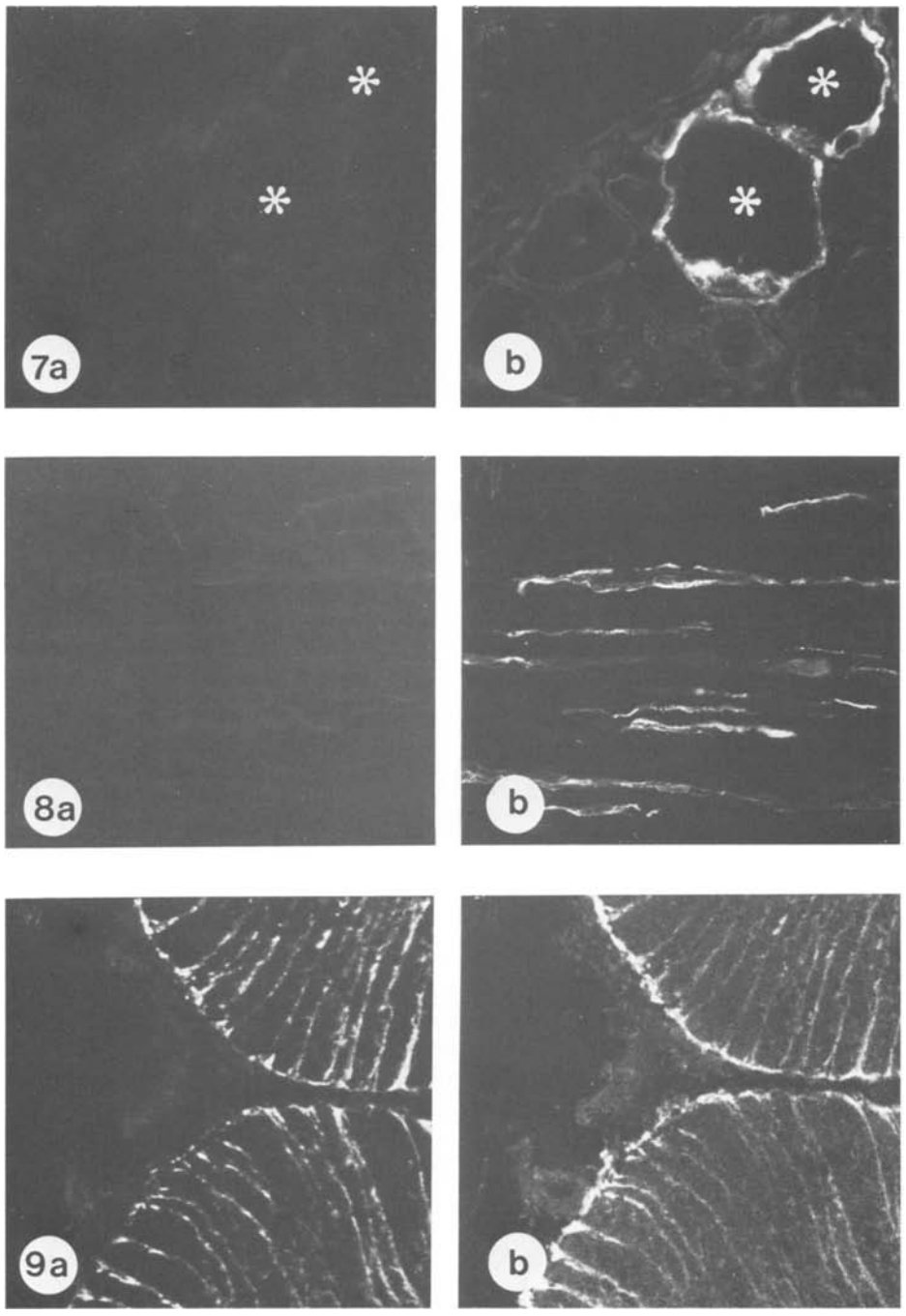
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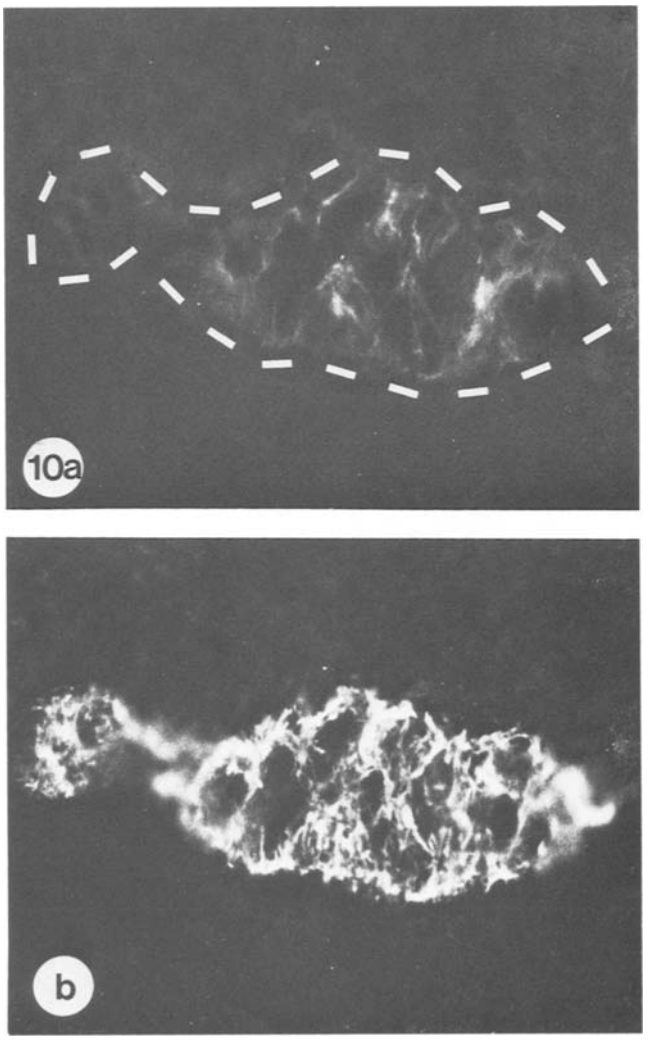

Fig. 10. Double-label GFAP immunofluorescence with monoclonal and polyclonal antibodies to GFAP in 7 μm frozen section of rat proximal colon treated as described in Fig. 7. Viewed with (a) fluorescein, (b) rhodamine optics. × 250. Polyclonal antibodies to GFAP show labelling in the glial cells of the myenteric plexus in b. Neurons in the plexus and the circular and longitudinal muscle and serosa are unlabelled. In the same section, mouse monoclonal anti-GFAP-3 shows selective labelling of some glial cells within the plexus in a although many glial cells, all neurons, muscle and serosa are unlabelled. Ganglion outlined in a by stippled line.

conditions used, the antisera do not cross-react significantly with other intermediate filament classes such as desmin, vimentin, neurofilaments or cytokeratins (Lazarides, 1980; Anderton, 1981), and that the immunostaining was restricted to glial filaments.

GFAP in peripheral glia is not identical to GFAP in astrocytes

The application of a monoclonal antibody directed against GFAP to sections of

Fig. 11. Immunoblot using mouse monoclonal antibody to GFAP. Procedure as described in the Methods. (a and c) Sciatic nerve extract, 400 μg, (b) brain extract, 75 μg. Note the band at 49 kd in brain b, and absence of a band in sciatic nerve (a and c).

peripheral nerves, sensory, sympathetic and enteric ganglia and brain, revealed a distribution of immunoreactivity very different from that seen with the sera. In spite of the strong and typical immunofluorescence reaction in astrocytes and Bergmann glia, no immunohistochemically detectable reactivity was found in Schwann or satellite cells in any location examined, while in the enteric plexuses only a few glia in the myenteric plexuses were positive (Figs. 7–10). Support for these immunohistochemical results was obtained by using SDS–polyacrylamide gel electrophoresis and immunoblotting of rat brain and sciatic nerve extracts with monoclonal anti-GFAP-3 (Fig. 11). To visualize the GFAP in rat brain extracts with the monoclonal antibody it was necessary to load relatively high concentrations of protein (75 μg) on to the SDS gels. When up to fifty times as much sciatic nerve extract (400 μg) as brain extract (8 μg) was loaded on the gels, no detectable GFAP immunoreactivity was seen in the sciatic nerve after 3 weeks exposure to X-ray film, whereas the brain GFAP could be visualized, suggesting that the GFAP polypeptide in the sciatic nerve was not recognized by the monoclonal antiserum.

Discussion

The present results establish that peripheral glia from a wide variety of locations in the P.N.S., including the myenteric plexus, sciatic nerve, sympathetic trunk and both sensory and sympathetic ganglia, contain an immunochemically detectable GFAP-like protein with a molecular weight identical to that of rat brain GFAP.

The immunohistochemical results further suggest, that in some nerves at least, this protein is found in most, possibly all, non-myelinating Schwann cells. This raises the interesting possibility that, in general, adult rat Schwann cells *in situ* either make myelin or contain GFAP, but not both, showing a similar division of molecular expression to that seen between oligodendrocytes and astrocytes in the C.N.S. In this study, many satellite cells, both in sympathetic and sensory ganglia, appeared GFAP-negative, even in animals more than 6 months old. Since GFAP immunoreactivity is present in most or all enteric glia (Jessen & Mirsky, 1980, 1983) and the glia of the olfactory nerve, satellite cells remain, at present, the only category of peripheral glia which, in substantial numbers, have been shown to express neither GFAP nor myelin. Those satellite cells that express GFAP in the DRG are preferentially associated with the largest neurons, which presumably give rise to axons enveloped by myelin-forming, GFAP-negative Schwann cells, while the Schwann cells that express GFAP surround the smaller non-myelinated axons. Therefore, it would seem that cell bodies surrounded by GFAP-containing satellite cells frequently give rise to axons enveloped by GFAP-negative Schwann cells, and vice versa.

In a separate study we have found that, in contrast with astrocytes and enteric glia, GFAP develops relatively late in Schwann and, especially, satellite cells. Little or no GFAP immunoreactivity is detectable in sciatic nerve sections before postnatal days 5 to 7. This is consistent with several reports by us and others (e.g. Raff *et al.*, 1979), that Schwann cells in cultures made from the sciatic nerve of newborn animals are GFAP-negative. The increase in GFAP staining of satellite cells up to about 6 months old and the relative paucity of GFAP immunoreactive satellite cells in ganglia from thoracic levels, even in old rats, probably accounts for our failure to detect GFAP in these cells in a previous study (Jessen & Mirsky, 1980) in which thoracic ganglia from 2 to 3 month old animals were used.

Despite the widespread occurrence of GFAP in the glial cells of the P.N.S., the results obtained with the monoclonal anti-GFAP antibodies also demonstrate an apparent lack of complete molecular identity between the peripheral and brain GFA polypeptides. This point was previously raised by the work of Davison & Jones (1981), who found dissimilarities between the peptide maps of brain GFAP and a sciatic nerve protein, which nevertheless had the same molecular weight as GFAP and showed GFAP-like solubility characteristics. The simplest interpretation of the present results is that polyclonal GFAP sera recognize two or more closely related glial filament proteins of similar molecular weight, at least one of which is not recognized by the monoclonal antibody. While the protein recognized by the monoclonal antibody is abundant in astrocytes in

the C.N.S., it is apparently not present in Schwann or satellite cells. On the other hand, it is present in low but variable amounts in enteric glial cells. It is possible that the difference between the GFAP found abundantly in astrocytes and that found in most P.N.S. glia is related to a post-translational modification. Alternatively, and as suggested by the peptide mapping (Davison & Jones, 1981), the antigenic difference may derive from different amino acid sequences between GFAP subunits, as has been shown for another family of intermediate filaments, the cytokeratins (Lee & Baden, 1976). These findings do not preclude the possibility that the protein in peripheral glia that is recognized by the polyclonal GFAP antiserum, but not by the monoclonal antibody, is also present in astrocytes. Taken together, the above observations suggest that GFA polypeptide filaments may be a heterogeneous group, showing a widespread and complex distribution in the peripheral and central nervous systems.

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References

ALBRECHTSEN, M., VON GERSTENBERG, A. C. & BOCK, E. (1983) Mouse monoclonal antibodies reacting with human brain glial fibrillary acidic protein. *Journal of Neurochemistry* (in press).

ANDERTON, B. H. (1981) Intermediate filaments: a family of homologous structures. *Journal of Muscle Research and Cell Motility* **2**, 141–66.

BARBER, P. C. & LINDSAY, R. M. (1982) Schwann cells of the olfactory nerves contain glial fibrillary acidic protein and resemble astrocytes. *Neuroscience* **7**, 3077–99.

BIGNAMI, A., ENG, L. F., DAHL, D. & UYEDA, C. T. (1972) Localization of the glial fibrillary acidic protein in astrocytes by immunofluorescence. *Brain Research* **43**, 429–35.

COGGESHALL, R. E., APPELBAUM, M. L., FAZEN, M., STUBBS, T. B. III & SYKES, M. T. (1975) Unmyelinated axons in human ventral roots, a possible explanation for the failure of dorsal rhizotomy to relieve pain. *Brain* **98**, 157–66.

DAHL, D., CHI, N. H., MILES, L. E., NGUYEN, B. T. & BIGNAMI, A. (1982) Glial fibrillary acidic (GFA) protein in Schwann cells: fact or artifact. *Journal of Histochemistry and Cytochemistry* **30**, 912–18.

DAVISON, P. F. & JONES, R. N. (1981) Filament proteins in central, cranial and peripheral mammalian nerves. *Journal of Cell Biology* **88**, 67–72.

DYCK, P. J. & HOPKINS, A. P. (1972) Electron microscopic observations on degeneration and regeneration of unmyelinated fibres. *Brain* **95**, 223–34.

JESSEN, K. R. & MIRSKY, R. (1980) Glial cells in the enteric nervous system contain glial fibrillary acidic protein. *Nature* **286**, 736–37.

JESSEN, K. R. & MIRSKY, R. (1983) Astrocyte-like glia in the peripheral nervous system: an immunohistochemical study of enteric glia. *Journal of Neuroscience* **3** (in press).

JESSEN, K. R., SAFFREY, M. J. & BURNSTOCK, G. (1983) The enteric nervous system in tissue culture I. Cell types and their interactions in explants of the myenteric and submucous plexuses from guinea pig, rabbit and rat. *Brain Research* **262**, 17–35.

LAEMMLI, U. K. & FAVRE, M. (1973) Maturation of the head of bacteriophage T4.1.DNA packaging events. *Journal of Molecular Biology* **80**, 575–99.

LANGFORD, L. A. & COGGESHALL, R. E. (1981) Branching of sensory axons in the peripheral nerve of rat. *Journal of Comparative Neurology* **203**, 745–50.

LAZARIDES, E. (1980) Intermediate filaments as mechanical integrators of cellular space. *Nature* **283**, 249–56.

LEE, L. D. & BADEN, H. P. (1976) Organization of the polypeptide chains in mammalian keratin. *Nature* **264**, 377–79.

RAFF, M. C., FIELDS, K. L., HAKAMORI, S.-I., MIRSKY, R., PRUSS, R. M. & WINTER, J. (1979) Cell-type-specific markers for distinguishing and studying neurons and the major classes of glial cells in culture. *Brain Research* **174**, 283–308.

SCHACHNER, M., HEDLEY-WHYTE, E. T., HSU, D. W., SCHOONMAKER, G. & BIGNAMI, A. (1977) Ultrastructural localisation of glial fibrillary acidic protein in mouse cerebellum by immunoperoxidase labelling. *Journal of Cell Biology* **75**, 67–73.

THORPE, R., DELACOURTE, A., AYERS, M., BULLOCK, C. & ANDERTON, B. H. (1979) Polypeptides of isolated brain 10 nm filaments and their association with polymerized tubulin. *Biochemical Journal* **181**, 275–84.

TOWBIN, H., STAEHLIN, T. & GORDON, J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proceedings of the National Academy of Sciences USA* **76**, 4350–54.

YEN, S. & FIELDS, K. L. (1981) Antibodies to neurofilament, glial filament and fibroblast intermediate filament proteins bind to different cell types in the nervous system. *Journal of Cell Biology* **88**, 115–26.