Cell Tissue Res (1996) 283:39–49

Cell & Tissue Research

Immunocytochemical characterization of a new marker of fibrous and reactive astrocytes

Jean-Luc Ridet[1], **Gérard Alonso**[1], **Norbert Chauvet**[1], **Jacqueline Chapron**[2], **Jeanine Koenig**[2], **Alain Privat**[1]

[1] INSERM U. 336, Université Montpellier II, case 106, Place Eugène Bataillon, F-34095 Montpellier Cedex 05, France
[2] CNRS URA 1126, Laboratoire de Neurobiologie Cellulaire, Université Bordeaux II, Avenue des Facultés, F-33405 Talence Cedex, France

Received: 2 May 1995 / Accepted: 31 July 1995

Abstract. A specific monoclonal antiserum (Mab 6.17) inducing a strong immunostaining of the neuromuscular junction has been used to detect the possible occurrence of the corresponding antigen throughout the intact or lesioned central nervous system of adult rats. In intact animals, 6.17-immunolabeling was essentially detected in astrocyte-like structures located in white matter fasciculi of the brain, such as the optic tract, corpus callosum, fornix, and in the white matter of the spinal cord. The astroglial nature of such 6.17-immunolabeled profiles was verified by performing double or triple immunofluorescent labeling with Mab 6.17 and with specific antisera against astrocytic markers, such as S100 protein, glial fibrillary acidic protein and vimentin. In the white matter, all the structures reactive to Mab 6.17 were also reactive to antibodies against S100 protein, glial fibrillary acidic protein and vimentin. On the other hand, astrocytes of the grey matter that were immunoreactive to S100 and glial fibrillary acidic protein but negative to vimentin, were devoid of 6.17-immunoreactivity. After lesions including stab wound through the diencephalon or transection of the spinal cord, a marked increase of 6.17-immunostaining was noted in the regions surrounding the lesions. In these regions, 6.17-immunolabeling was associated with S100-, GFAP- and vimentin-positive astrocytes constituting the glial scar. The ultrastructural localization of 6.17-immunoreactivity indicated that, similar to glial fibrillary acidic protein and vimentin, the recognized antigen was mainly associated with gliofilaments. These observations indicate that, in the central nervous system of adult rats, Mab 6.17 recognizes a molecule associated with gliofilaments, which is essentially associated to reactive astrocytes expressing high levels of vimentin.

This work received financial support from the Institut National de la Santé et de la Recherche Médicale (INSERM), the Institut de Recherche sur la Moelle Epinière (IRME), and the Association Française contre les Myopathies (AFM).

Correspondence to: G. Alonso

Key words: Mab 6.17 – Reactive astrocyte – Diencephalon – Spinal cord – Confocal microscopy – Ultrastructure – Rat (Sprague Dawley)

Introduction

Mab 6.17 is a mouse monoclonal antibody that has been raised against homogenates of embryonic rat muscle (Koenig et al. 1988). Using this antibody, intense immunostaining was firstly localized at the neuromuscular junction of adult rats. Although the nature of this molecule is still unknown, it is suspected that it is synthesized by the Schwann cell and concentrated in the synaptic cleft (Koenig et al. 1988). In a preliminary unpublished study, we observed a marked 6.17-immunostaining in the white matter of the spinal cord. Since the 6.17-immunoreactive (IR) profiles detected in the spinal cord were morphologically similar to astrocytes, we decided to characterize thoroughly the nature of these labeled structures within several regions of the intact and lesioned CNS of adult rats by performing double immunofluorescent labeling of the 6.17 antigen and of specific immunocytochemical markers of astrocytes.

The classical astrocytic markers 1) the glial fibrillary acidic protein (GFAP), a constituent of gliofilaments, and 2) the S100 protein (S100), a cytoplasmic calcium-binding protein, are both detected in virtually all mature astrocytes (Eng et al. 1971; Bignami et al. 1972; Matus und Mughal, 1975; Bignami et Dahl, 1976; Ludwin et al. 1976). In addition vimentin (VIM), a constituent of the filaments present in immature astroglia during CNS development (Dahl et al. 1981; Schnitzer et al. 1981), is present in fibrous astrocytes located in fasiculi and is highly expressed in reactive astrocytes that appear after traumatic lesion of the CNS (Dahl et al. 1981; Schnitzer et al. 1981; Pixley and De Vellis, 1984; Schiffer et al. 1986, 1993; Takamiya et al. 1988; Calvo et al. 1991). In the present study, sections cut through intact or lesioned brains or spinal cords

were thus double or triple immunostained for Mab 6.17 and for S100, GFAP or VIM.

Materials and methods

Male adult Sprague-Dawley rats (Iffa-Credo, France) weighing 250 g were used in this study. They included intact animals (*n*=4), and animals receiving a surgical lesion in the diencephalon (*n*=3) or the spinal cord (*n*=3). All the experimentations were performed according to the "Principles of laboratory animal care" (NIH publication N° 86-23) and to the guidelines issued by the French ethical committee.

Surgical procedures

For the brain lesions, rats under deep anesthesia with equithesin (0.3 ml/100 g body weight, i.p.) were fixed in a stereotaxic device. Surgical lesions were performed by means of a metallic rectangular knife which was lowered in the vertical plane from the cortex to the ventral surface of the diencephalon.

For the spinal cord lesions, the rats were deeply anesthetized with equithesin and a laminectomy was performed at a low thoracic level (T8). The dorsal hemivertebra was removed and complete section of the cord, including the dura mater, was performed. Back muscles and skin were then sutured.

All lesioned animals were then housed individually for survival periods of seven days. They received a daily intramuscular injection of gentamycin (Gentalline®, Shering-Plough, France, 0,2 mg/100 g body weight).

Immunocytochemical procedures

Production of 6.17 antibodies

Mab 6.17 was obtained from hybridoma cultures, as already described by Koenig and colleagues (1988). Balb C mice were immunized by intravenous injections of an embryonic rat muscle homogenate. Cell fusion was then performed with the immunized Balb C mouse splenic lymphocytes and myeloma cells of the Balb C NS1. The supernatant of these hybridoma cultures was used as Mab 6.17.

Preparation of tissues

Both control and operated animals were anesthetized with pentobarbital (30 mg/kg body weight, intraperitoneally), and sacrified by intracardiac perfusion of PBS followed by a solution containing 4% paraformaldehyde and 0.5% glutaraldehyde in phosphate-buffered saline (PBS, pH 7.5). The brains and spinal cords were then removed and postfixed in the same fixative but without glutaraldehyde overnight at 4°C. Transverse sections (40 µm thick) were performed with a Vibratome. For lesioned brains, sections were collected around the site of lesion. For lesioned cords, sections were performed at a distance of 2 mm or 1 cm and 3 cm from the transection site.

Fluorescent immunocytochemistry. Sections were directly transferred into 0.04% sodium borohydride for 10 min. After rinsing in PBS, incubations with the primary antibodies were performed with 0.1% Triton X-100 and 1% bovine serum albumin (BSA) in PBS buffer for 48 h at 4°C under gentle agitation. The antisera used were (1) mouse monoclonal IgM anti-6.17 (Koenig et al. 1988) diluted 1:400, (2) rabbit polyclonal IgG anti-S100 (Sigma) diluted 1:1000, (3) rabbit polyclonal IgG anti-GFAP (Dakopatts) diluted 1:1000, (4) mouse monoclonal IgG anti-vimentin (Sigma) diluted 1:1000. After rinsing in PBS, sections were incubated for 2 h at 4°C with the corresponding secondary antiserum: goat anti-mouse IgM for 6.17, goat anti-rabbit IgF for S100, or goat anti-mouse IgG for GFAP and vimentin, conjugated with rhodamine (for 6.17) or with fluorescein (for S100, vimentin and GFAP). All secondary antibodies were diluted 1:200 in PBS containing 1% BSA and 0.1% Triton X-100.

Series of double immunofluorescent labeling were performed by incubating the sections with two primary antibodies including the monoclonal (IgM) 6.17 and either the polyclonal antisera to GFAP, S100 or the monoclonal (IgG) antiserum to vimentin. After rinsing, sections were incubated with the corresponding secondary antibodies. Some sections were additionally treated for triple immunofluorescent labeling by incubating them 1) 48 h at 4°C with three primary antibodies including the mouse monoclonal IgM anti-6.17, the mouse monoclonal IgG anti-vimentin and the rabbit polyclonal IgG anti-GFAP, and 2) 2 h at 4°C with the corresponding secondary antibodies including a goat anti-mouse IgM conjugated with rhodamine (for 6.17), a goat anti-mouse IgG conjugated with fluorescein (for vimentin) and a goat anti-rabbit IgG conjugated with Cy5 (for GFAP). After rinses in PBS, sections were then mounted in Mowiol (Calbiochem, La Jolla, USA), and observed with a confocal laser scanning microscope (BIORAD MRC-600) equipped with a krypton/argon mixed gas laser. Three laser lines emitting at 488 nm, 568 nm and 647 nm were used for exciting the fluorescein-, the rhodamine- and the Cy5-conjugated secondary antibodies, respectively, providing a minimum overlap of the emission spectra of the three fluorochromes. The specificity of antibodies anti-S100, anti-GFAP and anti-vimentin was certified by the manufacturers. The controls consisted of omitting the primary antibodies or applying each antibody sequentially and then reacting them with the inappropriate secondary antibody.

Peroxidase immunocytochemistry. Sections were placed in 0.04% sodium borohydride for 10 min at room temperature. After several rinses in TRIS buffer saline (TBS, pH 7.5), they were incubated with the mouse monoclonal IgM 6.17 antibody (diluted 1:400) in TBS containing 1% BSA for 48 h at 4°C. Sections were then thoroughly washed in TBS and incubated for 4 h at 4°C under gentle agitation with the secondary goat anti-mouse IgM antibody peroxidase-conjugated diluted 1:500 (Sigma). Finally, the peroxidase product was revealed with 0.05% 3-3'-diaminobenzidine and 0.01% H_2O_2 in the same buffer. Sections were then rinsed in phosphate (0.12 M) buffer (pH 7.5) and postfixed in 1% osmium tetroxide in the same phosphate buffer for 30 min. After a rapid rinse in distilled water, sections were dehydrated in graded ethanol and in acetone before being flat-embedded in Araldite. Ultrathin sections (100 nm thick) were cut with a Microm RMC-MT7 ultramicrotome through brain regions exhibiting strong 6.17-immunostaining. They were collected on copper grids, contrasted with uranyl acetate and examined with a Zeiss 900 electron microscope.

Results

We tested several sets of monoclonal antibodies raised against 6.17, including ascite fluids. The same immunostaining patterns were obtained with all these antibodies in both control and lesioned tissues.

Intact animals

Profiles immunoreactive to 6.17 were observed in the white matter of the diencephalon and spinal cord (Fig. 1).

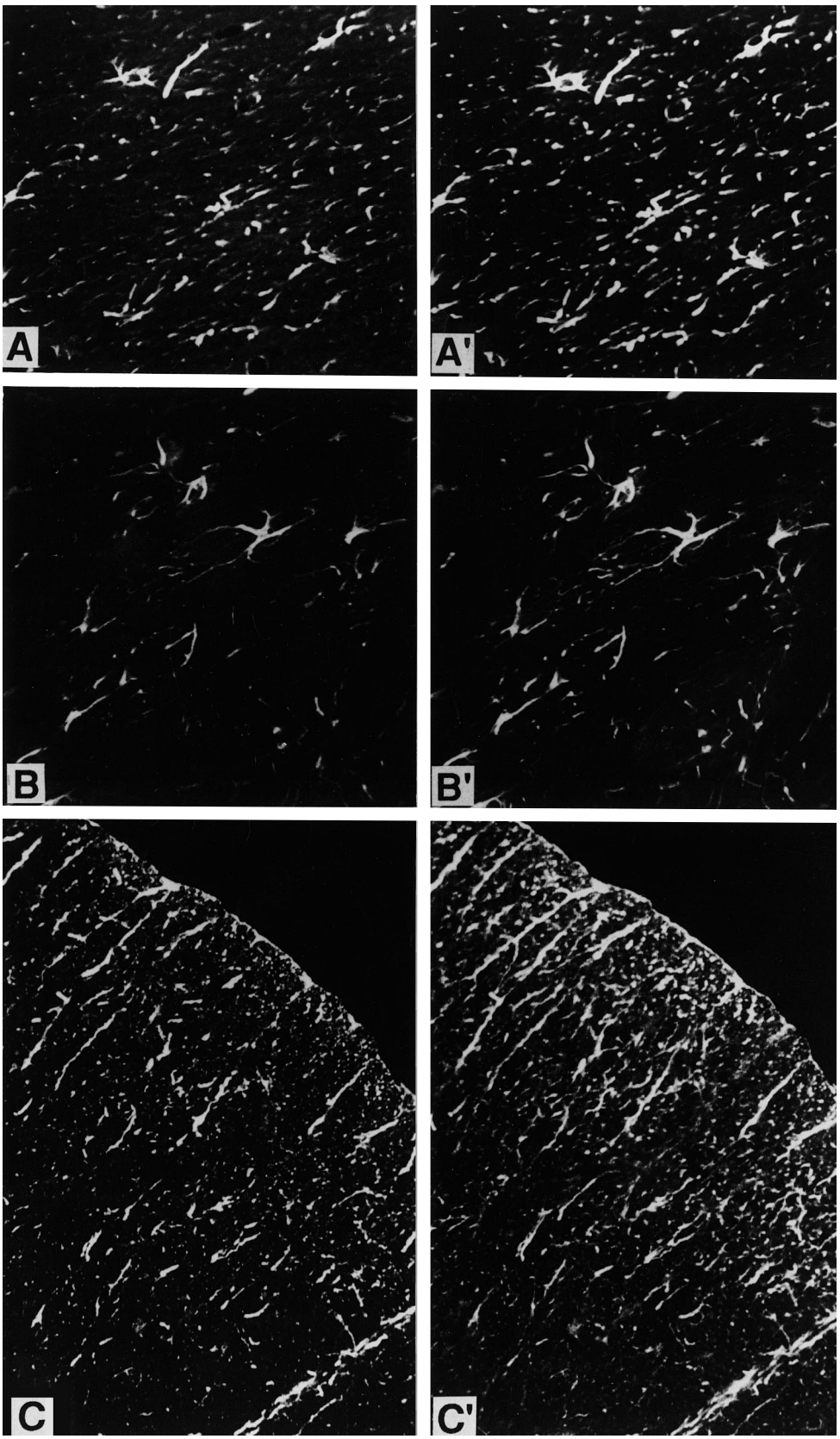

Fig. 1. Double immunostaining for 6.17 (**A-C**) and for GFAP (**A'-B'**) or vimentin (**C'**) in the control CNS white matter. In the fimbria (**A-A'**) as in the corpus callosum (**B-B'**), the majority of the 6.17-IR cell bodies and processes also exhibit GFAP immunostaining. In the white matter of the spinal dorsal horn (**C-C'**), the majority of 6.17-IR cell bodies and radially oriented processes are also vimentin-IR. **A-A'**, **B-B'**: ×420; **C-C'**: ×214

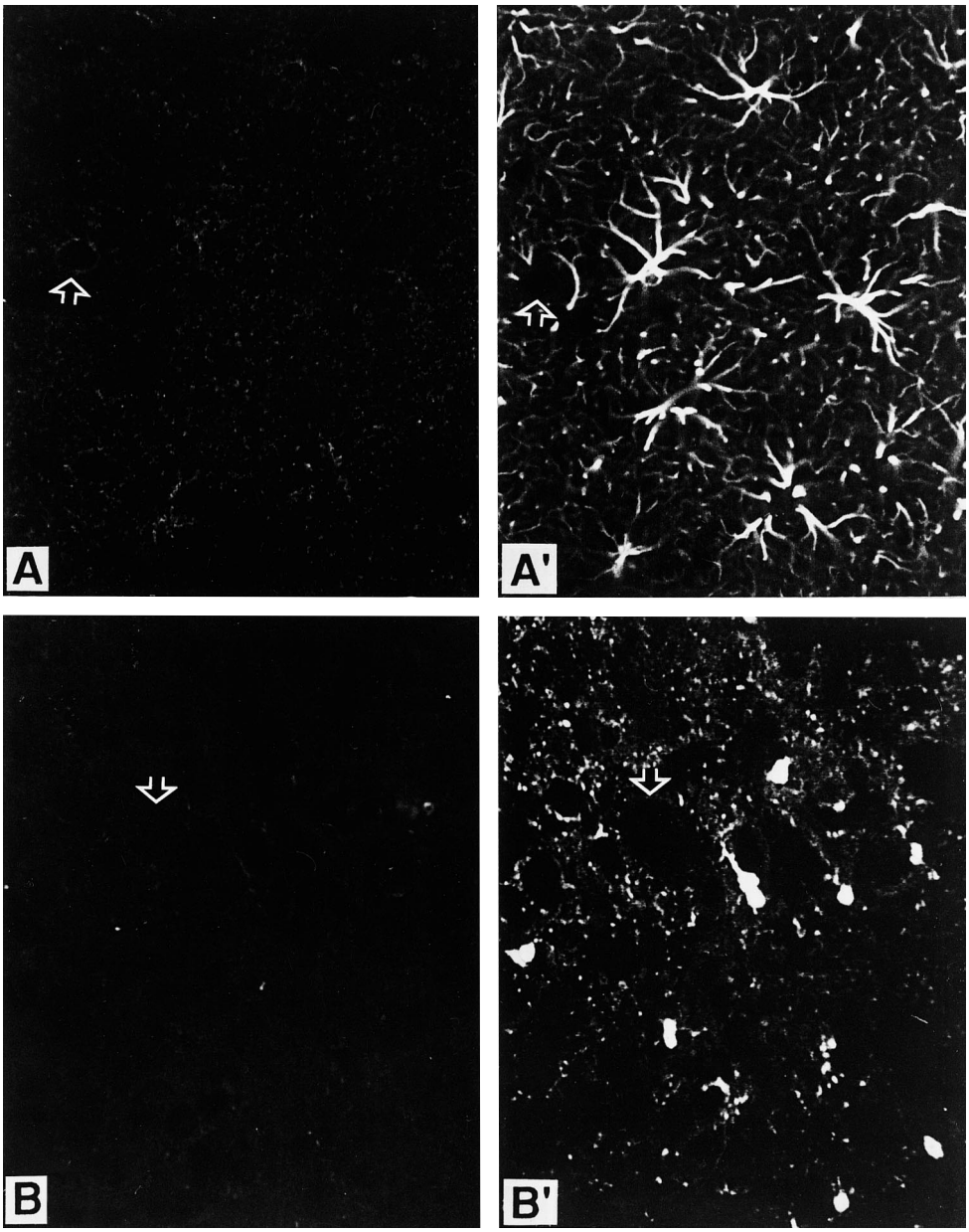

Fig. 2. Double immunostaining for 6.17 (**A-B**) and for GFAP (**A'**) or for S100 (**B'**) in the control CNS grey matter. In the amygdala (**A-A'**) and the spinal cord grey matter (**B-B'**), no 6.17-immunostaining is associated with the astrocytes of the brain grey matter (**A**), identified by their immunostaining to GFAP (**A'**) or S100 (**B'**). The *empty arrows* point to the same blood vessels in **A-A'** and in **B-B'**. **A-A'**, **B-B'**: ×420

In the diencephalon, 6.17-IR structures were detected within the different myelinated axon fasciculi including the optic tract, the fornix (Fig. 1A) and the corpus callosum (Fig. 1B), where they mainly appeared as dispersed profiles exhibiting short processes. In addition, 6.17-immunoreactivity was associated with some ependymocytes, bordering the third ventricle (Fig. 3A), and with processes located close to the dorsal and ventral brain surface. On the other hand, ependymocytes bordering the lateral ventricles were 6.17-negative. In the white matter of the spinal cord, immunoreactivity was observed in radially oriented processes or dot-shaped profiles, whereas no 6.17-IR perikarya could be observed (Fig. 1C). Scarce 6.17-IR processes were also observed in the peri-ependymal region of the grey matter of the spinal cord as well as along the entire spinal cord surface.

Double-immunostaining experiments indicated that all the 6.17-IR structures detected throughout the white matter of the diencephalon or spinal cord were also immunostained for S100, GFAP or VIM. On the other hand, 6.17-immunolabeling was rarely associated with S100- or GFAP-IR astrocytes located in the grey matter of these two CNS regions (Fig. 2). In the diencephalon, 6.17-immunostaining was associated with VIM-IR ependymocytes bordering the third ventricle, whereas the other VIM-IR ependymocytes detected throughout the brain and spinal cord were 6.17-negative (Fig. 3B-B'). In the outer border of the diencephalon and spinal cord, 6.17-IR was associated with profiles immunoreactive to S100, GFAP or VIM that were identified as astrocytes constituting the glia limitans, whereas the VIM-IR and S100- and GFAP-negative meningeal cells located along the brain surface were 6.17-negative.

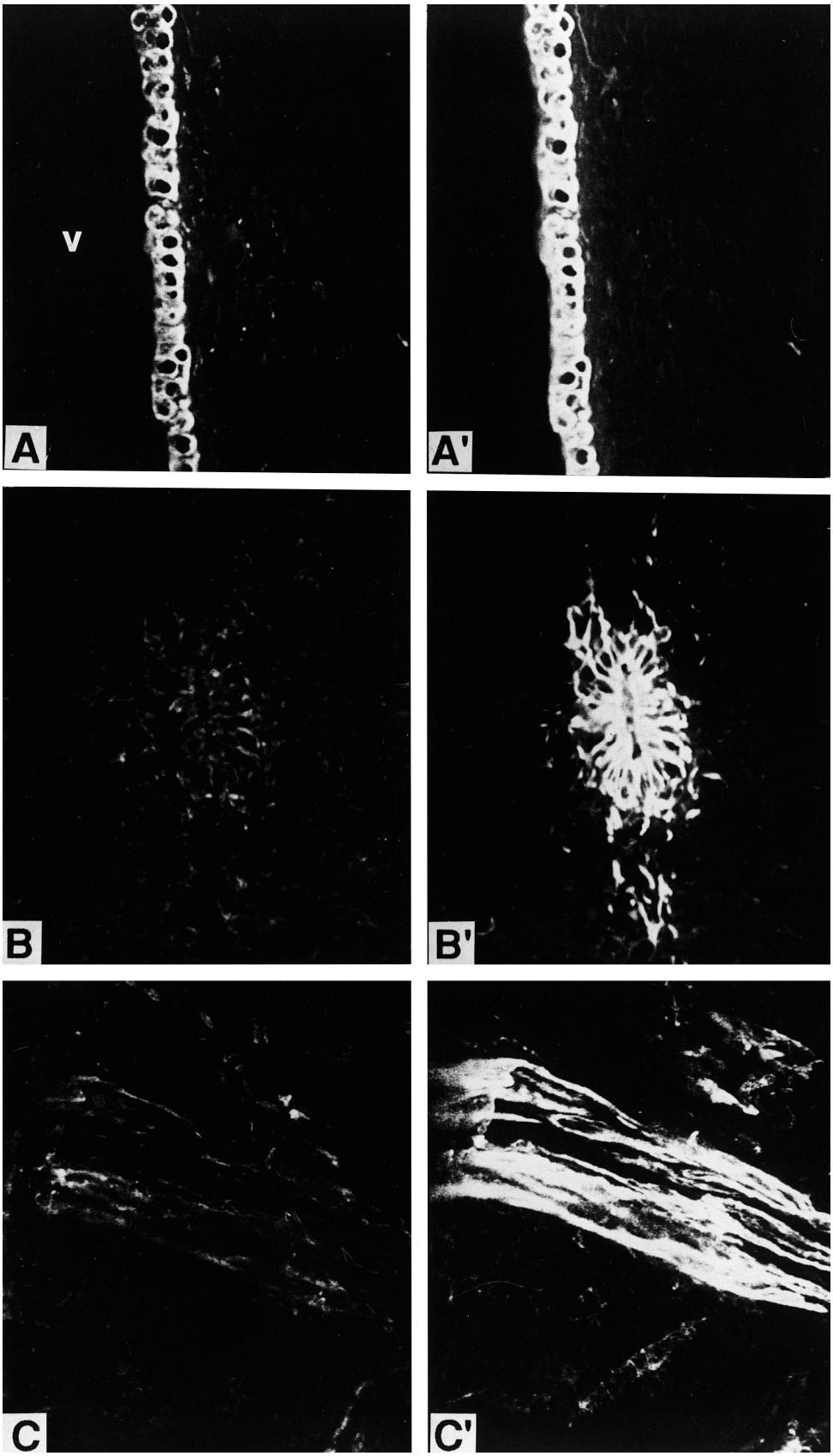

Fig. 3. Double immunostaining for 6.17 (**A**-**C**) and for vimentin (**A**'-**C**') in bordering regions of the control CNS. Intense 6.17-immunostaining is associated with the vimentin-IR ependymocytes bordering the third ventricle (*v*) (**A**-**A**'), whereas only faint if any 6.17-immunoreactivity is associated with the intensely vimentin-IR ependymocytes bordering the central canal in the spinal cord (**B**-**B**') or with the vimentin-IR meningeal cells located along the external brain surface (**C**-**C**'). **A**-**A**', **B**-**B**', **C**-**C**': ×460

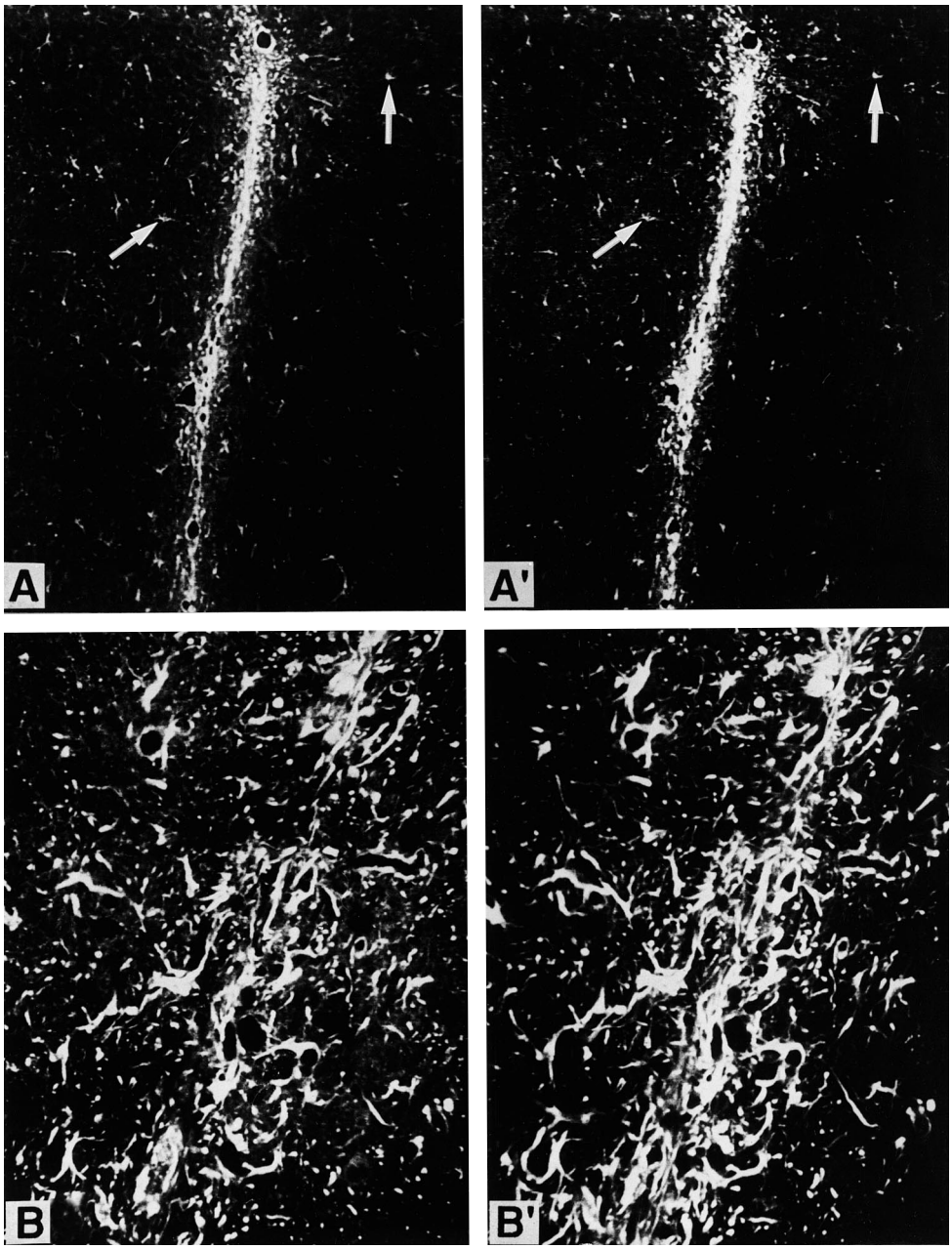

Fig. 4. Double immunostaining for 6.17 (**A-B**) and for GFAP (**A'**) or vimentin (**B'**) in the lesioned diencephalon. Within the lesional scar formed along the entire surgical lesion, reactive astrocytes are immunoreactive to 6.17 and to either GFAP (**A-A'**) or vimentin (**B-B'**). Note that 6.17-immunostaining is also detected within some reactive astrocytes located at some distance from the lesion (*arrows* in **A-A'**) **A-A'**: ×114; **B-B'**: ×420

Lesioned animals

As in control animals, intense 6.17-immunostaining was detected throughout the white matter of both brain and spinal cord sections.

In the brain, the surgical lesion performed by the metallic knife could be detected throughout the cerebral cortex, the hippocampus, the thalamus and the hypothalamus up to its ventral surface. In all the animals examined, a strong 6.17-immunostaining was associated with astrocytic-like profiles located along the entire length of this wound (Fig. 4).

In the spinal cord, numerous 6.17-IR profiles were detected throughout the entire grey matter, including the dorsal (Fig. 5) and ventral horns, and in the intermediate region. The organization of these 6.17-stained profiles appeared very similar to that of the reactive astrocytes observed in this region. 6.17-IR profiles were detected in the grey matter as far as several centimeters from the lesion, although their numerical density appeared lower than in regions located close to the lesion. In double-immunostained sections, all the 6.17-IR structures detected in the lesioned brain or spinal cord were found to exhibit strong immunostaining for either GFAP, S100 or VIM. In the lesioned brain, such reactive astrocytes were detected along the whole length of the surgical cut and always remained restricted close to the lesion (Fig. 4). In the lesioned spinal cord, the sections examined generally did not include the glial scar, due to the difficulty of Vibratome sectioning this region. The surrounding regions

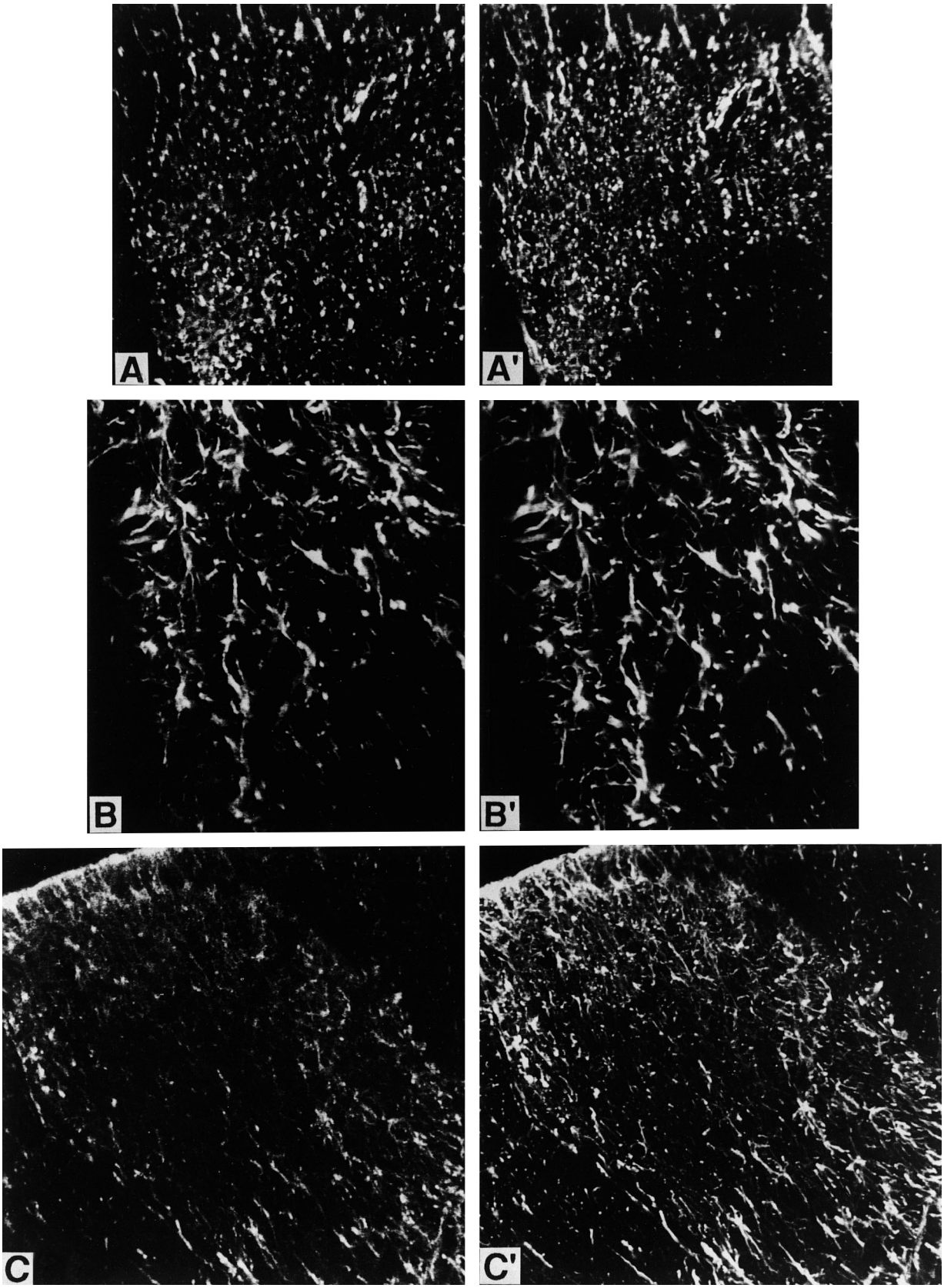

Fig. 5. Double immunostaining for 6.17 (**A-C**) and for vimentin (**A'-C'**) in the lesioned spinal dorsal horn. In spinal cord portions proximal (2 mm) to the lesion (**A-A'** and **B-B'**), the majority of the reactive astrocytes located in the grey matter of the dorsal horn are immunoreactive to both 6.17 (**A-B**) and vimentin (**A'-B'**). In spinal cord regions located more distally from the lesion (3 cm) (**C-C'**), some of the GFAP-IR reactive astrocytes located in the grey matter of the dorsal horn also exhibit 6.17-immunostaining. **A-A'**: ×220; **B-B'**: ×440; **C-C'**: ×220

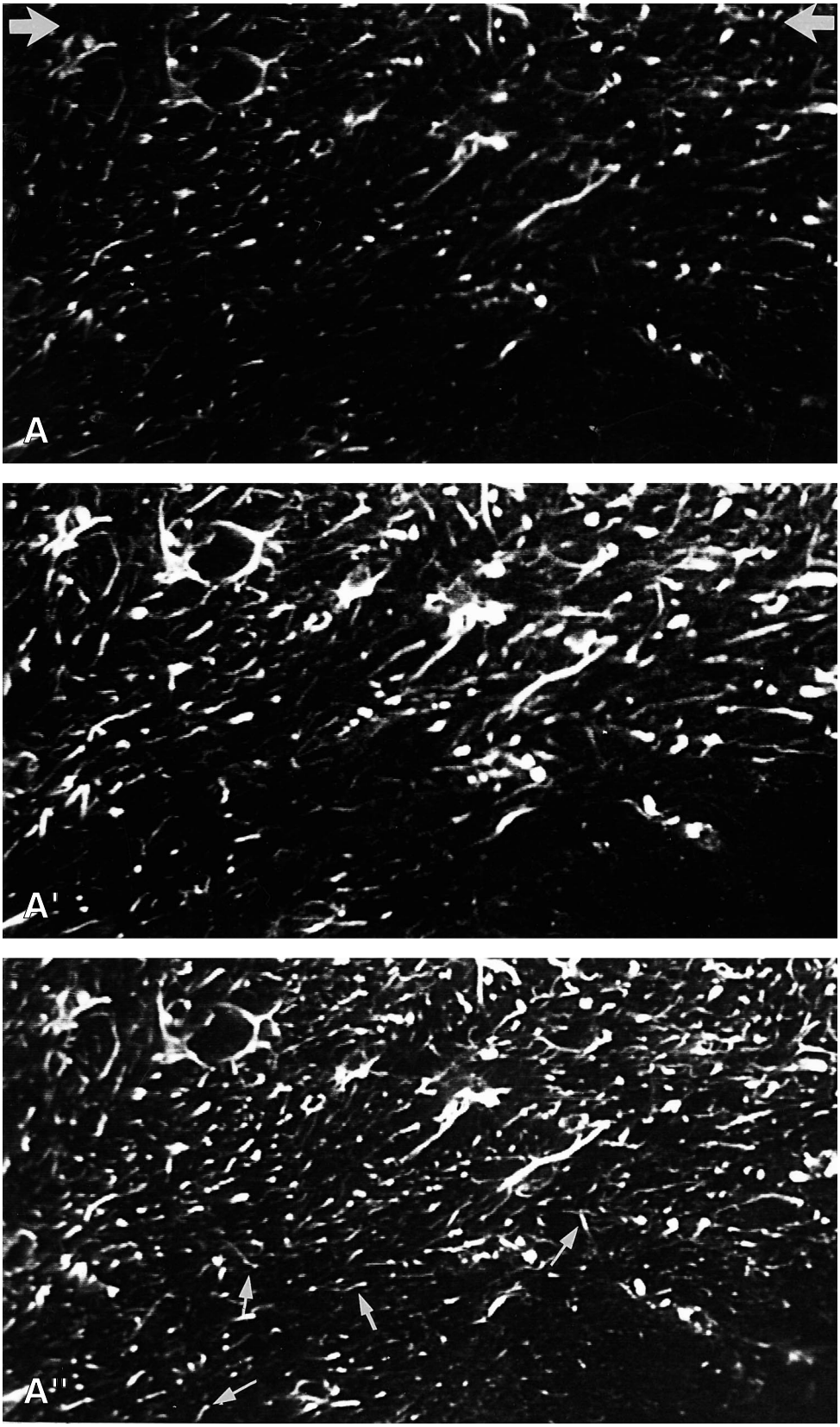

Fig. 6. Triple immunostaining for 6.17 (**A**), vimentin (**A**') and GFAP (**A**") in the lesioned diencephalon. In the vicinity of the surgical cut (*large arrows* in **A**) 6.17-IR is associated with structures immunoreactive to both VIM and GFAP. At a distance of the lesion, thin GFAP-IR processes that appear VIM-negative (*small arrows* in **A**") are devoid of 6.17-IR. **A-A**": ×570

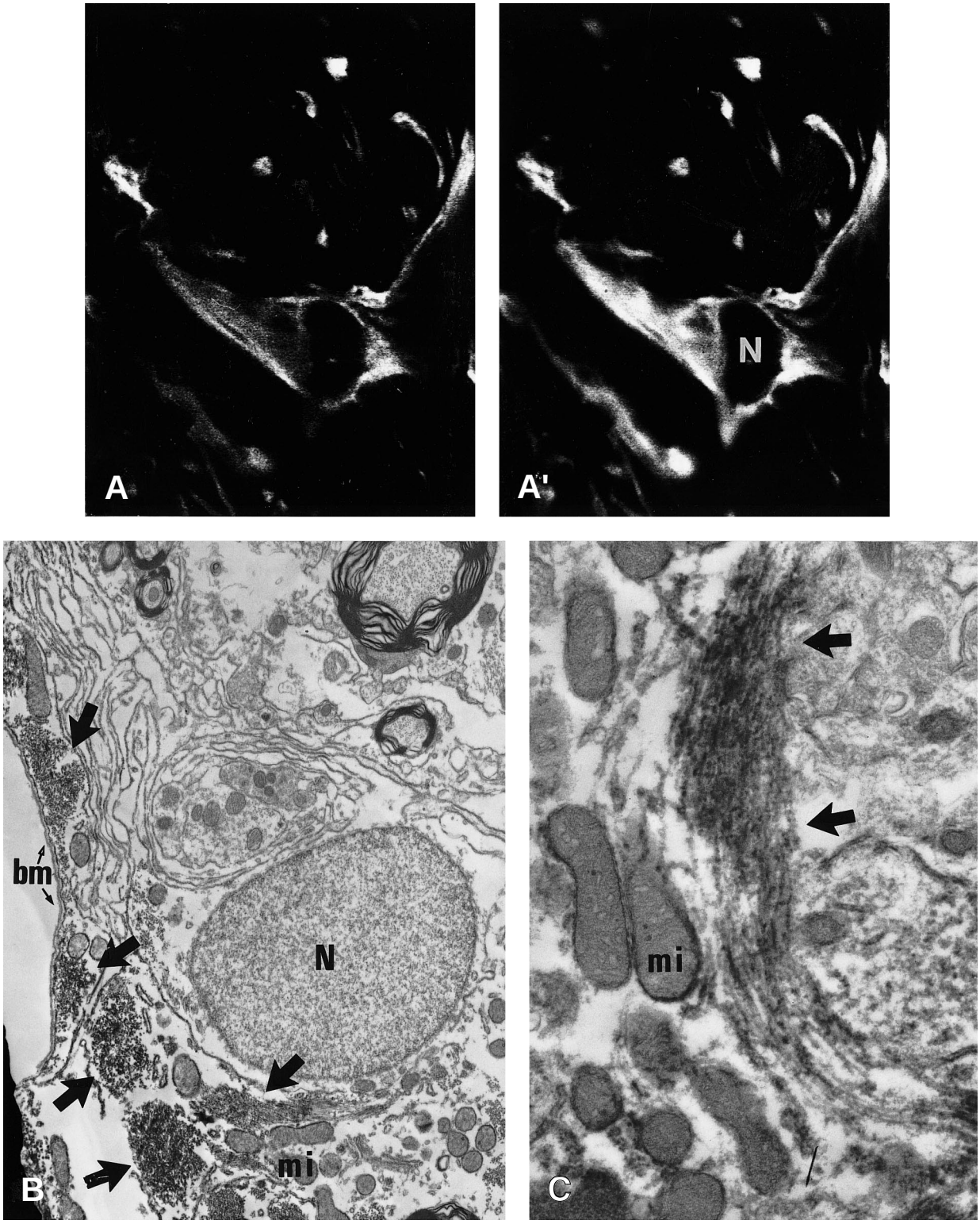

Fig. 7. Intracellular localization of 6.17-immunostaining within reactive astrocytes of the lesioned hypothalamus. **A-A'** Confocal image of a reactive astrocyte double immunostained for 6.17 (**A**) and for VIM (**A'**) observed at high direct magnification. As for VIM-immunostaining, 6.17-immunostaining is mostly localized to the cytoplasm of the cell body and processes. Note that VIM-immunostaining appears more intense and more widely distributed within the cellular structures than 6.17-immunostaining. **B**, **C** Electron micrographs of 6.17-peroxidase immunostaining within reactive astrocytes. The reaction product its mainly associated with bundles of gliofilaments (*arrows*). In **B** note that the border of the lesion is limited by astrocytic processes containing dense 6.17-immunostaining and secreting a basement membrane (*bm*). *mi* Mitochondria; *N* nucleus. **A-A'**: ×1610; **B**: ×9500; **C**: ×25650

examined were thus located at some distance (1–2 mm) from the lesion. They nevertheless contained numerous 6.17-IR profiles that were dispersed throughout the grey matter in both the dorsal (Fig. 5) and ventral horns and were found to also exhibit either GFAP-, S100- or VIM-immunostaining.

In triple-immunostained sections, all the 6.17-IR structures detected in the area closely surrounding the lesion were found to exhibit strong immunostaining for both VIM and GFAP. In this region however, all the GFAP-IR structures that were VIM-negative were also 6.17-negative (Fig. 6). The observation of thin optical sections (less than 0,5 mm) at high magnification indicated that similar to GFAP- or VIM-immunostaining, 6.17-immunostaining was mainly localized to the cytoplasm of labeled structures (Fig. 7A-A'). This was confirmed by electron-microscopic observation which demonstrated that in the reactive astrocytes detected in the lesioned brains or spinal cords, 6.17-immunostaining was essentially associated with bundles of gliofilaments (Fig. 7B, C).

Discussion

The exact nature of the antigen recognized by the Mab 6.17 is still unknown. A first attempt to purify the antigen was made from rat muscle. Tissues were ground in high salt buffer. Proteins insoluble in 0.5 M NaCl and soluble in 0.02 M NaCl were selected, and fractionated by chromatography on hydroxyapatite, DEAE cellulose and HPLC. Unfortunately, the purification, which was followed by Western blotting, failed because of the lack of specificity of the antibody under these conditions. Using the immunocytochemical approach, 6.17-IR was never found to be associated either with muscle or with any other tissue outside of the nervous system. Similarly, 6.17-IR was never found to be associated with cultured spinal cord neurons or pure spinal cord motoneurons. In contrast, a strong 6.17-labeling was associated with cultured Schwann cells. In order to clarify if 6.17 antigen was a component of the basal lamina, we have compared the localization of 6.17-immunoreactivity with that of two major components of the basal lamina on cryostat sections of adult rat lens. Intense labeling of the conspicuous basal lamina around the external surface of lens was obtained with antibodies against heparan sulfate proteoglycan and laminin, whereas no labeling was obtained with the Mab 6.17 (Koenig et al. 1988).

The present data demonstrate that both in the control and lesioned brain and spinal cord, the Mab 6.17 labels astrocytes which exhibit a strong immunoreactivity to GFAP, S100 and VIM. Although the 6.17 epitope is unknown, it is clear that it is not contained by GFAP, S100 or VIM molecules. Indeed, double-labeling experiments showed that throughout the control CNS, a number of intensely GFAP-IR and S100-IR astrocytes were 6.17-negative (Fig. 2A-A'), whereas no 6.17-IR was associated with structures exhibiting strong VIM-IR, such as the ependymocytes bordering the lateral cerebral ventricle or the central canal and the meningeal cells located on the brain surface (Fig. 3B-B').

GFAP and S100 are virtually produced by all mature astrocytes (Eng et al. 1971; Bignami et al. 1972; Matus and Mughal, 1975; Ludwin et al. 1976; Dahl et al. 1981; Schnitzer et al. 1981). The fact that these two markers were found to be associated with the large majority of 6.17-IR structures detected in the intact and lesioned CNS indicates that this antigen is essentially produced by astrocytes. The only exception concerns the localization of 6.17-IR within ependymocytes bordering the third ventricle. Contrasting with GFAP, VIM is absent from most mature astrocytes present in the grey matter throughout the intact CNS (Schnitzer et al. 1981). However, intense VIM-immunoreactivity is detected within the fibrous astrocytes present in the white matter (Schnitzer et al. 1981) and within reactive astrocytes present in the vicinity of a lesion which also exhibit increased expression of GFAP (Calvo et al. 1991; Eddleston and Mucke, 1991). The present finding that in both the intact and lesioned CNS, 6.17-immunoreactivity was systematically associated with astrocytes exhibiting intense VIM-immunoreactivity thus indicates that this protein is specifically produced by astrocytes in which the metabolic machinery is highly stimulated.

Several years ago, Malhotra and colleagues developed a monoclonal antibody (Mab J1-31), which labels reactive astrocytes located close to a surgical lesion site (Malhotra et al. 1984; Singh et al. 1986; Predy et al. 1988; Malhotra et al. 1993). The present labeling patterns obtained with Mab 6.17 appear therefore very similar to that obtained by Malhotra and colleagues with the Mab J1-31. However, after a lacerated-type surgical lesion of the spinal cord, it has been shown that Mab J1-31 recognizes reactive astrocytes in the immediate vicinity of the lesion, whereas reactive astrocytes located at some distance were not immunostained (Predy et al. 1988; Malhotra et al. 1993). In contrast, Mab 6.17 labels reactive astrocytes that are both close and at a distance from a surgical lesion. This was the case in the lesioned spinal cord where 6.17-IR astrocytes were detected as far as several centimeters from the edge of the lesion. It can thus be assumed that, in addition to the reactive astrocytes that form the glial scar, 6.17 antigen is also expressed by reactive astrocytes that develop as a consequence of axotomy. It is thus likely that 6.17 and J1-31 represent distinct molecules that are overexpressed by reactive astrocytes. The present data clearly show that, within reactive astrocytes, the 6.17 antigen is mainly associated with bundles of gliofilaments. Its role in the various metabolic and morphologic modifications that affect these astrocytes warrants further investigation.

Acknowledgements. The authors acknowledge Françoise Sandillon and Ariane Legrand for their excellent technical assistance, Jean-René Teilhac for artwork and Dr. Brian P. Lockhart for critical reading of the manuscript.

References

Bignami A, Dahl D (1976) The astroglial response to stabbing. Immunofluorescence studies with antibodies to astrocyte-specific protein (GFA) in mammalian and submammalian vertebrates. Neuropathol Appl Neurobiol 2:99–111

Bignami A, Eng L, Dahl D, Uyeda C (1972) Localization of the glial fibrillary acidic protein in the astrocytes by immunofluorescence. Brain Res 43:429–435

Calvo JL, Carbonell AL, Boya J (1991) Co-expression of glial fibrillary acidic protein and vimentin in reactive astrocytes following brain injury in rats. Brain Res 566:333–336

Dahl D, Rueger DC, Bignami A (1981) Vimentin, the 57,000 molecular weight protein of fibroblast filaments, is the major cytoskeletal component in immature glia. Eur J Cell Biol 24: 191–196

Eddleston M, Mucke L (1993) Molecular profile of reactive astrocytes – implication for their role in neurologic disease. Neuroscience 54:15–36

Eng LF, Vanderhaeghen JJ, Bignami A, Gerstl B (1971) An acidic protein isolated from fibrous astrocytes. Brain Res 28: 351–354

Hatten ME, Liem RKH, Shelanski ML, Mason CA (1991) Astroglia in CNS injury. Glia 4:233–243

Koenig J, Chapron J, Vigny M (1988) Does the Schwann cell synthesize a molecule concentrated at the neuromuscular synapse? Neurosci Lett 89:265–270

Ludwin SK, Kosek JC, Eng LF (1976) The topographical distribution of S-100 and GFA proteins in the adult rat brain: an immunohistochemical study using horseradish peroxidase-labeled antibodies. J Comp Neurol 165:197–208

Malhotra SK, Wong F, Cumming P, Ross SD, Snitka TK, Manickavel V, Warren KG, Jeffrey V (1984) A monoclonal antibody for cytoskeletal antigenic determinant(s) distinguishable from glial fibrillary acidic protein in astrocytes. Microbios Lett 26: 151–157

Malhotra SK, Svensson M, Aldskogius H, Bhatnagar R, Das GD, Shnitka TK (1993) Diversity among reactive astrocytes: proximal reactive astrocytes in lacerated spinal cord preferentially react with monoclonal antibody J1-31. Brain Res Bull 30: 395–404

Matus A, Mughal S (1975) Immunohistochemical localization of S-100 protein in brain. Nature 258:746–748

Pixley SKR, De Vellis J (1984) Transition between immature radial glia and mature astrocytes studied with a monoclonal antibody to vimentin. Dev Brain Res 15:201–209

Predy R, Malhotra SK, Das GD (1988) Enhanced expression of a protein antigen (J1-31 antigen, 30 kilodaltons) by reactive astrocytes in lacerated spinal cord. J Neurosci Res 19:397–404

Schiffer D, Giordana MT, Micheli A, Giaccone G, Pezzotta S, Mauro A (1986) Glial fibrillary acidic protein and vimentin in the experimental glial reaction in the rat. Brain Res 374:110–118

Schiffer D, Giordana MT, Cavalla P, Vigliani MC, Attanasio (1993) A Immunohistochemistry of glial reaction after injury in the rat: double stainings and markers of cell proliferation. Int J Dev Neurosci 11:269–280

Schnitzer J, Franke WW, Schachner M (1981) Immunocytochemical demonstration of vimentin in astrocytes and ependymal cells of developing and adult mouse nervous system. J Cell Biol 90:435–447

Singh R, Singh B, Malhotra SK (1986) A new “marker” protein for astrocytes. Biosci Rep 6:73–79

Takamiya Y, Kohsaka S, Toya S, Otani M, Tsukada Y (1988) Immunocytochemical studies on the proliferation of reactive astrocytes and the expression of cytoskeletal proteins following brain injury. Dev Brain Res 38:201–210