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# Coexpression of glial fibrillary acidic protein and vimentin in astrocytes within the newly-formed subarachnoid space after an experimental brain wound

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## Abstract

The immunohistochemical distribution of glial fibrillary acidic protein (GFAP) and vimentin (VIM) has been studied in the glial cords appearing within the newly-formed subarachnoid space after an experimental brain wound in rats. Consecutive Epon-embedded semithin tissue sections were immunostained alternatively for GFAP and VIM. This method allowed the demonstration of coexpression of both antigens in adjacent sections of the glial cords. In contrast, regenerated meningeal cells exclusively exhibited VIM immunoreactivity.

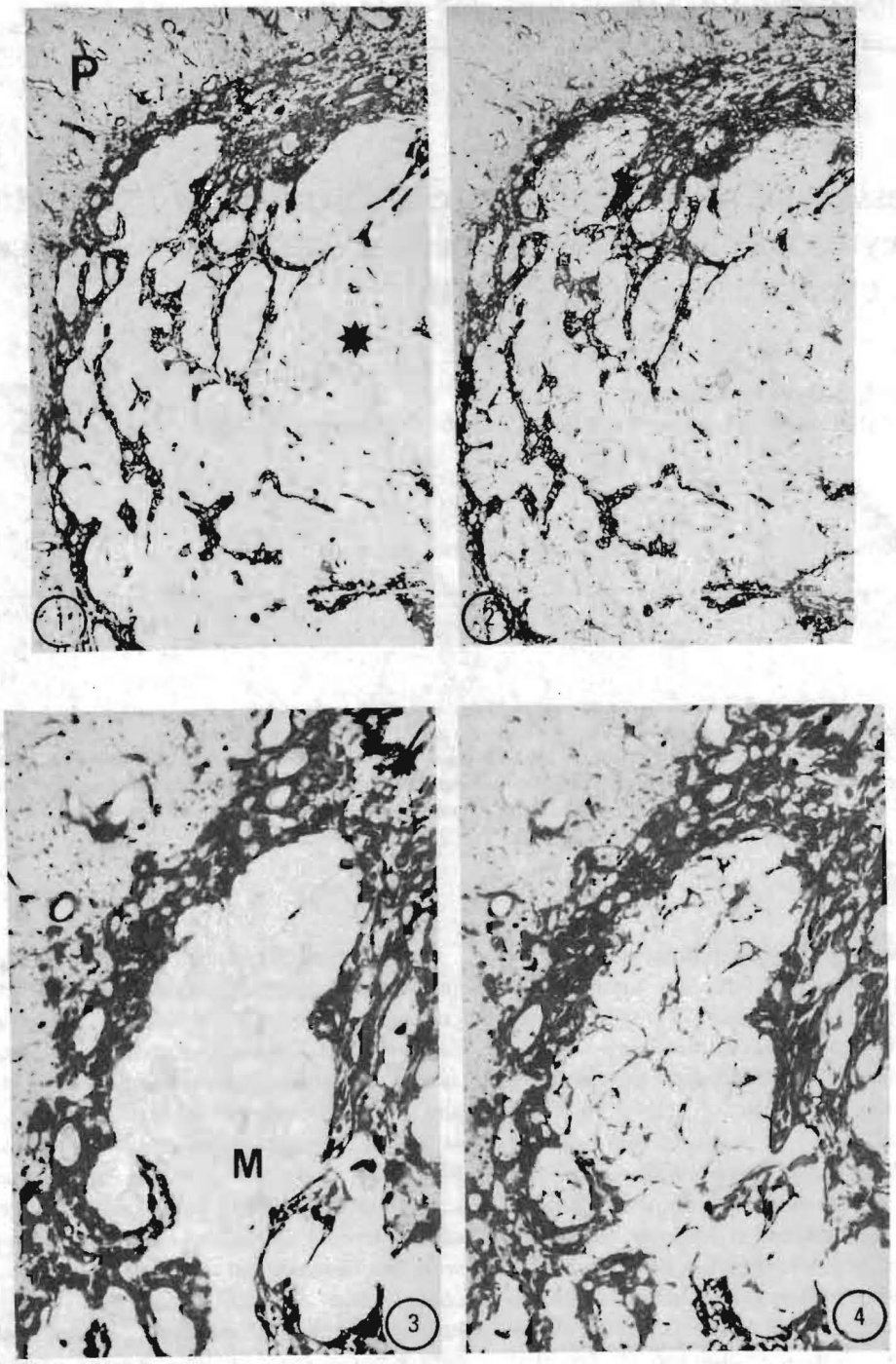
## Introduction

The presence of astrocyte cell processes entering the meningeal territory in traumatic brain lesions has been described by Penfield (1927) in his classic studies using metallic impregnations. However, the presence of astrocyte cell processes and somata in meningeal territory has been reported ultrastructurally only in experimental allergic encephalitis (Moore and Raine 1986) and after a wide cerebral wound showing both meningeal and glia limitans regeneration (Carbonell and Boya 1988). To date, little attention has been rendered to the expression of intermediate filament proteins in these heterotopic glial elements of the subarachnoid space. Thus, immunopositivity for glial fibrillary acidic protein (GFAP) has been described (Moore and Raine 1986), but other immunocytochemical glial markers such as vimentin protein (VIM), characteristic for immature and reactive astrocytes (Schnitzer et al 1981; Schiffer et al 1986; Takamiya et al 1988), have not been studied.

Recent previous studies from our group using immunohistochemical techniques to detect the coexpression of GFAP and VIM have been performed on consecutive Epon-embedded semithin sections of normal optic nerves (Calvo et al 1990), and 5-day old experimental brain wounds (Calvo et al 1991). The extreme thinness of these sections (0.5 $\mu$m) makes it possible to compare the immunostaining for both glial antigens in consecutive sections of the same cell. In the present work we have applied this method to demonstrate coexpression of both GFAP and VIM in the heterotopic glial elements located in the subarachnoid space in fully scarred experimental brain wounds.

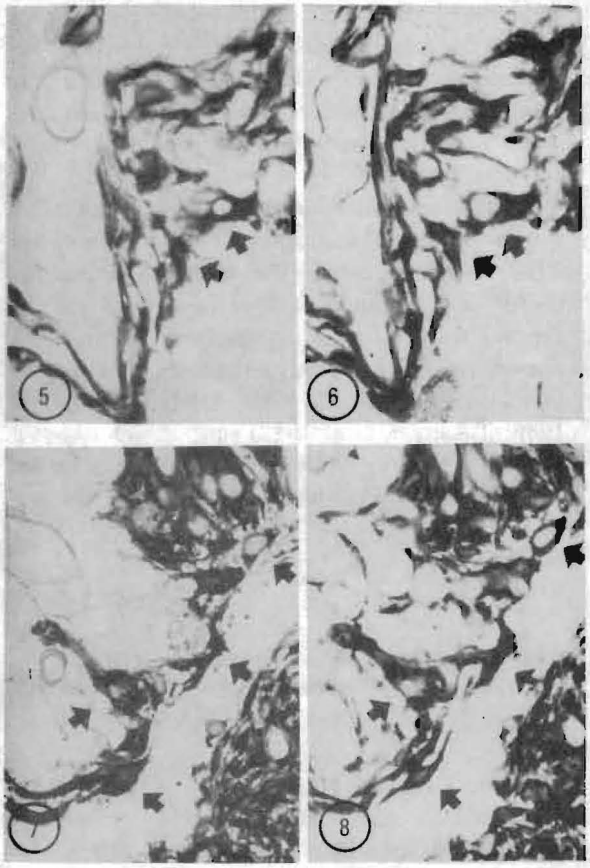
## Materials and methods

Five Wistar rats aged 4 months were used for our study. After drilling the skull on a point located 5 mm behind the bregma and 3 mm to the right of




**Figs. 1 and 2.** Low-power magnification of consecutive semithin sections showing the newly-formed subarachnoid space (asterisk) and the solid glial cords displaying immunopositivity for GFAP (Fig. 1) and VIM (Fig. 2). P = nervous parenchyma. x165.

**Figs. 3 and 4.** Detail of Figs. 1 and 2. The loose meningeal network (M) is immunonegative for GFAP (Fig. 3) and immunopositive for VIM (Fig 4). x470.




**Figs. 5-8.** High-power magnification of astrocyte somata close to the glial cords showing the expression of both, GFAP (Figs. 5 and 7) and VIM (Figs. 6 and 8), in consecutive sections of the same cell profiles (arrows). x700.

the midline, a brain stab wound 3 mm deep and 1 mm thick was made on the right cerebral hemisphere. The wounds obtained left wide cavities that were very suitable for the study of the regeneration of glia limitans and meninx (Carbonell and Boya 1988). Animals were killed 30 days after the lesion. By then, the scarring process is known to be concluded and the relationships between meninx and glia limitans fully established (Carbonell and Boya 1988). Under deep ether anesthesia, rats were perfused with 2% glutaraldehyde and 2% paraformaldehyde in 0.1 M, pH 7.4 phosphate buffer. Small blocks of tissue were obtained including the walls of the needle path. Such samples were embedded in Epon without postfixation in osmium tetroxide, and oriented with the wound track perpendicular or parallel to the section plane. Sets of 10 consecutive semithin sections 0.5 $\mu$m thick were obtained from each block with a LKB Ultramicrotome. Sections were "etched" for 20 min with sodium ethoxide, incubated in non-immune swine serum and immunostained alternatively for GFAP and VIM. A peroxidase-antiperoxidase (PAP) technique (Taylor 1986) was used to detect GFAP using a polyclonal anti-bovine GFAP antiserum (Dakopatts, Denmark). VIM was detected by an indirect immunoperoxidase method (Taylor 1986), using a monoclonal anti-human VIM antibody (Dakoppats, Denmark) as the first reagent. The substitution of the primary antibody for non-immune swine serum resulted in an absence of immunostaining. Positive controls were processed at the same time following the respective immunostaining methods.

## Results

In all cases, the solid glial cords within the newly-formed subarachnoid space displayed coexpression of both glial antigens, GFAP and VIM, as was stated in consecutive sections (Figs. 1 and 2). Frequently, connections of glial cords with the newly-formed glia limitans were found (Figs. 1 and 2). Delicate star-shaped meningeal cells negative for GFAP and positive for VIM were seen filling up the hollow of the lesion and forming a loose reticular network (Figs. 3 and 4). Occasionally, scattered bizarre astrocytes showing wide somata and thick cell processes were noted within this meningeal network. These glial cells were very close to the glial cords and coexpressed both GFAP and VIM (Figs. 5-6 and 7-8).

## Discussion

Our results demonstrate that solid glial cords appear in the newly-formed subarachnoid space at the lesion cavity after an experimental brain wound. These solid cords consist of astrocyte somata and cell processes and can be considered expansions of the newly-formed glia limitans. The glial nature of these cords had been demonstrated through their immunopositivity for GFAP and their characteristic ultrastructure (Moore and Raine 1986; Carbonell and Boya 1988). However, our study is the first in describing the coexpression of both astrocytic antigens, GFAP and VIM, in these structures.

According to our previous studies (Carbonell and Boya 1988) glial cords were fully differentiated at the end of the first postlesional month. Therefore, the present investigation has been performed 30 days after the brain lesion. Ultrastructurally (Carbonell and Boya 1988), these formations consisted of bundles of astrocyte cell processes stuffed with glial filaments and wrapped up by a basal lamina. Astrocyte somata could also be detected making up the glial cords (Carbonell and Boya 1988). Moore and Raine (1986) named glial elements in the subarachnoid space as "heterotopic astrocytes" or "leptomeningeal gliosis". However, in our view, this terminology appears misleading since these elements are an intrinsic component of the newly-formed glia limitans rather than being alien to it. This is reinforced by the presence of a complete basal lamina separating these glial elements from the regenerated meningocytes. In addition, it was also possible to demonstrate ultrastructurally the continuity of the glia limitans with these formations (Carbonell and Boya 1988).

Glial cords appearing in the newly-formed subarachnoid space should be "de novo" formations produced by the proliferation and migration of reactive astrocytes at the lesion border entering the cavity of the wound. The following facts support this view: a) continuities of the glial cords and the glia limitans are found; b) the immunostaining pattern of the glial cords (i. e., positive for GFAP and VIM) is similar to the regenerated glia limitans (Pixley and De Vellis 1984; Schiffer et al 1986; Abnet et al 1991); c) experimentally, migratory ability has been described for astrocytes (Emmett et al 1991); d) in earlier stages of the evolution of the lesion, we were unable to detect ultrastructurally neither glial cords within the magma of necrotic tissue nor among the macrophage clusters bordering the lesion. Furthermore, glial cords appeared simultaneously and never before the establishment of the new glia limitans (Carbonell and Boya 1988).

As has been described elsewhere (Pixley and De Vellis 1984; Schiffer et al 1986; Takamiya et al 1988; Calvo et al 1991), reactive astrocytes recover their ability to synthesize VIM. VIM is considered an immunocytochemical marker for immature astrocytes. Moreover, VIM immunoreactivity is lost during the normal astroglial development and only persists in astrocytes located in specific areas (Schnitzer et al 1981; Shaw et al 1981; Calvo et al 1990).

In our study, the coexpression of GFAP and VIM in the glial cords of the subarachnoid space may be due either to a certain degree of immaturity of the astrocytes that make up these structures or to singular environmental conditions close to the glial cords i. e., contact with meningeal cells and/or cerebrospinal fluid.

This contact with the meninx may be responsible at least for the maintenance of the positivity for VIM. Actually, while the immunostaining for GFAP is seen in a well-defined band of the nervous tissue surrounding the brain lesion, VIM immunoreactivity keeps restricted to the glia limitans (Pixley and De Vellis 1984; Schiffer et al 1986; Takamiya et al 1988) and, as described in the present work, to the solid glial cords in the newly-formed subarachnoid space.

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