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# Coexpression of vimentin and glial fibrillary acidic protein in astrocytes of the adult rat optic nerve

J.L. Calvo, A.L. Carbonell and J. Boya

*Department of Histology, Faculty of Medicine, University Complutense, Madrid (Spain)*

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The localization of vimentin (VIM) and glial fibrillary acidic protein (GFAP) was studied immunohistochemically in adult rat optic nerve. Consecutive Epon semithin tissue sections were immunostained respectively for VIM or GFAP. With this method, both antigens were detected in consecutive sections of the same cell. According to our results, most astrocytes in the adult rat optic nerve showed coexpression of VIM and GFAP.

Two intermediate filament (IF) proteins have been described in astrocytes. GFAP is considered a characteristic marker for mature astrocytes[1,2,7,10]. Vimentin (VIM), initially described as characteristic for mesenchymal cells, has been detected later on in different miscellaneous cells. In the central nervous system VIM has been detected in the radial glia, immature astrocytes and ependymal cells[2,5,12]. As reflected by the shared immunoreactivity for VIM and GFAP, maturing astrocytes show a transition pattern in the expression of both antigens. The immunoreactivity for VIM vanishes, while appears and increases for GFAP. In rodents, such transition takes place within the first two postnatal weeks. Thereafter, astrocytes in the adult nervous tissue remain $GFAP^+$ and $VIM^-$ (ref. 9).

Former beliefs about the expression of a single IF protein per cell type, broaden to admit that the coexpression of two or more proteins in the same cell is not exceptional[16]. Respect to astrocytes, GFAP and VIM have been co-detected in immature[9,17], mature[12,13], and reactive astrocytes[11,14].

In the course of immunocytochemical studies on the rat optic nerve, we happened to observe a brisk cell positivity for VIM. These cells showed morphological features and a distribution similar to that observed when stained for GFAP. The coexistence of VIM and GFAP has been demonstrated biochemically in the optic nerve of the adult rat[5] and other mammals[6]. However, the cellular localization of both proteins cannot be assessed with these methods. An overlapping immunohistochemical pattern was described on cryostat sections of the adult rat optic nerves immunostained for both VIM and GFAP[13]. Technical limitations, particularly the thickness of tissue sections, only allowed an approximative comparison.

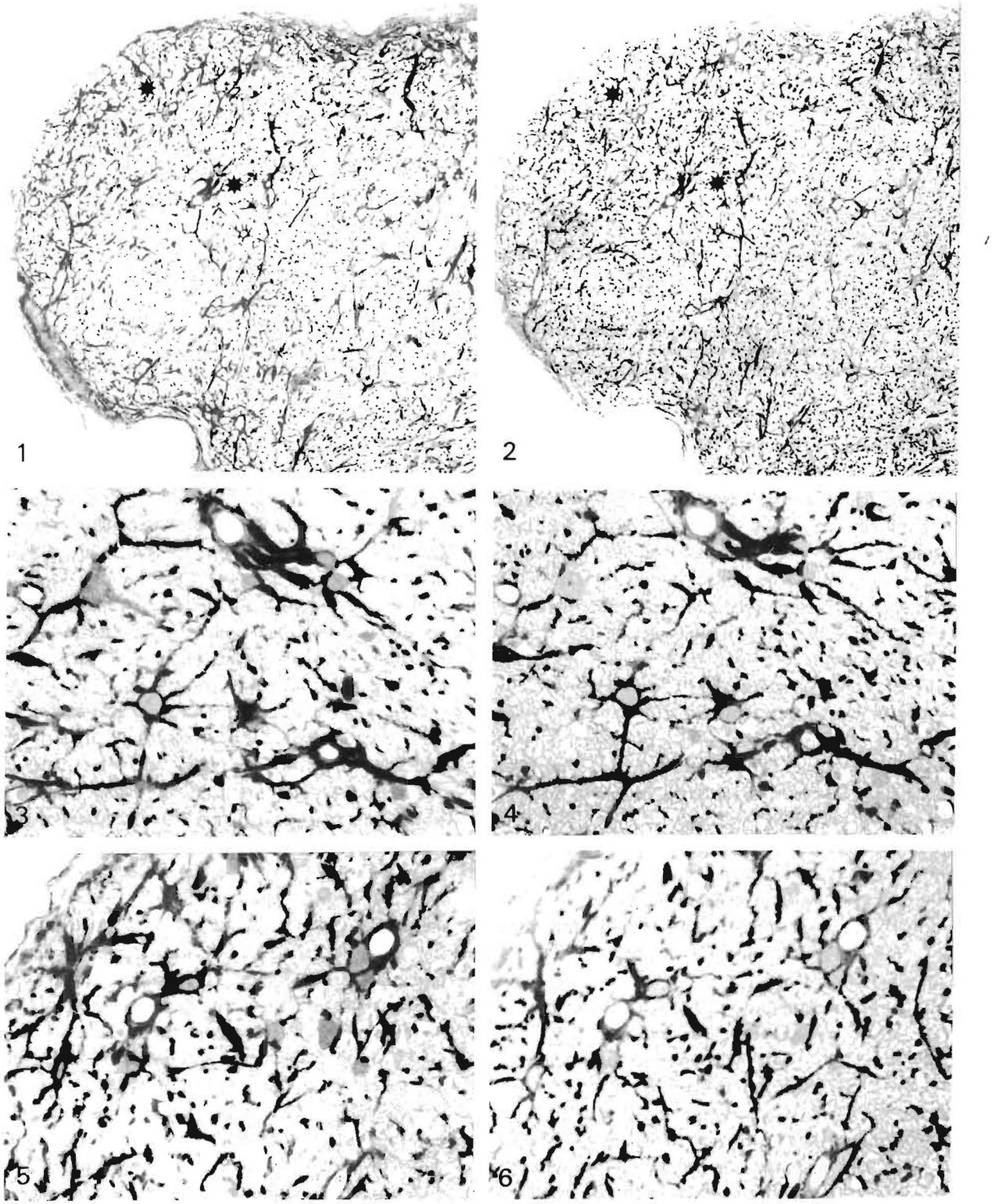
In our work, we have immunostained for VIM and GFAP, semithin sections (0.5 $\mu$m) of Epon-embedded rat optic nerves, which allow to study the same cell in consecutive sections. In this way, we could determine accurately coexpression phenomena. Our results demonstrate that most astrocytes in the rat optic nerve coexpress VIM and GFAP in adult animals.

Six Wistar rats 6 months old were perfusion-fixed with 2% glutaraldehyde, 2% paraformaldehyde in 0.1 M phosphate buffer pH 7.4. The optic nerves were embedded in Epon without previous osmication. Tissue samples were oriented to reveal transverse sections of the optic tracts. Sets of 10 semithin (0.5 $\mu$m) sections were prepared from each tissue block. Sections were 'etched' for 20 min with sodium ethoxide, incubated in non-immune swine serum and immunostained alternatively for VIM and GFAP. VIM was detected by an indirect immunoperoxidase method[15], using a monoclonal anti-human VIM antibody as the first reagent. A peroxidase–antiperoxidase (PAP) technique[15] was used to detect GFAP using a polyclonal anti-bovine GFAP antiserum. The substitution of the primary antibody for non-immune swine serum resulted in an absence of immunostaining in both methods used. Positive controls were processed at the same time following the respective immunostaining method. After the immunostaining, sections were slightly counterstained with Toluidine blue.

All sections studied showed an overlapping immunostaining pattern for both VIM and GFAP (Figs. 1 and 2).

*Correspondence:* J. Boya Vegue, Department of Histology, Faculty of Medicine, University Complutense, 28040 Madrid, Spain.

Numerous immunostained cell processes were seen throughout transverse sections of the optic nerve, somewhat concentrated at the nerve periphery (Figs. 1 and 2). Positivity for both antigens was disclosed for the same




Figs. 1 and 2. Low-power magnification of consecutive semithin tissue sections of the rat optic nerve immunostained for VIM (Fig. 1) and GFAP (Fig. 2). The immunostaining pattern is very similar in both figures. Asterisks: areas shown magnified in Figs. 3–6. ×95.
Figs. 3 and 4. High-power view of the central area of the rat optic nerve immunostained for VIM (Fig. 3) and for GFAP (Fig. 4). Overlapping morphological appearance of positive starshaped astrocytes and numerous positive cell processes in both figures. ×670.
Figs. 5 and 6. High-power view of the peripheral area of the rat optic nerve immunostained for VIM (Fig. 3) and for GFAP (Fig. 4). Coincidental pattern of immunostaining. ×670.

astrocyte in consecutive sections (Figs. 3 and 4). Glial cell processes positive for both antigens were frequently seen cross sectioned, appearing as minute positive dots (Figs. 5 and 6).

Some astrocyte cell processes appeared stained only for one of the antigens. In most cases, they corresponded to processes sectioned longitudinally which may only appear in one of the sections (Figs. 5 and 6). Occasionally, some cell body profiles with astroglial features only showed immunostaining for an antigen, commonly VIM. These somata accounted for less than a 5% of all immunostained cell bodies in a transverse section of the optic nerve.

Our results demonstrate the coexpression of both VIM and GFAP in the adult rat optic nerve. These results confirm previous reports based on biochemical[5] and immunohistochemical techniques[13].

The use of semithin sections allows us to demonstrate the presence of both IF proteins in the majority of the optic nerve astrocytes in adult rats. Accordingly, this coexpression can be viewed as a generalized phenomenon rather than as an occasional occurrence. Only a small fraction of the astrocytes of the optic nerve in adult rats appear to express only VIM or GFAP. With the techniques employed, we cannot discern whether these cells actually contain only one of the IF proteins or if the concentration of the second component is under the detection level of our techniques. The presence of immunohistochemically different astrocyte types has been described in the optic nerve[8]. Perhaps, the difference we have highlighted respect to the expression of VIM and GFAP are one more signal of the heterogeneity of these astrocytes.

The coexpression of VIM and GFAP in the adult rat optic nerve could be interpreted as a sign of cell immaturity or incomplete maturity. Certainly, along the transition VIM-GFAP, maturing astrocytes may coexpress both proteins[9,17]. The coexpression has been also described as a generalized phenomenon in fetal tissues[16]. However, our study was developed in adult animals. In addition, known ultrastructural features of astrocyte immaturity such as the presence of microtubuli have not yet been described in the astrocytes of the optic nerve.

In the adult, the coexpression of VIM and GFAP has been determined in astrocytes in contact with the nerve tissue surface, such as Bergmann cells, and astrocytes in large myelin bundles[5,12,13]. Coexpression is also common in reactive astrocytes surrounding wounds and lesions[11,14]. Cell processes of astrocytes could be in contact with pial elements downward proliferated into the wound, as has been demonstrated ultrastructurally[4]. We consider that the coexpression of VIM and GFAP in the astrocytes of the adult rat optic nerve is not related to immaturity. Rather, it could reflect a phenotypical adaptation of astrocytes to peculiar environmental conditions such as contact with pial envelops, relationship with myelin fibers or other factors.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