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Astrogliosis in Culture: III. Effect of Recombinant Retrovirus Expressing Antisense Glial Fibrillary Acidic Protein RNA

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Injury to the central nervous system (CNS) either from trauma or due to demyelinating/degenerating diseases results in a typical response of astrocytes, termed astrogliosis. This reaction is characterized by astrocyte proliferation, extensive hypertrophy of nuclei, cell body, and cytoplasmic processes and an increase in immunodetectable glial fibrillary acidic protein (GFAP). GFAP accumulation may cause a physical barrier preventing the reestablishment of a functional environment. Our studies have aimed at modulating astrogliosis by inhibiting or delaying GFAP synthesis in damaged and reactive astrocytes. The present study investigates the use of a recombinant retrovirus expressing antisense GFAP RNA in controlling the response of mechanically injured astrocytes. A 650 bp fragment from the coding region of mouse GFAP cDNA was cloned in the antisense orientation under the control of long terminal repeat (LTR) promoter of Moloney murine leukemia virus. Increase in GFAP as detected by immunocytochemical staining in injured astrocytes was inhibited by treatment with retrovirus expressing antisense GFAP RNA. Also, astrocytes at the site of injury in these scratched cultures did not show cell body hypertrophy compared to control cultures. These observations demonstrate that the increase in GFAP at the site of injury can be inhibited using retroviral treatment and indicate the potential of retrovirus-mediated gene transfer in modulating scar formation in the CNS in vivo. These studies also shed light on the role of GFAP in maintaining the morphology of astrocytes. © 1994 Wiley-Liss, Inc.

Key words: astrocytes, CNS injury, in vitro model, gliosis

INTRODUCTION

Gliosis is the most frequently observed reaction of astrocytes at the site of injury following trauma to the central nervous system (CNS). It is also associated with CNS demyelinating and degenerating diseases such as multiple sclerosis and Alzheimer's disease (Reier, 1986; Eng, 1988a,b; Eng et al., 1992). Astrogliosis is characterized by hyperplasia, hypertrophy of nuclei, cell bodies, cytoplasmic processes and extensive synthesis of glial fibrillary acidic protein (GFAP) intermediate filaments in the injured cells (Eng, 1988a; Condorelli et al., 1990; Hozumi et al., 1990; Vijayan et al., 1990; Eng et al., 1992; Yu et al., 1993). The precise function of this intermediate filament protein is yet unknown, although metabolic turnover studies and those with the astrocytoma cell line U251 support a structural role for GFAP (Chiu and Goldman, 1985; DeArmond et al., 1986; Weinstein et al., 1991). Also, neither the functional significance for, nor the effect of increase in GFAP following injury is known. The lack of axonal regeneration following injury has been attributed to several factors, viz. presence of inhibitory molecules from postinjury myelin breakdown, absence of requisite growth factors or formation of a gliotic scar by reactive astrocytes (for review, see Eng et al., 1992). We have hypothesized that the rapid gliotic response of astrocytes may interfere with the function of residual neuronal circuits by formation of a physical barrier, thus preventing remyelination or regeneration (Eng et al., 1987; Reier and Houle, 1988).

Antisense RNA treatment has been used in a number of studies to successfully decrease the expression of a specific protein in a target cell (for review, see Eng, 1993). Ongoing studies in our laboratory have focussed on using antisense GFAP RNA to inhibit the increase in GFAP synthesis following injury, thereby modulating astrogliosis. We have previously demonstrated successful inhibition of GFAP using antisense oligonucleotides in primary astrocyte cultures stimulated with dibutyryl cAMP as well as in mechanically injured cultures (Yu et al., 1991, 1993).

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To overcome the transient nature of inhibition associated with the use of oligonucleotides, we have constructed a recombinant retrovirus containing the coding region of GFAP in the antisense orientation under the control of a retroviral long terminal repeat (LTR) promoter. Using this vector we have continued our ongoing investigation on whether antisense treatment, in addition to inhibition of GFAP synthesis, will modulate other astrogliotic responses such as hypertrophy and hyperplasia. Present studies show that retroviral infection of mechanically injured astrocytes inhibit the increase in GFAP synthesis associated with injury along with controlling hypertrophy. Use of recombinant retrovirus, along with providing stable integration of the transfected gene, offers the advantage that astrocytes at the site of injury rapidly proliferate and retroviral infection is targeted only at dividing cells (Miller et al., 1990; Culver et al., 1992).

MATERIALS AND METHODS

Cell Culture

Primary astrocyte cultures of rat cerebral cortices from newborn Sprague-Dawley rats (VAMC animal facility, Palo Alto, CA) were prepared as described previously with modifications (McCarthy and DeVellis, 1980; Yu et al., 1989, 1991, 1993). Briefly, neopallia freed of meninges were cut into small cubes (< 1 mm^3) in modified Dulbecco's modified Eagle's medium (DMEM; Applied Scientific, San Francisco, CA). The tissue was disrupted by vortexing for 1 min and the resulting suspension was passed through two sterile nylon Nitex® sieves (L. and S.H. Thompson, Ontario, Canada) of pore sizes 80 and 10 μm. Cells were plated at a density of 15 × 10^6 cells per 75 cm^2 plastic tissue culture flask. Cultures were incubated at 37°C in a 95%:5% (vol:vol) mixture of atmospheric air and CO_2 with 95% humidity and grown until the cells reached confluency, usually 6–8 days. After reaching confluency, the cultures were shaken on an orbital shaker to remove oligodendrocytes. Following the shaking period, the adherent astrocytes were trypsinized and the secondary astrocytes were plated on 35 mm tissue culture dishes at a density of 1 × 10^5 cells per dish. Culture medium was changed three days after initial seeding and 3 times a week thereafter with DMEM containing 10% fetal calf serum (FCS). Cultures were allowed to reach confluency before using for experiments.

Scratch Wound Model

The scratch wound model has been recently established in our laboratory as an in vitro system to study astrocytic response to mechanical injury in a culture environment lacking interactions with neurons (Yu et al., 1993). Confluent cultures of astrocytes were scratched with a sterile plastic pipette tip and the culture medium was changed to remove most of the detached cells and debris. Injured cultures were immediately incubated with viral supernatant (10^5–10^6 cfu/ml) from packaging cells, either containing parent virus (LXSN) or virus expressing antisense GFAP sequences (AS) for 24–72 hr in the presence of polybrene (5 μg/ml final concentration; Miller and Rosman, 1989). Following incubation, cultures were fixed in 5% glacial acetic acid-95% ethanol for 15 min at 4°C and immunostained with polyclonal anti-GFAP or monoclonal anti-vimentin antiserum. Each time point was performed on at least 2–3 independent cultures.

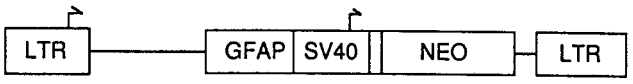

Fig. 1. Structure of the LXSN recombinant retrovirus containing the GFAP cDNA in the antisense orientation. Antisense GFAP is synthesized under the control of the viral long terminal repeat (LTR) promoter while SV40 promoter drives synthesis of the neomycin phosphotransferase (NEO) gene.

Construction of Antisense GFAP Retrovirus

Murine GFAP cDNA was cloned in the retroviral vector LXSN (a gift from Dr. Miller) (Miller and Rosman, 1989) by directional cloning using fragments with heterologous ends (Fig. 1). A 650 bp fragment of the coding region of the murine cDNA was amplified using Bam HI and Eco RI linked primers (Operon Technologies, Inc., Alameda, CA). The sense strand oligomer was selected from the initiation site of the mouse GFAP cDNA and was linked to the Bam HI site. The second oligomer, linked to Eco RI site was complementary to nucleotides 717–731 (Lewis and Cowan, 1985). The amplified polymerase chain reaction (PCR) product was cloned into LXSN downstream from the LTR promoter of the Moloney murine leukemia virus. Ecotropic packaging cell line Ψ CRE cells (a gift from Dr. Mulligan; Danos and Mulligan, 1988) were transfected with the resultant plasmid (antisense-AS) or the parent plasmid (LXSN) by calcium phosphate coprecipitation (Graham and van der Eb, 1973; Mann et al., 1983).

RNA Analysis

Reverse transcriptase-PCR (RT-PCR) was used to analyze the expression of neomycin phosphotransferase (neo) and GFAP in the Ψ CRE clones. Total RNA was isolated by a simple modification of Peppel/Baglioni method (Salvatori et al., 1992). Briefly, cells were lysed in a SDS solution followed by addition of potassium acetate and chloroform-isoamyl alcohol mixture and

placed on ice for 2 min. The tube was then centrifuged which resulted in DNA-protein precipitate to form at the aqueous/organic interface. The aqueous phase was collected and RNA precipitated with isopropanol, 1 μg of RNA was used in a RT reaction using random hexamers. The resultant cDNA was amplified using the GENEAMP® RNA PCR kit (PERKIN ELMER CETUS, Norwalk, CT). GFAP cDNA was amplified using the Bam HI and Eco RI primers used in cloning of the 650bp fragment. To amplify the neo gene, the following primers were used: nucleotides 1566–1587 and those complementary to nucleotides 2302–2323 from the nucleotide sequence of Tn5 DNA (Beck et al., 1982).

In situ hybridization was performed using a nonradioactive method which utilized digoxigenin-dUTP-labelled probe (Genius kit from Boehringer Mannhein Biochemicals, Indianapolis, IN). Confluent scratched astrocyte cultures treated with LXSN or AS expressing viral supernatants for 48 hr were fixed in 4% paraformaldehyde for 5 min at room temperature (room temp). After fixation, the cells were treated with 0.2 N HCl for 20 min at room temp, washed in 2× SSC-5 mM EDTA for 15 min at room temp followed by a proteinase K (1 μg/ml) digestion for 15 min at 37°C. The proteinase K treatment was neutralized with 0.2% glycine solution for 10 min at room temp. The cells were postfixed in 4% paraformaldehyde for 30 min at room temp and then prehybridized for 30 min at 42°C in a solution containing 45% formamide, 6× SSC, 5× Denhardt's solution and 100 μg/ml sonicated salmon sperm DNA. Following prehybridization, the cells were incubated with 1 ng/μl of random primed digoxigenin-labelled sense strand probe in a mixture containing 45% formamide, 6× SSC, 5× Denhardt's solution, 10% dextran sulfate and 100 μg/ml sonicated salmon sperm DNA overnight at 42°C. The cells were washed twice with 45% formamide, 6× SSC at 42°C for 15 min each, twice with 2× SSC at RT for 5 min each, and twice with 0.2× SSC at 50°C for 15 min each. The cells were then incubated for 20 min with 0.5% blocking reagent (supplied with the Genius kit) followed by 1:500 dilution of antibody conjugated with alkaline phosphatase for 2 hr at room temp. After three washes to remove the unbound antibody, the alkaline phosphatase substrate was added along with the dye (nitroblue, tetrazolium salt) and incubated in the dark for 2–4 hr at room temp. The reaction was stopped by the addition of 10 mM Tris.HCl-1 mM EDTA, pH 7.6, and the cells were only slightly counterstained with saffranin so that the reaction product could be easily visible. The cultures were then mounted in a 70% glycerol solution.

Immunocytochemistry

Polyclonal rabbit antibody to multiple sclerosis plaque GFAP (Eng and DeArmond, 1983) or a commercially available monoclonal antibody to vimentin (Dako Corp, Carpinteria, CA) was used for immunostaining [at a dilution of 1:1,000 (GFAP) or 1:500 (vimentin)] scratched astrocyte cultures using peroxidase-antiperoxidase (PAP) method (Sternberger et al., 1970; Eng and DeArmond, 1983; Yu et al., 1993). Pig antiserum to rabbit IgG was prepared in our laboratory. Goat anti mouse IgG and rabbit and mouse PAP were purchased from Sternberger-Monoclonals Incorporated (Baltimore, MD). Following immunostaining, all cultures were counterstained with hematoxylin and mounted in a 70% glycerol solution.

RESULTS

Production of GFAP Antisense Retrovirus

The parent retrovirus (LXSN) or the GFAP antisense retrovirus (AS; shown in Fig. 1) was transfected into the packaging cells Ψ CRE and G418 resistant colonies were assayed for virus production by infecting NIH 3T3 cells (Miller and Rosman, 1989). The viral titer for both clones (LXSN and AS) ranged between 5×10^5 to 1×10^6 cfu/ml. LXSN and AS clonal lines were tested for presence of neo and GFAP RNA synthesis using RT-PCR amplification. RNA was prepared and reverse-transcribed with random hexamers and then amplified by adding GFAP and neo primers. As shown in Figure 2 (lanes 5 and 6), amplified fragments of the expected sizes were produced with the neo and GFAP primers for the AS line (750 and 650 bp, respectively) and only the neo primer for the LXSN line (Fig. 2, lanes 3 and 4); no product was obtained with RNA prepared from the untransfected Ψ CRE cells (Fig. 2, lanes 1 and 2). In lane 7, DNA of the GFAP antisense retroviral construct was used to generate a reference fragment.

Scratch Wound of Astrocyte Cultures

We have previously demonstrated that scratching of a confluent culture of astrocytes results in a denuded area due to removal of cells from a substratum (Yu et al., 1993). As shown in Figure 3A, the edge of the scratch is lined with irregularly shaped cells which are all positively stained for GFAP. Following injury, by 6 hr, most astrocytes respond by sending out cytoplasmic processes toward the site of injury, and by 3 days after the injury, the denuded areas are completely covered with cytoplasmic processes. This astrogliotic response is also characterized by nuclear hypertrophy and an increase in immunodetectable GFAP in the cytoplasmic processes of injured cells as shown before by Yu et al. (1993) and also in Figure 3B.

Effect of GFAP Antisense Retroviral Treatment

Astrocyte cultures were immediately treated with LXSN or AS viral supernatant for increasing periods of

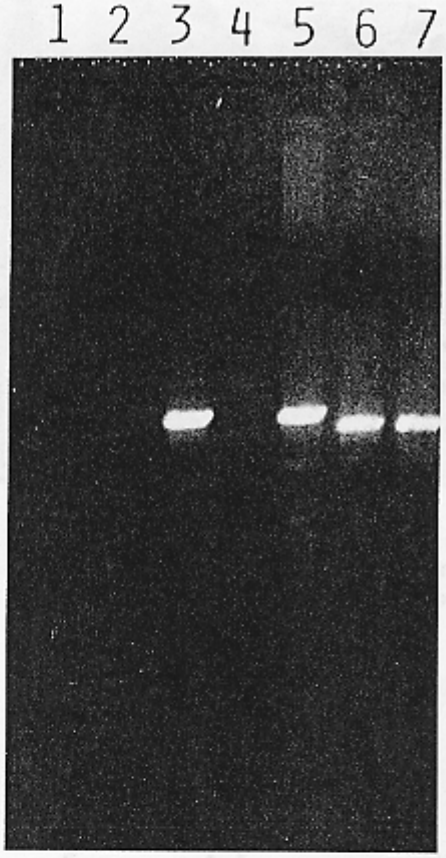

Fig. 2. Characterization of the packaging cells Ψ CRE producing the GFAP antisense virus. PCR products from the amplification of cDNA reverse transcribed from RNA prepared from untransfected Ψ CRE cells **(lanes 1, 2)**, LXSN **(lanes 3, 4)** and AS **(lanes 5, 6)** clonal lines. Lanes 1, 3 and 5 show products obtained using the neo primers and lanes 2, 4 and 6 show products obtained using GFAP primers. **Lane 7** shows amplification of GFAP antisense retroviral vector DNA.

time following scratch injury. One day after antisense viral treatment (Fig. 4B), cells along the edge of the scratch appeared restricted in sending out cytoplasmic processes towards the denuded area. In contrast, in cultures treated with LXSN, astrocytes near the wound sent out elongated cytoplasmic processes which were extremely positive for GFAP staining (Fig. 4A). This response was comparable to that seen in control injured cultures by 24 hr (data not shown). By 72 hr the antisense effect was most pronounced, and very few astrocytic processes were seen extending into the scratched area (Fig. 4D). Morphologically, these cells showed an epithelial rather than a fibrous appearance. The suppressive effect on GFAP in these cells was evident by immunocytochemical staining (Fig. 4, cells marked with a star). Occasionally, a few thin processes were sent out by cells treated with the antisense virus which did not extend very far in the site of scratch wound. Figure 4C shows a sister culture 3 days after LXSN treatment in which extensive hypertrophy of astrocytic cytoplasmic processes was observed along with a prominent positive reaction for GFAP. The gliotic response of these cells was comparable to that shown by control cultures. Nuclear hypertrophy (characterized by changes in size and shape of the nucleus) in injured astrocytes was seen in control as well as antisense-treated cultures. In some cells along the scratch edge, the astrocytic nucleus appeared to have doubled in size. Previously, we have shown lobulated nuclei in injured astrocytes (Yu et al., 1993).

In order to determine whether the reduced cytoplasmic hypertrophy of astrocytes at the edge of the wound was due to the continued presence of the antisense virus in the cultures, after 24 hr of incubation, the viral supernatants were removed, the cultures washed once and incubated for additional 24 hr in fresh medium. As shown in Figure 5B, even after removal of antisense virus, astrocytes at the edge of the scratch were unable to extend their cytoplasmic processes. Some cells along the scratch also showed reduced GFAP staining. This response was similar to that seen when cultures were treated with AS supernatant for 48 hr (Fig. 5D). However, cultures treated with control LXSN virus retained their normal response to injury, showing the major characteristics of astrogliosis: nuclear and cytoplasmic hypertrophy and increase in immunostainability for GFAP (Fig. 5A,C). Similar hypertrophic responses were seen in scratched cultures treated with another retrovirus expressing the β-galactosidase marker gene (Price et al., 1987; data not shown). The morphology and extent of GFAP staining was similar in cells in the confluent areas of all cultures studied (data not shown).

In order to verify that antisense GFAP RNA was being expressed in the astrocytes at the edge of the wound, scratched cultures treated with LXSN and AS expressing viral supernatants were processed for in situ hybridization using sense-strand specific oligonucleotide probe. Many cells along the edge of the scratch treated with the AS supernatant showed strong hybridization to the probe (Fig. 6B). Cells treated with control supernatant did not show significant hybridization with the oligonucleotide (Fig. 6A). Cells expressing the antisense GFAP RNA appeared to show a flat morphology. However, not all cells along the wound showed high levels of hybridization with the GFAP sense strand probe, which may reflect heterogeneity of expression due to the site of proviral insertion or the lack of uptake of the virus by these cells.

To confirm that the antisense GFAP treatment was specific in inhibiting only the expression of GFAP, scratched astrocyte cultures treated with LXSN and AS supernatants for 24 hr were analyzed for vimentin ex-

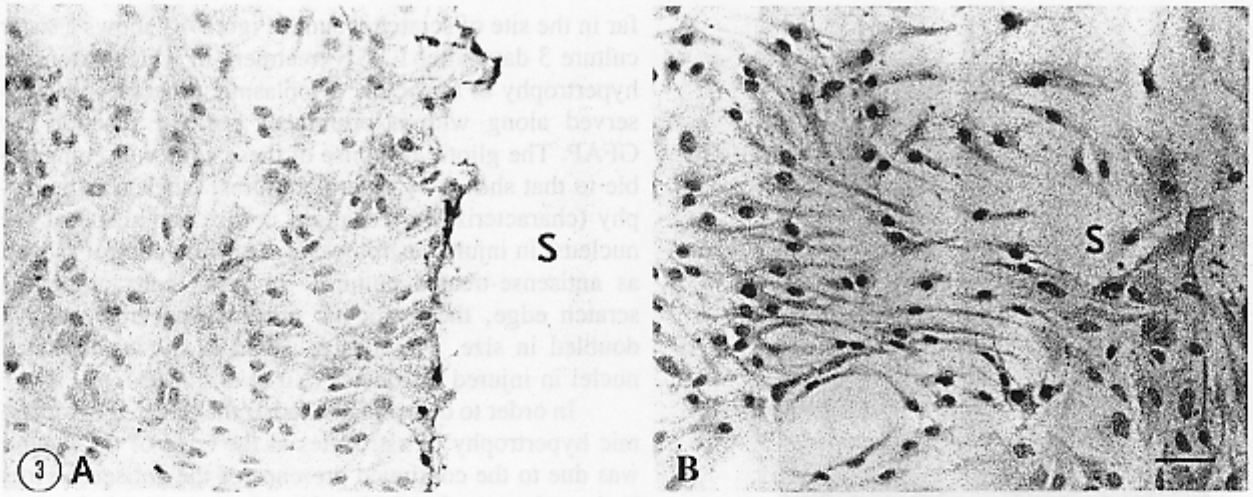

Fig. 3. Immunostain for GFAP in control cultures. (A) 0 hr, (B) 72 hr after astrocyte cultures were scratched. Note the long cytoplasmic processes grown toward the wound and strongly stained for GFAP in B compared to A. S denotes the denuded area for reference. Bar = 50 μ for A and B.

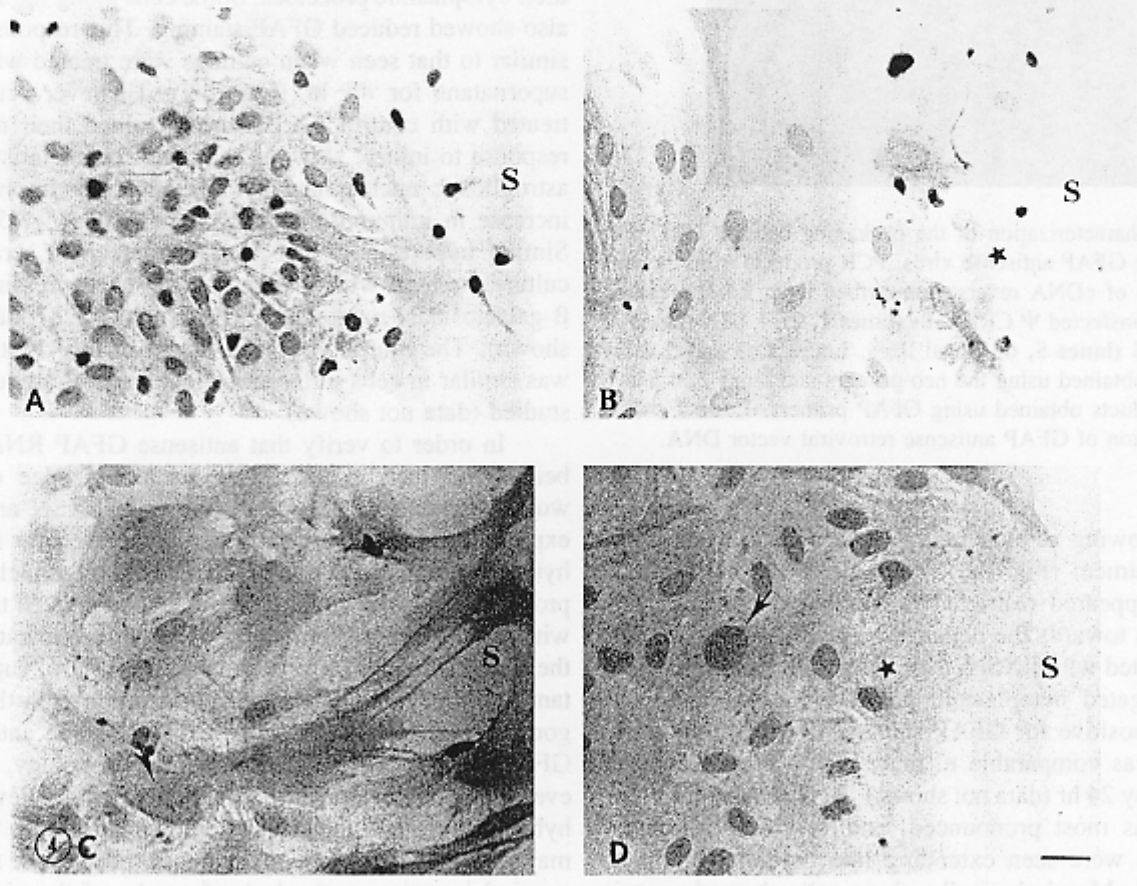

Fig. 4. Immunostain for GFAP in retrovirus-treated cultures. LXSN- **(A,C)**, AS- **(B,D)** treated cultures were stained for GFAP after 24 hr (A,B) and 72 hr (C,D). Astrocytes in A have sent out pale and flat cytoplasmic processes which are less prominent in B. In C, the site of scratch wound is filled with hypertrophic processes of astrocytes which show a strong reaction to GFAP. In D, cells at the edge of the scratch are restricted from sending out processes. Stars denote cells showing reduced GFAP staining. Arrows indicate cells with nuclear hypertrophy. S denotes the denuded area for reference. Bar = 25 μ for A–D.

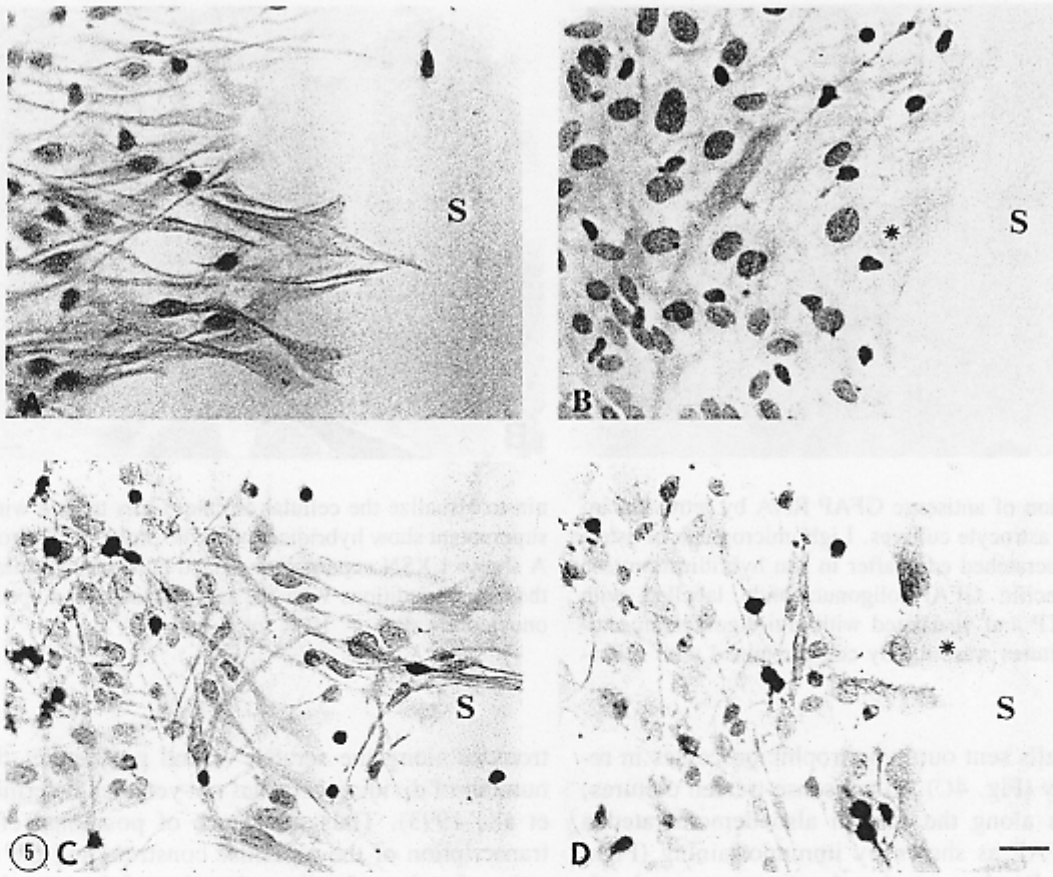

Fig. 5. Immunostain for GFAP in retrovirus treated cultures. LXSN- (A,C), AS- (B,D) treated cultures were stained for GFAP after 24 hr virus/24 hr media (A,B) and 48 hr (C,D). Astrocytes in B fail to send out processes even after the removal of virus. Similar cytoplasmic outgrowth is observed in A and C. In B and D, cells at the edge show a flat morphology and reduced GFAP staining (asterisks in B and D). S denotes the denuded area for reference. Bar = 25 μ for A–D.

pression by immunostaining using a monoclonal antibody. Vimentin and GFAP have been shown to coexist in cultured astrocytes (for review, see Eng and Lee, 1994). As shown in Figure 7C and D, cells at the site of injury in LXSN- and AS-treated cultures showed comparable levels of vimentin expression. The staining in these cultures also appeared similar to that seen in a control scratched culture (Fig. 7B).

DISCUSSION

Reactive astrogliosis in the CNS is the most prominent reaction of astrocytes adjacent to the site of injury which is characterized by extensive synthesis of GFAP and by hypertrophy of astrocytic nuclei, cell bodies and cytoplasmic processes. Controlling astrogliosis, which leads to scar formation in vivo following injury, may aid in the recovery and regeneration of neurons in the CNS. In an in vitro model for gliosis, we have previously demonstrated the feasibility of antisense nucleotides in inhibiting the overexpressed GFAP associated with injury (Yu et al., 1991, 1993). Although antisense oligomers have proved to be a powerful tool in controlling gene expression, their effect is ultimately transient due to the limited stability of antisense RNA (Eng, 1993; Stein and Cheng, 1993). In this report, using a recombinant retrovirus expressing antisense GFAP RNA, we show that following antisense retroviral treatment, along with reduction in GFAP, the hypertrophy of astrocytic processes normally seen at the site of injury is greatly diminished. However, nuclear hypertrophy was not affected by the presence of antisense GFAP RNA. Only a single exposure to virus containing medium was sufficient to reduce the cell body hypertrophy of astrocytes which was most obvious at 72 hr (Fig. 4D). This response was specific to antisense virus treatment since in the presence of control virus

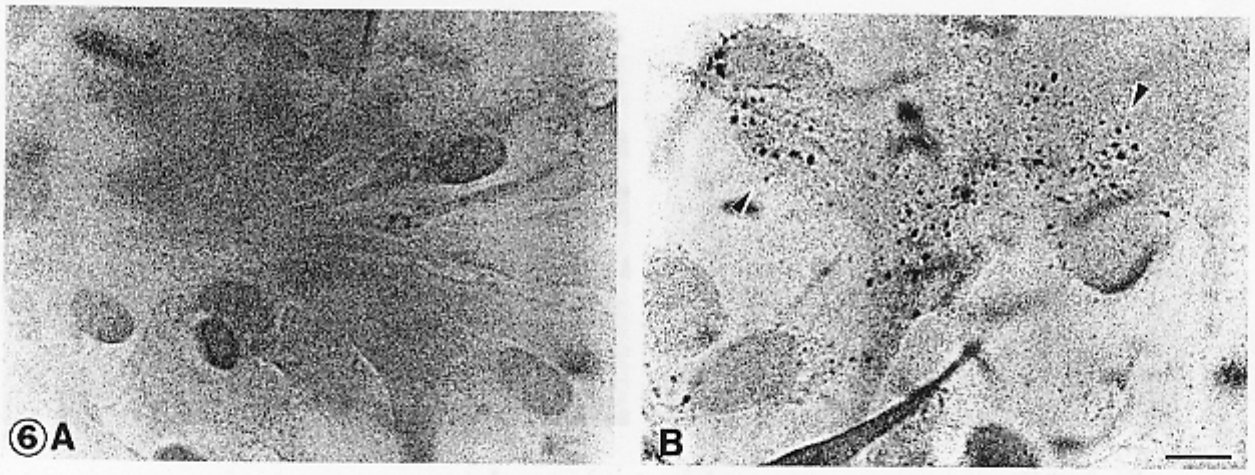

Fig. 6. Expression of antisense GFAP RNA by retroviral infected scratched astrocyte cultures. Light micrograph of astrocytes along the scratched edge after in situ hybridization to a sense-strand specific GFAP oligonucleotide labelled with digoxigenin-dUTP and visualized with anti-digoxigenin antibodies. Both cultures were lightly counterstained with saffranin to visualize the cellular outline. Cells treated with the AS supernatant show hybridization to the probe in B (arrowheads). A shows LXSN supernatant-treated cultures hybridized under the same conditions with the same sense-strand specific oligonucleotide. Bar = 10 μ for A and B.

(LXSN), the cells sent out hypertrophic processes in response to injury (Fig. 4C). In antisense-treated cultures, numerous cells along the scratch also demonstrated a decrease in GFAP as shown by immunostaining (Figs. 4B, D and 5B, D). In control and antisense-treated cultures, cells in the vicinity of the scratch showed increase in nuclear size (Figs. 4C, D). The presence of the antisense GFAP RNA in the cells along the scratch was evident by in situ hybridization (Fig. 6B). Even after removal of the virus following a 24 hr incubation period, reduced cytoplasmic hypertrophy in cells along the scratch was seen demonstrating the effectiveness of retroviral infection on the targeted cells (Fig. 5B). Figure 7 shows that the expression of another intermediate filament protein, vimentin, was not affected following antisense GFAP treatment. These results suggest that continuous suppression of GFAP overexpression in astrocytes following injury may be required to prevent the hypertrophy of cytoplasmic processes of astrocytes.

The variable level of antisense GFAP RNA expression along the edge of the scratch was not an unexpected observation. In other in vitro and in vivo studies where inhibition of the sense transcript was attempted with antisense constructs, only some of the transfected cells showed inhibition (Kim and Wold, 1985; Nishikura and Murray, 1987; Katsuki et al., 1988; Wong et al., 1989; Owens and Bunge, 1991). Although integration of the retroviral genome in cellular DNA is a well established phenomenon, the exact site of proviral insertion following infection is unclear (Varmus and Brown, 1989). Although we have previously demonstrated that many astrocytes along the scratch wound proliferate, the exact number of dividing cells has not yet been determined (Yu et al., 1993). Thus, influence of positional effects on transcription of the antisense construct and the fact that only a fraction of cells at the edge of the scratch divide, may contribute to the variable expression of the antisense RNA.

Retroviral-mediated gene transfer for expressing antisense RNA into mammalian cells has been well documented (Miller et al., 1988; Spampinato and Ferri, 1989; Honma et al., 1991; Ye and Wu, 1992). Using this approach, the requirement for myelin-associated glycoprotein (MAG) in initiation of peripheral myelination and that of P_0 protein in subsequent compaction of myelin has been demonstrated (Owens and Bunge, 1991; Owens and Boyd, 1991). Our data, which show the inability of astrocytes to send out processes following infection with retrovirus expressing antisense GFAP RNA, suggest a role for GFAP in cytoplasmic process formation and structural stability of astrocytes. These results are in agreement with those of Weinstein et al. (1991) who demonstrated that a permanently transfected human astroglioma cell line with an antisense GFAP DNA construct could no longer synthesize GFAP nor form processes in response to neurons in culture. The relatively slow turnover rate for GFAP is also consistent with a possible structural role for the glial filament (Trimmer et al., 1982; DeArmond et al., 1983, 1986; Smith et al., 1984).

Until recently, the use of retroviral vectors was considered limiting because of the requirement for rep-

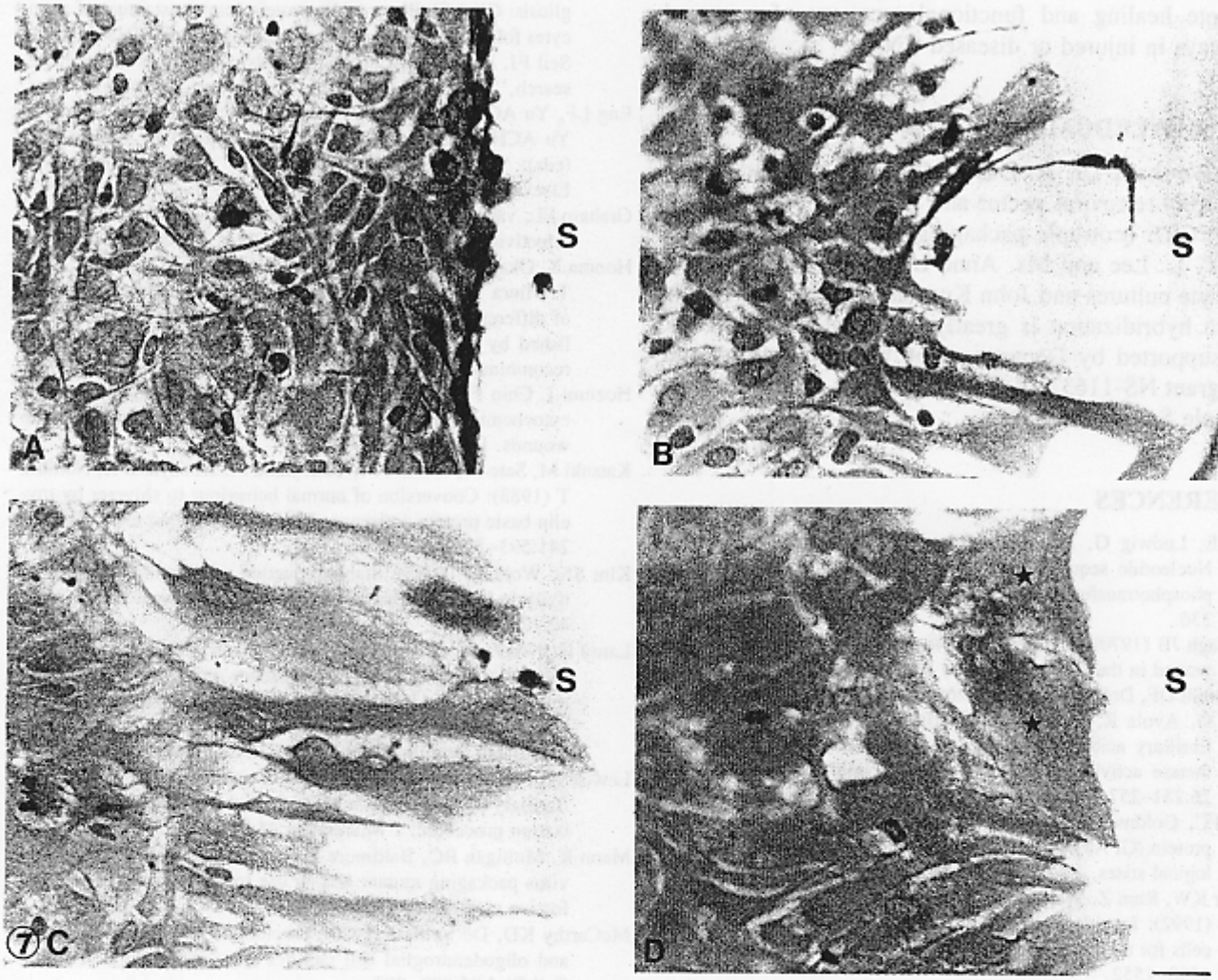

Fig. 7. Immunostain for vimentin in control and retrovirus-treated cultures. (A) 0 hr, (B) 24 hr control, (C) LXSN, (D) AS 24 hr treatment. Astrocytes in B and C show cytoplasmic processes extending toward the wound which are positively stained for vimentin. Cells in D showing a flat morphology (stars) also appear comparably stained for vimentin as astrocytes in B and C. Astrocytes immediately after scratching (A) show uniform cytoplasmic staining for vimentin. S denotes the denuded area for reference. Bar = 25 μ for A–D.

lication of their target cells (Miller et al., 1990). However, this property of retroviruses was exploited in some studies where in vivo gene transfer with retroviral vector producer cells was used for treatment of experimental brain tumors (Culver et al., 1992; Takamiya et al., 1992). In vitro and in vivo studies have shown proliferation of astrocytes at the site of injury (Cavanagh, 1970; Latov et al., 1979; Yu et al., 1993). These dividing astrocytes which contribute to the scar formation would serve as an ideal target for introducing antisense GFAP construct by retroviral-mediated gene transfer to modulate astrogliosis. Thus, the retroviral method offers an advantage over antisense oligonucleotide treatment since only a selected population of cells at the site of injury will be targeted. Transcription of the inserted cDNA driven by the strong viral promoter LTR would ensure production of high levels of antisense GFAP RNA in infected cells (Miller et al., 1993).

We have demonstrated that following injury in vitro, along with reduction in immunodetectable GFAP, hypertrophy of astrocytic cytoplasmic processes can be decreased with a recombinant retrovirus expressing antisense GFAP RNA. Hypertrophy of astrocytic nucleus, cell body and cytoplasmic processes and an increase in biosynthesis of GFAP are characteristic of astrogliosis. Modulation of astrogliosis with retroviral vectors may

promote healing and functional recovery of neuronal pathways in injured or diseased CNS.

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