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NG2 positive cells of rat spinal cord activated during experimental autoimmune encephalomyelitis are spatially associated with radially oriented astroglia and express p75 receptor: a role for nerve growth factor in oligodendrocyte progenitor migration?

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ABSTRACT

Data have been provided from several studies that support the proposal that the adult oligodendrocyte progenitors migrate into the lesioned areas under conditions of experimental autoimmune encephalomyelitis (EAE). However, the routes of migration of these cells and the governing mechanisms are not clear. In the present studies, we have examined the effect of EAE upon activation of endogenous oligodendroglia progenitors and their spatial distribution in the spinal cord of Lewis rats using immunocytochemical procedures. Antibodies against the marker chondroitin sulfate proteoglycan NG2, are used for identification of oligodendroglia progenitors. We find that the activated elongated subpopulation of NG2 positive oligodendroglia progenitors of white matter is spatially associated with the radially-oriented astroglia during the acute phase of EAE. The latter re-expressed the phenotypic embryonic marker nestin while still expressing the mature astroglial marker GFAP. The elongated oligodendroglia progenitors express p75 receptor. In addition, colocalization of NG2 and p75 is observed also in ependymal neural cells of the central canal and the subventricular zone. This raises the possibility that the activated NG2+/p75+ parenchymal cell pool may also be recruited from multipotent neural cells of the germination areas. Our data suggest that, under EAE conditions, the radially oriented astroglia of juvenile phenotype may serve as scaffolding for migrating activated endogenous oligodendroglia progenitors just like radial glia provide a path for neuronal and oligodendroglia progenitor cells in embryonic stage. The expression of p75 receptor in oligodendroglia progenitors associated with radially oriented astroglia during EAE may implicate a role for NGF in the regulation of migration of oligodendroglia progenitors.

Key words

Experimental autoimmune encephalomyelitis • Spinal cord • Oligodendroglia progenitors Radially oriented astroglia • Migration • p75 receptor

Introduction

The presence of putative oligodendroglia progenitor cells (OPCs) in the adult central nervous system (CNS) identified with antibodies against the chondroitin sulfate proteoglycan (NG2), has been repeatedly reported in the last years (Levine et al., 2001; Dawson et al., 2003). As a result of a variety of CNS insults, including autoimmune demyelinating diseases, these cells become activated which is evidenced by an increase in NG2 immunoreactivity, morphological changes and proliferation. Thus far,

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NG2 positive cells have been thought to consist essentially of "adult OPCs" that persist in the adult CNS and whose only function is to generate oligodendrocytes throughout life. This view was recently challenged when NG2 expressing cells were identified as multipotent progenitor cells or intrinsic CNS adult stem cells (Butt et al., 2005). Thus the term OPC has more recently been reserved to describe NG2 positive cells which give rise to oligodendrocytes. Substantial evidence supports the generation of oligodendrocytes from NG2 positive cells both *in vivo and in vitro* (Baracskay et al., 2007). In experimental autoimmune encephalomyelitis (EAE), the recruitment of progenitor cells from the subventricular zone (SVZ) and the central canal (CC) into areas of demyelination has been described to take place in addition to the activation of the resident population (Calzà et al., 2003; Petratos et al., 2004). This suggests that both populations may represent a source of new oligodendrocytes for remyelination.

Data have been provided from several studies in support of the proposal that, in EAE, the adult oligodendrocyte progenitors migrate into the lesioned areas (Keirstead et al., 1998; Levine et al., 2001; Picard-Riera et al., 2002; Dawson et al., 2003). The mechanisms and routes of migration are not clear. Progenitors do not migrate randomly, but follow the discrete paths. Migration of oligodendrocyte precursors along axons is considered a prerequisite for myelination (Chatterjee et al., 2008), however other routes for progenitor migration are also possible. Tourbah et al. (1997) found that the CG4 cells of the progenitor line that are transplanted into the spinal cord of EAE rats about two weeks prior to induction of EAE, were often associated with radially oriented GFAP-positive cells. According to the authors, this observation may be an indication that this parenchymal migratory pathway represents at least one of the possible routes for migration of transplanted progenitors. However, the question of whether the endogenous oligodendroglia progenitors in the spinal cord would follow such a migratory route under EAE conditions has not yet been addressed.

During development and in the early postnatal period, radial glia are well known to be used as scaffolding for migration of neurons (Hatten, 2002) but they were also suggested to act as guides for migration of glial progenitors (Zerlin et al., 1995; Goldman et al., 1997; Kakita and Goldman, 1999; Diers-Fenger et al., 2001). On the other hand, it has been postulated that the radial glia phenotype can be reinduced in the astroglia of the adult CNS under certain pathological conditions (Hunter and Hatten, 1995; Leavitt et al., 1999; Chanas-Sacre et al., 2000; Shibuya et al., 2002; Talbott et al., 2005).

It has been reported that the nerve growth factor (NGF) plays multiple roles in ammeliorating various symptoms of EAE (Calzà et al., 1998; Micera et al., 2000; Ransohoff and Trebst, 2000; Arrendono et al., 2001; Flugel et al., 2001; Althaus, 2004; Villoslada and Genain, 2004) and it has been postulated that NGF may be implicated in migration of resident brain progenitor cells in EAE (Calzà et al., 1998, 2002, 2003; Triaca et al., 2005). NGF is known to induce and/or promote migration of several cell types (Anton et al., 1994; Dollè et al., 2005; De Simone et al., 2007). On the other hand, the low affinity NGF receptor-p75 itself has been reported to be involved in some cell migratory processes (Anton et al., 1994; Bentley and Lee, 2000). A subpopulation of resident oligodendroglia progenitors in the brains of MS patients has recently been shown to express p75 (Chang et al., 2000; Petratos et al., 2004).

The present studies have been undertaken to throw more light upon the potential migratory routes of endogenous oligodendroglia progenitor cells and migratory signals in the spinal cord of EAE rats in the acute phase of the disease. In particular, the experiments were designed to investigate the activation of endogenous oligodendroglia progenitors, their spatial relationship to the radially oriented astroglia and the possibility of re-induction of juvenile radial glia phenotype in the latter. Finally, the experiments were also designed to verify whether the oligodendroglia progenitors in the EAE spinal cord are capable of expression of p75 receptor.

Methods

Animals, EAE induction and clinical assessment

Adult (2 months old) female Lewis rats were used for immunization. Animal care procedures, both in Warsaw and Rome laboratories, were conducted in conformity with Intramural Committee and Institutional guidelines in accordance with National

and International laws and policies. Two modes of EAE induction were applied.

Mode I of EAE induction

Rats were injected intradermally into each footpad with 0.1 ml of the following emulsion: 1:1 (1 g - 1 ml) guinea pig spinal cord – Complete Freund's Adjuvant (CFA, Sigma, St. Louis, MO) containing 2 mg/ml of *Mycobacterium tuberculosis*. Rats were weighted and examined for grading of clinical signs. Rats were sacrificed at 14 day after immunization (acute phase of EAE). As controls, rats injected with CFA or untreated rats of the same sex and of age matching the EAE rats were used. The number of rats analyzed in each experimental group in each experiment was 5-6. Three independent sets of experiments were performed. The average clinical score was 3.8 at 14th day postimmunization. Virtually no signs of disease were observed in CFA injected group of rats at 14th day, other than inflammation in both foot pads. Animals were sacrificed on the 14th day postimmunization.

Mode II of EAE induction

CD4+ T cells (anti-MBP or anti-OVA) modified with permanent expression of green fluorescent protein (GFP) (kindly provided by Dr. A. Flügel and Prof. H. Wekerle from Max-Planck Institute of Psychiatry, Martinsried, Germany) were re-stimulated by co-culture with irradiated (5000 rad) thymocytes, as antigen presenting cells, in a medium containing MBP antigen (10 μg/ml) for two days. Activated blasts (4 million per animal) were injected intravenously. All animals receiving anti-MBP T cells developed monophasic autoimmune encephalomyelitis (EAE) and the course of the disease was typical, as described previously (Kurkowska-Jastrzębska et al., 2007). The number of rats analyzed in each experimental group in each experiment was 3-4. Three independent sets of experiments were performed. The maximal clinical signs (on average 3.5) were observed on the 4th and 5th days after administration of T cells, and usually included tail and hind limb paralysis. Infiltration of T cells was monitored on the 5th day after administration. T cells infiltrate the white matter of spinal cord where they form few infiltration sites which are typically localized mainly in the vicinity of the blood vessels as described previously (Kurkowska-Jastrzębska et al., 2007). In a series where anti-OVA T cells were injected, only singular cells were detectable. Animals recovered completely during the next 5 to 7 days. Animals receiving anti-OVA T cells did not develop any signs of the disease. Animals were sacrificed on the 5th day after T cells administration.

Tissue preparation

The animals were sacrificed by transcardial perfusion (under ether anaesthesia) with 0.1 M PBS plus heparin (6250 units/l) followed by 2% paraformaldehyde. The lumbar part of the spinal cord was surgically removed, postfixed in the same fixative and cryoprotected in 30% sucrose in 0.1 M PBS for at least 48 h at 4°C. Frozen 20 μm sections were cut in coronal plane and collected in PBS with 0.1% sodium azide. Free floating, adjacent sections were then processed as described below.

Immunocytochemical and staining procedures

To identify inflammatory sites and gross cellular morphological alterations, the sections were stained with 0.1% toluidine blue.

Immunocytochemical reactions (both single and double staining) were performed using diaminobenzidine (DAB) or DAB, Ni^{++}, as a substrate for horseradish peroxidase or the fluorescent dyes: Fluorescein and Texas Red. All reactions were performed in 0.3% Triton X-100 in 0.1 M PBS. Free-floating slices were incubated with 0.75% H_2O_2 for 30 min (for DAB system only). The non-specific binding sites were blocked with 5% NHS (normal horse serum – for monoclonal antibodies) or 5% NGS (normal goat serum – for polyclonal antibodies). The sections were then incubated overnight at 4°C with primary antibodies. The following concentrations of primary antibodies were used: anti-NG2 – rabbit polyclonal, 1:300 (Chemicon, Temecula, CA); anti-GFAP mouse monoclonal, 1:1000 (Boehringer, Mannheim, Germany) or anti-GFAP rabbit polyclonal, 1:200 (Sigma); anti-nestin – mouse monoclonal, 1:800 (Chemicon, Temecula, CA); anti-p75 mouse monoclonal,1:20, clone IgG192, generously supplied to Dr. Aloe by Dr. E.M. Johnson (Washington University, St. Louis, WA). After washing, the slices were incubated at room temperature for 2 hours with biotinylated secondary antibodies (Vector Laboratories, Burlingame, CA) at a concentration of 1:200. Anti-mouse, anti-rabbit or

anti-goat secondary antibodies were used due to earlier reaction with monoclonal or polyclonal primary antibody, respectively. For DAB detection, slices were incubated for 1 hour with avidin-horseradish peroxidase complex (Vector) at a concentration of 1:100. The reactions were developed using DAB (brown) or DAB, Ni^{++} (black) substrates, coverslipped with DPX mounting medium (Sigma, St. Louis, MO, USA) and then analyzed with a light microscope. For the fluorescent mode of detection, slices were incubated for 1 hour with Fluorescein Avidin DSC complex (Vector) or Texas Red Avidin D (Burlingame, CA) at a concentration of 1:1000, coverslipped with mounting medium for fluorescence (Vector) and visualized using a fluorescent microscope. Reactions with omission of primary or secondary antibodies were performed as negative controls for all immunocytochemical assays.

Confocal imaging

The sections were double immunostained for: GFAP and NG2, nestin and NG2, and GFAP and nestin. The cells appearing to be double immunostained with appropriate cellular markers were identified and evaluated by confocal imaging. Using a confocal microscope and software (Leica Microsystems, Wetzlar, Germany), the images were acquired in adequate emission channels. The images were then viewed as stacked z-dimension images, both as single 0.5 μm optical sections or as merged images.

Results

Confirming the earlier data (Oderfeld-Nowak et al., 2001; Levine et al., 2001; Oderfeld-Nowak et al., 2003; Dawson et al., 2003) activation of resting NG2 positive oligodendroglia progenitors in spinal cord was observed during the acute phase of EAE in the parenchyma of the white and gray matter, independently of the way by which the disease was induced. Figg. 1B and E show the activation of NG2+ cells, respectively, in the white and gray matter of the spinal cord of rats in which EAE has been induced according to the Mode I (see Methods). Activated cells become thicker and their NG2 immu-

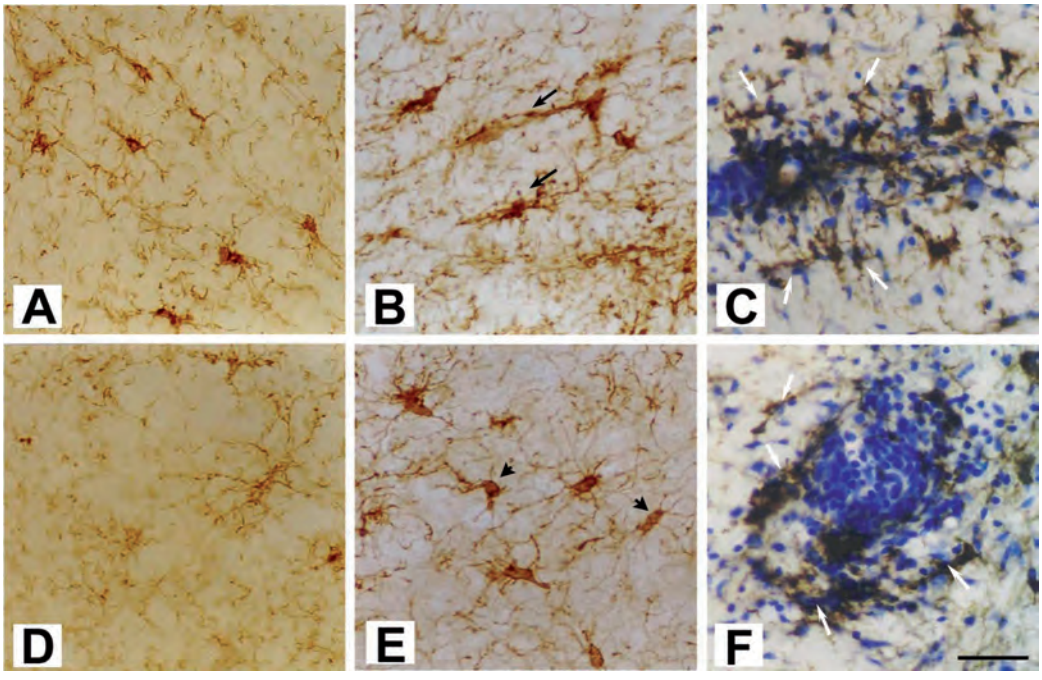

Fig. 1. - Activation of NG2 positive oligodendroglia progenitors in the white (A: control, B: EAE) and gray (D: control, E: EAE) matter of the spinal cord during the acute phase (14th day) of the disease, induced according to the Mode I (see Methods). Note: in the white matter activated NG2+ cells are of elongated shape and distributed in an arranged parallel fashion (black arrows in B) while in the gray matter are of stellate shape and distributed randomly (black arrowheads in E). NG2 immunoreactivity seen in the inflammatory foci in the white (C) and gray (F) matter of EAE spinal cord - double staining: NG2 (brown) and toluidine blue (blue), is mostly associated with vasculature (white thin arrows). Scale bar equal to: 38 μm. Color figure can be viewed in the online issue.

noreactivity increases. NG2 immunoreactivity seen in the inflammatory foci in the white (C) and gray (F) matter of EAE spinal cord, appears to be mostly associated with vasculature, possibly representing a continuous layer of activated NG2+ pericytes which tightly invest endothelial cells (Pouly et al., 2001). Interestingly, in addition to the observation that the shape of activated NG2+ cells was different in the white and gray matter, differences were also noted in the distribution of these cells in parenchyma. While the gray matter progenitor cells have a more rounded, stellate aspect and are distributed randomly (Fig. 1E), the white matter cells become elongated and distributed in an arranged parallel fashion (Fig. 1B), suggesting that they may follow specific routes. We have demonstrated that during the peak of EAE, the majority of activated elongated oligodendroglia progenitors were closely associated with activated radially oriented astroglia, independently on the way in which EAE has been induced. Figs. 2B and D illustrate this phenomenon in rats, in which EAE has been induced according to the Mode I and Figs. 3C and F in rats, in which EAE has been induced according the Mode II. Most interestingly, we have found that the activated radially oriented astroglia, while up-regulating GFAP (Figs. 2B and E, and Figs. 3A and G) re-express nestin (Figs. 2D, F and G, and Figs. 3D, H and I). The colocalization of GFAP and nestin is particularly well seen in confocal image (Fig. 3I). As shown in Fig. 3F, also activated NG2+ cells appear to express nestin.

Double immunostaining experiments with antibodies against NG2 and p75, performed only in conditions of EAE induced according to the Mode I, revealed the occurrence of colocalization of the two immunoreactivities in many of the elongated oligodendroglia progenitor cells associated with radially oriented astroglia in the spinal cord during acute

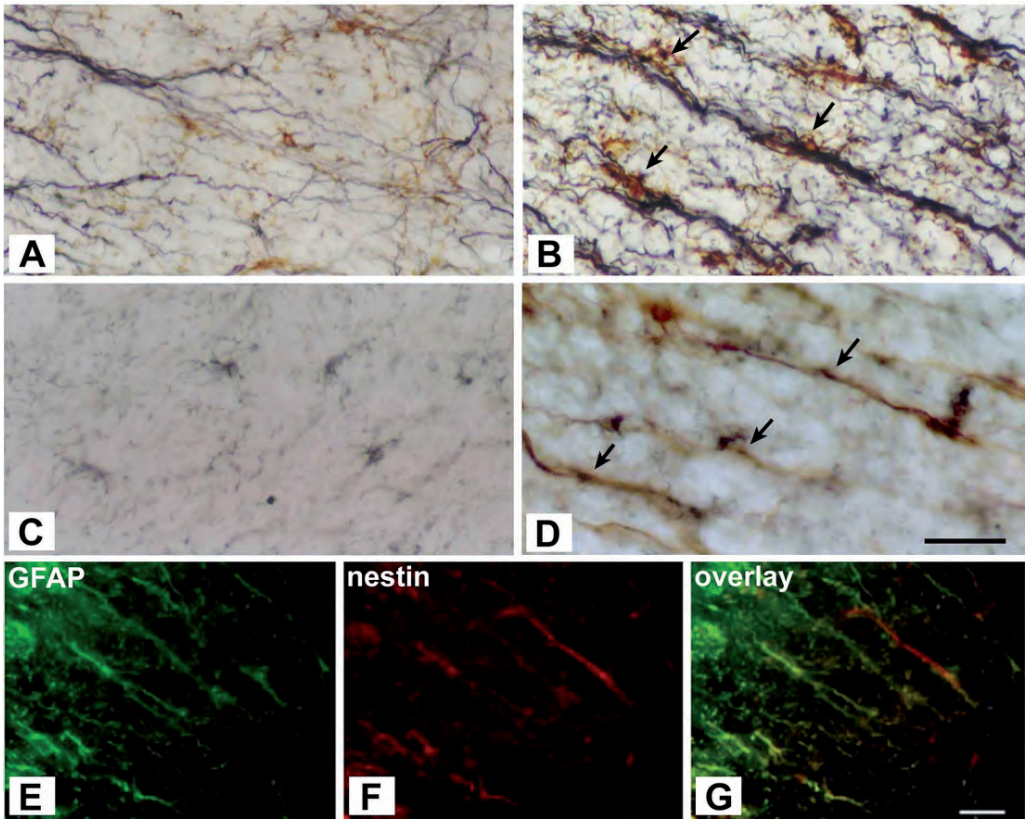

Fig. 2. - Spatial association of elongated white matter oligodendroglia progenitors with radially oriented astroglia in the spinal cord during the acute phase (14th day) of EAE induced according to the Mode I (see Methods). A-D: double immunostaining: NG2/GFAP, A: control, B: EAE, NG2 (brown - DAB), GFAP (black - DAB, Ni^{++}); NG2/nestin: C: control, D: EAE, NG2 (black - DAB, Ni^{++}), nestin (brown - DAB) (black arrows in B and D point to NG2+ cells (majority) associated with radially oriented astroglia). E-G: double immunofluorescent labeling using visualization with fluoresceine (green - GFAP) or Texas Red (red - nestin). Note colocalization of GFAP and nestin in radially oriented astroglial cells (G). Scale bar equal to 38 μm. Color figure can be viewed in the online issue.

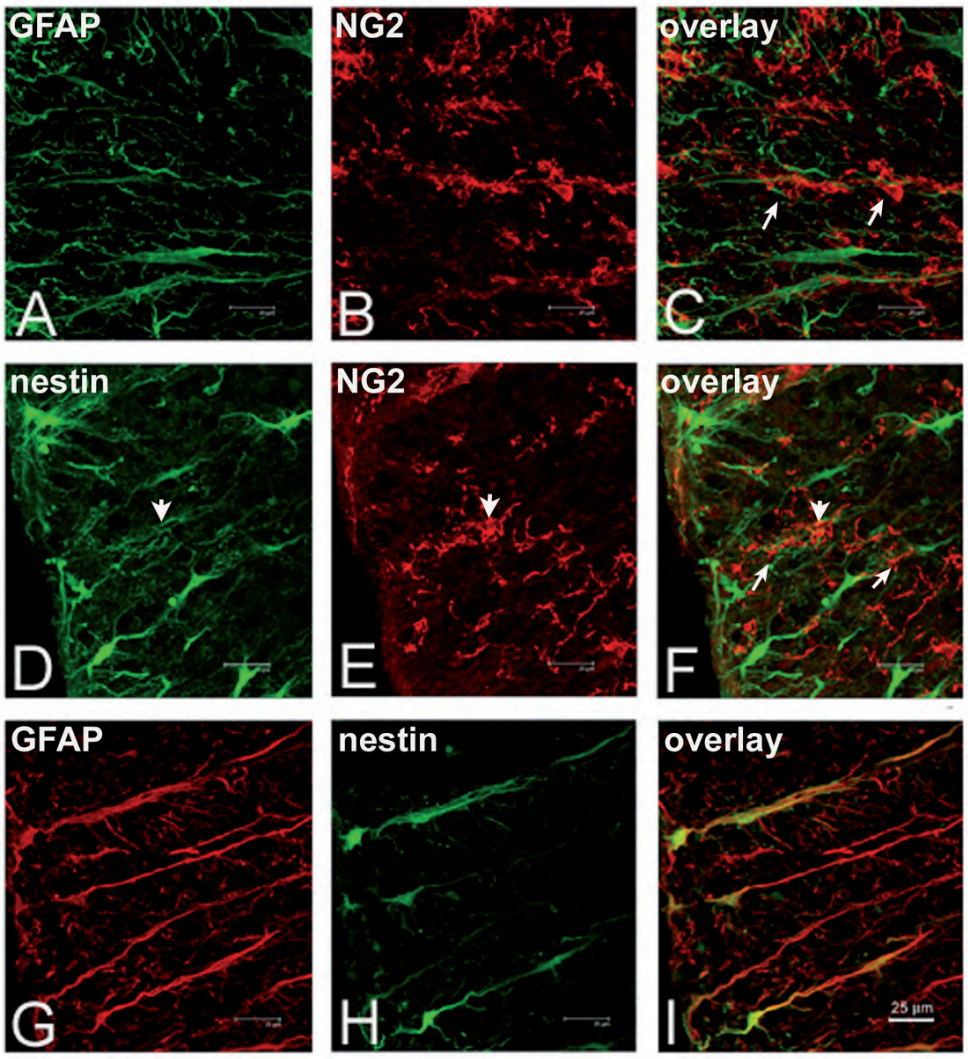

Fig. 3. - Spatial association of elongated white matter oligodendroglia progenitors with radially oriented astroglia in the spinal cord during the acute phase (5th day) of EAE induced according to the Mode II (see Methods). Double immunofluorescent labeling using visualization with fluoresceine (green) or Texas red (red) (confocal image showing maximum projection of the entire confocal stack). White arrows in C and F point to NG2+ cells (majority) associated to radially oriented astroglia; white arrowhead in D, E and F shows an example of NG2+ cell expressing NG2 and nestin (F). Note colocalization of GFAP and nestin in radially oriented astroglial cells (I). Scale bar, as indicated in I, equal to 25 µm. Color figure can be viewed in the online issue.

phase of EAE and, sporadically, in NG2+ cells in the white matter not closely associated to radially oriented astroglia (Figs. 4A-C). However, such colocalization was not observed in the stellate population of NG2 positive cells (not shown). In addition, confirming our earlier data (Oderfeld-Nowak et al., 2001, 2003), p75 expression was also observed in radially oriented astroglia cells and in round cells that are morphologically similar to oligodendrocytes (Figs. 4A-C).

As revealed by double immunostaining experiments, the ependymal cells of the central canal in EAE rats showed coexpression of NG2 and p75 immunoreactivity (Figs. 4D-F). In addition, such colocalization has also been observed in the ependymal cells of the subventricular zone (SVZ) (Figs. 4G-I).

Discussion

Our data which indicate a close association of NG2 – positive cells with radially – oriented astroglia of the spinal cord during the acute phase of EAE, suggest that these astroglia cells may serve as a scaffold for

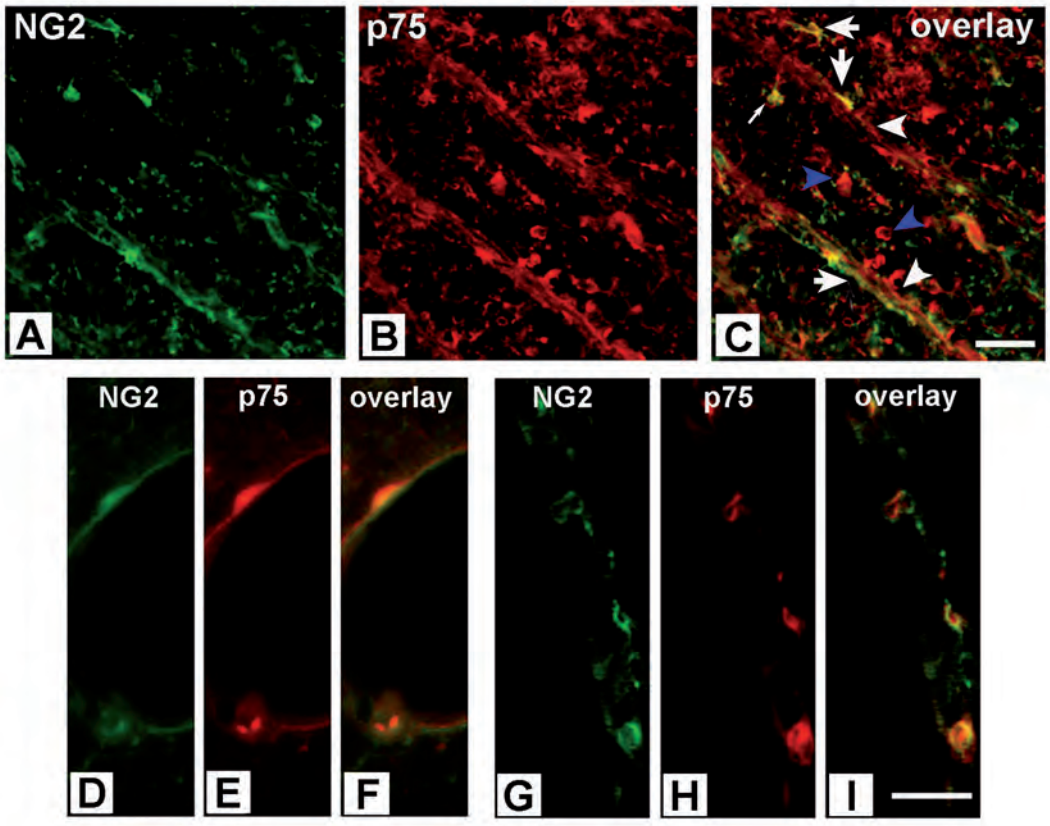

Fig. 4. - Colocalization of NG2 and p75 immunoreactivities in the cells during the acute phase (14th day) of EAE induced according to the Mode I (see Methods): A-C, in elongated oligodendroglia progenitors spatially associated with radially oriented astroglia in the spinal cord (white solid arrows in C); sporadically, p75 immunoreactivity was seen also in NG2+ cells not associated to astroglia (white thin arrow in C). Note also expression of p75 in radially oriented astroglia (white arrowheads in C) and in some round cells (blue arrowheads in C), most probably oligodendrocytes, confirming our earlier results (Oderfeld-Nowak et al., 2001, 2003); D-F, in the ependymal cells of the central canal and G-I, in the ependymal cells of the subventricular zone. Scale bar equal to 38 μm (A-C) and 28 μm (D-I). Color figure can be viewed in the online issue.

migration of activated endogenous oligodendroglia progenitors in a manner similar to the radial glia that provide a path for neuronal and oligodendroglial progenitor cells in embryonic stage. The data also raise the possibility that migrating progenitors may be derived not only from activated parenchymal pool but could also be recruited from cells of the germination areas such as the ependymal cells of the central canal. The expression of p75 receptor in elongated NG2+ cells associated to radially oriented astroglia may indicate a role for NGF in regulation of the process of migration of oligodendroglia progenitors.

Data have been provided from several studies in support of the proposal that, in EAE, adult oligodendrocyte progenitor cells migrate into the lesioned areas (Calzà et al., 1998; Nait-Oumesmar et al., 1999; Levine et al., 2001; Ben-Hur et al., 2003). Progenitors do not migrate randomly, but follow discrete paths. Several data indicate that, in embryonic and early postnatal phases, migration of glial progenitors may occur in conjunction with radial glia. The idea that glial progenitor cells use radial glial cells as scaffolding for migration in a manner similar to neuronal cells, was proposed some years ago (Goldman et al., 1997; Zerlin et al., 1995). *In vitro* studies suggest that radial glia contribute a permissive pathway along which migratory progenitors can travel and that contact with radial glia keeps progenitor cells in an immature, migratory state (Goldman et al., 1997). It has been reported that GFP-labeled progenitors from the SVZ migrate in a radial tract. This indicates that radial glial cells might be used as guidance cues (Kakita and Goldman, 1999). Diers-Fenger et al. (2001) described cells stained with antibodies to AN2 (an analog of NG2). AN2

is expressed by oligodendroglial progenitor cells in the murine embryonic spinal cord. These cells are closely apposed to and elongated along the radial glial processes. This study suggested that glial precursors, like neurons, may use radial glia as scaffolds for migration. Our results indicating that oligodendroglia progenitors in EAE spinal cord of adult rat are closely apposed to and elongated along the radially oriented astroglia seem to be in line with the above observations. In addition, Tourbah et al. (1997) found that the CG4 cells (of the progenitor cell line) transplanted into the spinal cord of EAE rats prior to induction of EAE were often associated with the radially oriented, GFAP positive cells which, according to the authors, may indicate a parenchymal migratory pathway.

It has long been known that radial glia cells which are present during development, at the end of the neuronal migration, are transformed in astrocytes and lose their specific antigens and the embryonic protein nestin. These proteins are considered to be required for these cells to play a role of path-defining cells (Chanas-Sacre et al., 2000). On the other hand, there are several indications that the processes of differentiation and trans-differentiation of the radial glia into astrocytes are bi-directional and that the transformation of the radial glia phenotype into the mature astroglia phenotype may, in certain conditions, be reversed (Chanas-Sacre et al., 2000). Grafting of embryonic Purkinje cells into the adult cerebellum or grafting of embryonic neocortical neurons into the adult somatosensory cortex induces the re-expression by host Bergmann glia or astrocytes, of nestin and of RC2 antigen, two elements of juvenile radial glial phenotype (Sotelo et al., 1994; Leavitt et al., 1999). The radial glia phenotype can be re-induced both *in vitro and in vivo*, in GFAP-positive adult cortical astrocytes in response to a diffusible factor released by embryonic forebrain (Hunter and Hatten, 1995). Sotelo et al. (1994) have shown that adult glial cells can change their phenotype expression in the presence of embryonic neurons, recapitulating transient phenotypes associated with specific stages of normal development that have been designated "adaptive rejuvenation". The appearance of embryonic markers in radially oriented astroglia of the adult spinal cord after the lesion was also demonstrated in a study by Talbott et al. (2005).

Our data that indicate the re-expression of nestin in radially oriented astroglia in the spinal cord during the acute phase of EAE seem to provide an example of such a reversal to the embryonic phenotype. This suggests that at least some of the juvenile properties of radial glia may be re-acquired. On the other hand, Shibuya et al. (2002, 2003) and Wu et al. (2005), while carrying investigations at various time points after spinal cord contusion, reported the emergence of radial glia that express nestin as well as RC2, marker of embryonic radial glia, with the peak of emergence at about four weeks. Whether in our conditions there was also some emergence of new post-natal radial glia cannot be answered without the studies of dynamic changes at different times of observations after evoking the disease.

There is now a great deal of evidence suggesting that the source of activated, and possibly, migrating oligodendroglia progenitors in EAE giving rise to new oligodendroglia, consist of cells of the resident population as well as those recruited from the SVZ and central canal zones (Calzà et al., 1998; Petratos et al., 2004). It was suggested that a population of precursor cells within the SVZ can be induced to express p75, to subsequently assume an oligodendroglial progenitor phenotype and to migrate in response to demyelination (Petratos et al., 2004). Our data which indicate that, during the acute phase of EAE, multipotential ependymal cells of the central canal and SVZ zones express NG2 and p75 immunoreactivity, provide support for the view that at least a portion of activated oligodendroglia progenitors could be recruited from the germination areas.

It has been reported that the proliferating rate of cells expressing p75 receptor in the SVZ increases during EAE (Calzà et al., 1998). As one of the possibilities the authors suggest that these proliferating cells can migrate and differentiate in glial or neural lineage. Our observation (unpublished data) that the NG2 positive oligodendroglia progenitor cells that are spatially associated with radially oriented astroglia proliferate is in line with such suggestion. In addition, the work of Aguirre and Gallo (2004) indicates the presence of migratory NG2-expressing progenitors in the subventricular zone in adult transgenic mice which can contribute to both gliogenesis and neurogenesis.

The molecular signals responsible for migration of oligodendroglia progenitors are not well known.

Several findings indicate a link between migration processes and growth factors, cytokines and adhesion molecules (Tourbah et al., 1997; Nait-Oumesmar et al., 1999; Milward et al., 2000). The data that provide support for a role for NGF in migration of glial progenitors in EAE were reported (Calzà et al., 1998, 2002, 2003; Triaca et al., 2003, 2005). NGF in EAE has been shown to promote differentiation of migrating oligodendroglia progenitors into oligodendrocytes (Calzà et al., 2003; Triaca et al., 2003). Interestingly, several authors indicate that NGF, (along with BDNF) promotes Schwann cell migration possibly via p75 receptor (Anton et al., 1994; Bentley and Lee, 2000; Cao et al., 2007).
Our present data demonstrate that expression of p75 receptor occurs on oligodendroglia progenitors during EAE. This observation suggests that glial p75 receptors could be a target for the action of neurotrophins such as NGF. In the CNS of multiple sclerosis patients, a subpopulation of elongated NG2 positive cells was also reported to contain p75 receptor (Chang et al., 2000; Petratos et al., 2004). The role of p75 in EAE has been reported (Cosgaya et al., 2002; Copray et al., 2004).
In conclusion: our data suggest that during EAE, oligodendroglia progenitor cells might migrate along the radially-oriented astroglia and that this process could be modulated by NGF via up-regulated p75 receptors in oligodendroglia progenitors. The latter would be of special interest in view of reports indicating the existence of a variety of beneficial effects of exogenously administered nerve growth factor in experimental autoimmune encephalomyelitis (Arrendono et al., 2001; Flugel et al., 2001, Ransohoff and Trebst, 2000; Villoslada and Genain, 2004). Further studies are needed to verify our assumptions.

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