Boundary Cap Cells are Highly Competitive for CNS Remyelination: Fast Migration and Efficient Differentiation in PNS and CNS Myelin-Forming Cells

V. ZUJOVIC,a,b,c J. THIBAUD,a,b,c C. BACHELIN,a,b,c M. VIDA,a,b,c F. COULPIER,d,e P. CHARNAY,d,e P. TOPILKO,d,e A. BARON-VAN EVERCOORENA,b,c,f

aUniversité Pierre et Marie Curie-Paris 6, Centre de Recherche de l’Institut du Cerveau et de la Moelle Epinière, UMR-S975, Paris, France; bInserm, U975, Paris, France; cCNRS, UMR 7225, Paris, France; dInserm, U784, Paris, France; eEcole Normale Supérieure, Paris, France; fAP-HP, Hôpital Pitié-Salpêtrière, Fédération de Neurologie

Key Words. Proliferation • Migration • Gliogenesis • Myelin repair

ABSTRACT

During development, boundary cap cells (BC) and neural crest cell (NCC) derivatives generate Schwann cells (SC) of the spinal roots and a subpopulation of neurons and satellite cells in the dorsal root ganglia. Despite their stem-like properties, their therapeutic potential in the diseased central nervous system (CNS) was never explored. The aim of this work was to explore BC therapeutic potential for CNS remyelination. We derived BC from Krox20Cre × R26RYfp embryos at E12.5, when Krox20 is exclusively expressed by BC. Combining microdissection and cell fate mapping, we show that acutely isolated BC are a unique population closely related but distinct from NCC and SC precursors. Moreover, when grafted in the demyelinated spinal cord, BC progeny expands in the lesion through a combination of time-regulated processes including proliferation and differentiation. Furthermore, when grafted away from the lesion, BC progeny, in contrast to committed SC, show a high migratory potential mediated through enhanced interactions with astrocytes and white matter, and possibly with polysialylated neural cell adhesion molecule expression. In response to demyelinated axons of the CNS, BC progeny generates essentially myelin-forming SC. However, in contact with axons and astrocytes, some of them generate also myelin-forming oligodendrocytes. There are two primary outcomes of this study. First, the high motility of BC and their progeny, in addition to their capacity to remyelinate CNS axons, supports the view that BC are a reservoir of interest to promote CNS remyelination. Second, from a developmental point of view, BC behavior in the demyelinated CNS raises the question of the boundary between central and peripheral myelinating cells. Stem Cells 2010;28: 470–479

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION

Animal models of demyelinating diseases have provided overwhelming evidence for Schwann cell (SC) capacity to regenerate and remyelinate central nervous system (CNS) axons (reviewed in [1–3]). However, the clinical translation of this strategy has been hindered so far by the poor capacity of SC to migrate in the lesioned CNS. This failure is attributed to a strong SC-astrocyte, cadherin-mediated interaction [4], which results in mutual exclusion of the two cell types and boundary formation [5–8]. In addition, grafted SC do not migrate in CNS white matter, suggesting the presence of inhibitory cues preventing their mobility in the CNS [5, 9, 10]. While most SC transplantation studies have focused on committed SC harvested from neonate or adult peripheral nerves, recent data indicate that more immature stages of the lineage, such as SC precursors, are more suitable for CNS transplantation in terms of survival and integration in the CNS [11]. However, the therapeutic potential of peripheral nervous system (PNS) stem cell for CNS repair has not been investigated so far.

During development, SC and their precursors originate from the multipotent neural crest cells (NCC), which generate the majority of dorsal root ganglia (DRG) neurons and glia during their first wave of migration. In addition, emigrating NCC give rise to boundary cap cells (BC), which arrest transiently at the dorsal root entry zone and motor exit point between embryonic day E10.5 and birth in the mouse [12].

Author contributions: V.Z.: Conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; J.T.: Collection and/or assembly of data, data analysis and interpretation; M.V.: Collection and/or assembly of data, data analysis and interpretation; M.V.: Collection and/or assembly of data, data analysis and interpretation; F.C.: Provision of study material or patients; P.T.: Provision of study material or patients, manuscript writing; F.C.: Provision of study material or patients, manuscript writing; P.T.: Provision of study material or patients, manuscript writing; A.B.-V.E.: Conception and design, data analysis and interpretation, financial support, administrative support, manuscript writing, final approval of manuscript.

Correspondence: A. Baron-Van Evercooren, PhD, INSERM UMR-S 975, CHU Pitié-Salpêtrière, 105 boulevard de l’Hôpital, 75634 cedex 13, Paris, France. Telephone: +33-1-4077-8129; Fax: +33-1-4077-8117; e-mail: anne.baron@upmc.fr. Received August 19, 2009; accepted for publication December 9, 2009; first published online in Stem Cells Express December 28, 2009. © AlphaMed Press 1066-5099/2009/$30.00/0 doi: 10.1002/stem.290

Stem Cells 2010;28:470–479 www.StemCells.com
BC behave as gatekeepers of the CNS-PNS boundary because they prevent motor neuron exit toward the periphery, while allowing axons to grow through [13]. Between E10.5 and E15.5, BC are the only cells in the PNS to express the transcription factor gene Krox20 (also known as Egr2) [13, 14]. Genetic tracing of BC using different knock-in of reporter gene in the Krox20 locus revealed that, during a second wave of migration, BC give rise to all spinal root SC and a subpopulation of DRG neurons and satellite cells [12]. Analysis of cell preparations containing BC suggested their multipotency and self-renewal ability [15]. Furthermore, when these preparations were primed to differentiate in SC in vitro and were transplanted in the axotomized sciatic nerve, they generated myelinating SC [16]. In this work, we used a Krox20-based Cre-lox fate mapping system to characterize precisely BC molecular signature in vitro and follow their fate after transplantation into the CNS, using two grafting methods to test their repair potential in the demyelinated spinal cord. We show that BC derivatives actively migrate toward the lesion, where they efficiently differentiate into SC, which leads to extensive remyelination. Interestingly, we found that BC progeny has the ability to generate myelin-forming oligodendrocytes. Our data substantiate BC potential in CNS myelin repair and raise novel issues concerning CNS-PNS trans-differentiation.

### MATERIALS AND METHODS

**Mouse Lines**

The mice used in this study were all of a mixed C57Bl6/DBA2 background. In the Krox20<sup>Cre</sup> line, the Krox20 coding sequence was replaced by knock-in of the Cre recombinase coding sequence [17]. In the R26<sup>RFP<sup>+</sup></sup> line, YFP is expressed in the ROSA locus after Cre recombination [18]. Three-month-old nude mice were purchased from JANVIER (Le Genest St Isle, France, http://www.janvier-europe.com). All animal protocols were performed in accordance with the guidelines published in the National Institute of Health Guide for the Care and Use of Laboratory Animals.

**Cell Preparation and In Vitro Characterization**

Cell preparations containing BC were prepared as described previously [12]. Briefly, meninges were microdissected from E12.5 Krox20<sup>Cre<sup>+</sup>/R26<sup>RFP<sup>+</sup></sup> embryos and dissociated in a collagenase (Sigma-Aldrich, St. Louis, http://www.sigma-aldrich.com) and trypsin (Invitrogen, Carlsbad, USA, http://www.invitrogen.com) mix. Cells were either seeded for cell culture or grafted in the spinal cord. For cell culture, the preparations were resuspended in NEF medium consisting of Dulbecco’s modified Eagle’s medium (DMEM)/F12 medium (1:1) supplemented with N2 (1%), B27 (0.5%), glucose (6 mg/ml), HEPES (5 mM; Invitrogen), insulin (25 μg/ml) fibroblast growth factor (FGF2) (20 ng/ml; Sigma) and epidermal growth factor (EGF) (20 ng/ml; Sigma). DMEM and F12 contain 1% of nonessential amino acids, penicillin/streptomycin, and sodium pyruvate (Invitrogen). For the negative fraction and committed SC preparation, see supplemental online information.

To characterize BC progeny phenotype, acute cell preparations were plated in 4-well plates (25,000 cells/well). After 4 h in Neurobasal media supplemented with FGF and EFG (NEF), cells were fixed with 2% paraformaldehyde (PFA), washed with phosphate-buffered saline, and processed for immunocytochemistry (supplemental online information).

**Demyelination and Cell Transplantation**

Nude mice were anesthetized with intraperitoneal injections of ketamine (100 mg/kg) and xylazine (10 mg/kg). Demyelination was performed by stereotaxic injection of 2 μl of 1% solution of lyso-phosphatidylcholine (LPC; Sigma) in saline in the dorsal spinal cord white matter funiculus. The demyelination site was marked with charcoal. Forty-eight hours after injection, acutely dissociated cells (2 μl, 5.10^6 cells/μl) were engrafted in the demyelinated lesion (<i>n</i> = 4 animals per time point). To evaluate BC migration potential, acutely dissociated cells corresponding to the total fraction or YFP-negative cells were engrafted one vertebra caudally to the lesion (<i>n</i> = 6 animals at 1 week; <i>n</i> = 12 animals at 4 weeks).

**RESULTS**

**BC Preparation and Characterization**

To perform transplantations of BC into the CNS, we have set up procedures to prepare these cells and to follow their migration and differentiation within the host. We previously developed a tracing system that takes advantage of the restricted expression of Krox20 in BC between E10.5 and E15.5. It is based on the use of a Krox20 allele carrying a knock-in of the Cre recombinase gene (Krox20<sup>Cre</sup>), leading to expression of Cre that parallels that of Krox20 [17]. The Krox20<sup>Cre</sup> mouse line was crossed with the reporter mouse strain R26<sup>RFP<sup>+</sup></sup> [18], leading to permanent, Cre-mediated expression of the YFP gene. This system allows persistent labeling of BC derivatives, although expression of Krox20 is usually not maintained in these cells [12]. We also have previously shown that BC are intimately associated with meninges, which can be obtained by microdissection and used as a source of BC [19]. In this work, meninges were microdissected from E12.5 Krox20<sup>Cre</sup>/R26<sup>RFP<sup>+</sup></sup> embryos, and dissociated cells were used either for cell culture or grafting experiments. Analysis of YFP expression after 4 hours in culture established that 7 ± 1% of cells were positive, indicating that they were either BC or BC derivatives. We used these short-term cultures to further characterize the precise molecular identity of YFP<sup>+</sup> cells by immunostaining (supplemental online information). Triple labelings for Hoechst, YFP, and cell stage–specific markers to visualize all cells showed that the majority of YFP<sup>+</sup> cells expressed the NCC markers Sox9 (81 ± 19%), nestin (99 ± 1%), Sox2 (99.3 ± 1%, a marker expressed until the immature SC stage), p75 (98.5 ± 1%), and Sox10 (79.5 ± 18%), markers expressed throughout the SC lineage. The majority of YFP<sup>+</sup> cells were negative for S100, a feature of immature and mature SC, but were positive (98.6 ± 1.3%) for polysialylated neural cell adhesion.
molecule (PSA-NCAM), which is not expressed by SC and their precursors. These data are consistent with BC constituting an intermediate stage between NCC and the remaining SC lineage. The majority of the YFP<sup>+</sup> cells present a phenotype similar to BC with the exception of Krox20 expression, suggesting that the negative fraction is mainly composed of immature NCC, the remaining 10% corresponding most likely to meningeal fibroblasts.

### Proliferation of BC Grafted in the Lesioned Spinal Cord

To investigate BC behavior after transplantation in the CNS, we used a demyelination model obtained by injection of LPC in the dorsal funiculus of nude mouse spinal cord. Although it was possible to purify YFP<sup>+</sup> cells by fluorescence-activated cell sorting (FACS) from the meninges preparation, we could not use them for transplantation because the number of collected cells was too small to allow engraftment of a sufficient number of animals and appropriate survival of the transplant. Therefore we performed grafts of the total cell preparations derived from E12.5 Krox20<sup>Cre</sup>;<R26RYFP<sup>+</sup> meninges. The cell preparations (2 μl, 5 × 10<sup>4</sup> cells/μl) were transplanted in the lesion 48 h after LPC injection. While only few YFP<sup>+</sup> cells were identified in the host parenchyma 2 days post-transplantation (p.t.) (Fig. 1A), the number of YFP<sup>+</sup> cells increased dramatically at 1 week (Fig. 1B) and 4 weeks p.t. (Fig. 1C) p.t. Because the spreading of YFP<sup>+</sup> cells in the lesion could have resulted from intense proliferation of the BC derivatives, we investigated the proliferation of YFP<sup>+</sup> cells by immunostaining for the mitotic marker Ki67 (Fig. 1D, 1E). Two days p.t., >50% of the YFP<sup>+</sup> cells were Ki67<sup>+</sup> (Fig. 1D, 1F). However, this proportion dropped to 20% after 1 week, and only rare YFP<sup>+</sup> cells were Ki67<sup>+</sup> at 4 weeks p.t. (Fig. 1F). These data are consistent with the expansion of the YFP<sup>+</sup> population being largely due to intense proliferation of BC and their derivatives early after grafting. However, this proliferative activity appeared to be highly regulated because it decreased dramatically with time and correlated with a change in morphology, evolving from a round or unipolar immature phenotype (Fig. 1A) to a more bipolar elongated shape (Fig. 1B, 1C), strongly suggesting BC commitment to a mature SC phenotype.

### Mobilization of BC by Distant Demyelinating Lesion

Committed SC do not migrate efficiently in the CNS [1]. The impressive expansion of BC, when grafted in spinal cord lesions, prompted us to investigate their potential for migration in this structure. Acute cell preparations were grafted one vertebra caudally from a lesion targeted to the dorsal funiculus, and migration was analyzed 1 week and 4 weeks p.t. (Fig. 2). After 1 week p.t., the majority of the YFP<sup>+</sup> cells were found away from the graft along the dorsal midline and at the level of the lesion (Fig. 2A–2C). On the basis of the migrating frontline, the migration distance was estimated at 6 mm, with numerous cells having reached the lesion. At 4 weeks p.t., YFP<sup>+</sup> cells formed a dense ribbon along the midline, joining the graft and lesion area and beyond (Fig. 2D–2F). Thus, BC derivatives, in contrast to committed SC, possess a high migratory potential.

To confirm that YFP<sup>+</sup> cells observed in the spinal cord are derived from BC, we repeated the transplantation experiments using cell preparations depleted of YFP<sup>+</sup> cells by FACS after isolation, and therefore depleted of BC and their derivatives (we refer to this cell preparation as the negative fraction, as opposed to the total fraction). Analysis of such grafts revealed that only rare YFP<sup>+</sup> cells were observed 1 week p.t. (supplemental online information), suggesting that at this stage the large majority of the YFP<sup>+</sup> cells observed after grafting of the total fraction is indeed derived from BC. Furthermore, most of the cells of the negative fraction were found away from the graft site and did not migrate over 1 mm (data not shown). These data undermine a possible influence of the negative fraction on the total fraction migration. At 4 weeks p.t., the number of YFP<sup>+</sup> cells in the negative fraction increased but stayed below that observed with the total fraction (supplemental online information). This indicates that, even at that stage, the majority of the YFP<sup>+</sup> cells detected after transplantation of the total fraction was derived from
The presence of YFP$^+$ cells in the case of the negative fraction might be due to intense proliferation of BC-derived cells that might have escaped the FACS selection or to de novo activation of the Krox20Cre locus after transplantation.

Integration of BC Derivatives with Astrocytes and White Matter

When grafted in or caudally from a lesion induced in the dorsal funiculus, committed SC show poor interaction with astrocytes and no interaction with CNS myelin [20]. We therefore questioned whether the migratory potential of YFP$^+$ cells was associated with improved interactions with astrocytes or white matter. At 1 week p.t., immunolabeling for the astrocyte marker GFAP showed that YFP$^+$ cells (green, A, B) intermingled with GFAP$^+$ astrocytes (red, A, C). At 4 weeks p.t. (D–F), YFP$^+$ cells (green, D, E) were clearly isolated and excluded from GFAP$^+$ area (red, D, F). At 1 week p.t., YFP$^+$ cells (green, G, I) were present in MOG$^+$ area (red, G, H). Dotted lines delineate the margin of the GFAP$^+$ (A–F) or MOG$^+$ area (G–I).

Figure 2. Efficient migration of boundary cap cells when grafted one vertebra away from the lesion. Spinal cords horizontal section 1 week post-transplantation (p.t.) (A–C) and 4 weeks p.t. (D–F). At 1 week p.t., YFP$^+$ cells were located in the graft (B), extended in the dorsal column, up to the lesion (C). At 4 weeks p.t., YFP$^+$ cells formed a thick ribbon along the dorsal column (E) and invaded the lesion (F).

Figure 3. Successful integration of boundary cap cells among astrocytes and white matter. At 1 week post-transplantation (p.t.) (A–C), YFP$^+$ cells (green, A, B) intermingled with GFAP$^+$ astrocytes (red, A, C). At 4 weeks p.t. (D–F), YFP$^+$ cells (green, D, E) were clearly isolated and excluded from GFAP$^+$ area (red, D, F). At 1 week p.t., YFP$^+$ cells (green, G, I) were present in MOG$^+$ area (red, G, H). Dotted lines delineate the margin of the GFAP$^+$ (A–F) or MOG$^+$ area (G–I).
information). Immunohistochemistry for chondroitin sulfate proteoglycan (CSPG), a marker of glial scar, showed that this observation was correlated with lower CSPG expression around BC grafts compared to SC grafts (supplemental online information), reflecting a decreased host response to BC grafts compared to SC grafts.

Furthermore, analysis of MOG expression, a specific marker of CNS myelin, highlighted the presence of numerous YFP+ cells in white matter tracts with 35 ± 2.8% YFP+/MOG+ area overlap 1 week p.t. (Fig. 3G–3I) but few (<1%) at 4 weeks (see below Fig. 6H). Interestingly, these periods of overlap followed by exclusion matched the successive migratory and differentiation phases of the transplanted cells.

Because, unlike SC, YFP+ cells express PSA-NCAM in vitro (supplemental online information), and ectopic expression of PSA-NCAM promotes SC integration in the injured [21] or demyelinated [20] spinal cord, we examined PSA-NCAM expression by the grafted cells at 1 and 4 week p.t. Although YFP+ cells did not express PSA-NCAM once differentiated in committed cells (4 weeks p.t.; Fig. 4E), several clusters of YFP+ cells expressing PSA-NCAM were identified during the migration phase (1 week p.t.; Fig. 4A–4D), suggesting the potential involvement of PSA-NCAM in BC derivatives improved integration in the CNS.

**Differentiation of the Grafted BC into Functional Myelin-Forming SC**

The progressive cessation of BC derivatives proliferation after transplantation of the total fraction in the lesion suggested their engagement in differentiation. Double staining for YFP and P0, a specific protein of peripheral myelin, showed large overlap between YFP+ and P0+ areas within the lesion 4 weeks p.t. (supplemental online information). These data indicate that most BC differentiated into SC (90% of YFP+ cells produce P0+ myelin, Suppl. Table 2) and achieved widespread remyelination of CNS axons.

Engraftment of SC remotely from a lesion causes a delay in their ability to compete with endogenous cells to repair the lesion [10]. In these conditions, and as a result of their slow migration in the CNS, committed SC participate minimally in CNS remyelination (<0.1%) [20]. Quantification of the P0+ and YFP+ areas within the lesion region revealed that the domains of overlap represented 75% of the P0+ areas (Fig. 5A–5E; supplemental online information), indicating that recruited BC derivatives largely outcompeted endogenous SC for myelin repair. This process was timely regulated. Indeed, at 1 week p.t., some of the YFP+ cells still expressed immature markers with 10% PSA-NCAM+ (Fig. 4), 5% Sox2+, 5%, Nestin+, and <50% p75+ (supplemental online information). However, none of these markers were detected at 4 weeks p.t., except for p75, which was detected in <5% of the YFP+ population, highlighting the absence of immature cells at later times.

To confirm that the myelin-forming YFP+ cells were of BC origin, we repeated these experiments using the negative fraction. Quantification of the P0+ and YFP+ areas within the lesion region revealed that the domains of overlap represented only 10% of the P0+ areas in the case of grafts performed with the negative fraction, as compared to 75% in the total fraction (supplemental online information). These observations indicate that the BC-depleted cell preparation is not able to provide cells that efficiently compete with endogenous cells for the formation of peripheral myelin in the lesion, implying therefore that the YFP+ cells of the total cell preparation, which outcompete the endogenous ones, are essentially BC derivatives.

Finally, we questioned the quality of myelin repair performed by BC derivatives, examining markers associated with mature nodes of Ranvier (Fig. 5F, 5G). Immunostaining for Nav, sodium channels present at the node, and for the paranodal protein Caspr, revealed YFP+ cells associated with normal organization of the node/paranode structure (Fig. 5F). Likewise, YFP+/P0+ internodes were normally flanked by Caspr+ paranode structures (Fig. 5G). Thus the progeny of the exogenous BC appears to efficiently participate in the restoration of structurally functional nodes of Ranvier on host axons.

**Differentiation of BC Progeny in Myelin-Forming Oligodendrocytes**

Because BC are candidate stem cells [15], we investigated whether the grafted cells could differentiate in cell types other than SC when grafted in the demyelinated CNS. We failed to detect YFP+ expressing the astrocyte marker GFAP, the smooth muscle antigen SMA, the neural markers III-tubulin, or the PNS neuronal marker peripherin (supplemental online information). However, some of the YFP+ cells expressed the post-mitotic oligodendrocyte marker CC1 (Fig. 6A–6D). Moreover, YFP+ cells expressed Olig2, a transcription factor specific of the oligodendrogial lineage (Fig. 6E–6G), and MOG, marker a specific of mature oligodendrocytes (Fig. 6H); this expression was confirmed with confocal microscopy (Fig. 6I). Triple immunolabeling for YFP, MOG, and βIII-tubulin to identify axons showed that YFP+/MOG+ processes surrounded βIII-tubulin+ axons, suggesting that the BC-derived oligodendrocytes ensheathed axons (Fig. 6J–6L). We questioned the presence of potential CNS contaminants in the negative fraction. However, the presence of MOG+ and YFP+ myelin was never observed in animals grafted with the negative fraction. These data strongly suggest that some of...
the transplanted BC progeny differentiated in myelin-forming oligodendrocytes.

BC-derived remyelination potential was further investigated by immuno–electron microscopy, visualizing YFP with BluoGal precipitates (Fig. 7, white arrows). The majority of BluoGal$^+$ cells presented specific SC features, with one cell associated to one axon and the presence of a basal membrane and collagen fibrils around the compacted myelin (Fig. 7A). However, few typical thin compacted CNS myelin sheaths labeled with BluoGal precipitate were also detected (Fig. 7B). These BluoGal$^+$ myelin sheaths were specific of the grafted cells because they were never observed outside of the lesion area. The CNS origin of BluoGal$^+$ myelin sheaths was determined by the absence of cell cytoplasm connected to myelin as well as that of a basement membrane and collagen, typical features of PNS myelin [22]. Moreover, these BluoGal$^+$ myelin sheaths were always thinner [23] than PNS BluoGal$^+$ myelin. Interestingly, BluoGal$^+$ CNS myelin sheaths were essentially localized in astrocyte-rich area.

**DISCUSSION**

BC are NCC derivatives, which contribute to generate PNS neurons and glia. While BC integrate within the normal PNS after grafting, their capacity to interact and respond to CNS environment was unexplored. To investigate their potential interest for CNS repair, we have used fate mapping and cell transplantation in the demyelinated spinal cord. We provide evidence that grafted BC integrated and migrated profusely in the demyelinated CNS. This capacity, mediated by improved mixing with astrocytes and myelin, allowed them to compete aggressively with endogenous myelin-forming cells for myelin repair, generating not only myelin-forming SC but also oligodendrocytes.

**BC Molecular Signature Defines BC as a Distinct Niche from NCC and SC Precursors**

On the basis of their contribution to both glial and neuronal lineages and their self-renewing capacity [15, 16, 24], it was suggested that BC are multipotent stem cells. Moreover, cellular preparations containing BC were isolated from E11 spinal nerve roots and were shown to express markers similar to NCC [15, 16]. However, the lack of tracing and clear identification of the microdissected population undermined the conclusion of these studies. Combining microdissection and cell fate mapping, we were able to isolate BC more efficiently based on their exclusive expression of Krox20 at E12.5. Short-term cultures (4 hours) provided an unambiguous demonstration that the molecular signature of Krox20 YFP$^+$ BC is unique but closely related to NCC. While BC express several markers common to the SC lineage (Sox10, p75), their molecular signature diverges from NCC by the expression of Krox20 and differs from the SC precursor stage because BC are immunolabeled for Sox9 and PSA-NCAM.

**Successful BC Migration Results from Favorable Interaction with Astrocytes**

A comparative study between SC and SC precursors suggested that precursors integrate and migrate more efficiently than SC when grafted in the CNS [11]. Because BC progeny integrated very efficiently among CNS myelin and astrocytes when grafted in the lesion (not shown), we speculated about their capacity to exit the glial boundary and migrate through white matter. Grafting BC remotely from the lesion, we found that BC progeny migrated profusely to the lesion site. This migratory process took place in an orderly sequence with cells first migrating from the graft to the spinal cord midline (data...
not shown), then migrating in a ribbon along the midline toward the lesion. BC migration was far more efficient than that of SC grafted in the same conditions, with cells migrating in multiple rows across white matter and at a speed of 36 μm/h (6 mm/week), compared to SC migrating in single rows and excluded from white matter, at a speed of 12 μm/h (2 mm/week) [20]. Multiple reasons may account for the successful migration of BC progeny in the CNS. First, while mature SC induce a strong astrocyte boundary that is likely to restrain their exit from the graft, BC progeny intermingled smoothly with astrocytes, which allowed them to spread beyond the lesion border and exit from the graft to initiate migration. Whereas SC-induced astrocyte response is characterized by upregulation of scar molecules such as proteoglycans [25], BC-improved interaction with astrocytes correlated with low levels of CSPG, compared to those induced by committed SC grafts. Second, BC progeny was dispersed in myelinated tracks, suggesting that, unlike SC, they did not sense the inhibitory cues of CNS myelin. Moreover, unlike SC and SC precursors [11], acutely isolated BC expressed PSA-NCAM, which is known to play an important role in SC migration [20, 26]. Thus, the high migratory performances of BC progeny might also be partly explained by their expression of PSA-NCAM. However, PSA-NCAM−forced expression in SC improved their integration among astrocytes but not among CNS myelin, and SC precursors, which do not express PSA-NCAM, migrate within white matter. We therefore speculate that mechanisms independent of PSA-NCAM expression may also account for the high motility of BC derivatives through CNS white matter.

**Demyelinating Conditions Favor BC Differentiation in Myelin-Forming Glia**

Stem-like cells often display a tendency to maintain an undifferentiated phenotype or differentiate into inappropriate cell types [27–29]. This is especially relevant to the NCC lineage, which shows high plasticity in terms of generating multiple lineages and capacity, once committed, to reverse into multipotent stem cells [30, 31]. While such plasticity could be undesirable for cell-based therapy, we provide evidence that BC proliferation and differentiation were timely regulated processes in demyelinating conditions.

We show that, after engraftment in the demyelinated lesion of the spinal cord, BC progeny not only survived up to 4 weeks but also colonized the lesion massively. In fact, YFP+ cells proliferated actively during the first week p.i. This proliferating activity was timely regulated because proliferation resumed in time before cell differentiation, and tumor formation was not observed. Similar observations were made.

**Figure 6.** Boundary cap cell progeny gives rise to mature oligodendrocytes. YFP+ cells (green, A–C, E, F) express CC1 (red, A, B, D) with a typical perinuclear labeling (red, B, D) and nuclear Olig2 (red, E, G), an oligodendroglial lineage marker. MOG+ (red)/YFP+ (green) cells were also detected (H, arrows). Three-dimensional reconstruction of the marked cell (I, arrows). YFP+ (green, J, K)/MOG+ (blue, J, L) processes surrounded βIII tubulin+ (red, J–L) axons.
when SC precursors were grafted in the demyelinated or injured spinal cord [11, 32]. This indicates that two different immature populations of SC lineage have the ability to respond to CNS cues when forced by transplantation in the injured CNS.

We then established that, even if BC are stem cell candidates, they did not undergo incomplete or inappropriate differentiation. Despite their very immature phenotype prior to grafting, BC downregulated their expression of immature markers such as Sox2, Nestin, p75, and PSA-NCAM in correlation with the emergence of P0, a marker of mature myelinating SC, 4 weeks p.t. Furthermore, being very closely related to NCC, BC could have harbored their multipotency and therefore differentiate in neurons and smooth muscle cells. While they were able to do so in vitro (data not shown), we failed to detect SMA+ smooth muscle cells or neuronal phenotypes among the grafted cells in vivo. Although we found no in vitro evidence of spontaneous differentiation of BC in myelin-forming cells, our in vivo experiments showed the presence of YFP+ mature myelinating SC and oligodendrocytes. These observations highlight that demyelinating conditions provide cues that drive BC essentially into the myelogenic pathway.

**BC Generate Functional SC and Oligodendrocytes**

When acute BC preparations were grafted in the intact rat sciatic nerve, they differentiated in myelin-competent SC only when primed to become SC in vitro prior engraftment [16]. However, their myelinating potential in the absence of priming has not been assessed to date. We show that in vitro priming is not necessary to induce BC differentiation in myelin-forming cells when grafted in the demyelinated spinal cord. Their direct involvement in the remyelination process was sustained by the presence of YFP+ cells associated in a 1:1 relationship with axons and with YFP+/P0+ PNS myelin, which have been shown by immuno-electron microscopy to be clearly compacted and surrounded by a basement membrane and collagen fibrils. The discrepancy between the two studies may be explained by different lesion environments eliciting different graft responses, different compositions of the grafted populations, or the difficulty in the former study to trace cells in the absence of a reliable fate mapping system.

We also provided evidence that, after migration >2 mm, BC progeny was able to participate massively to remyelination because it contributed to >70% of the newly formed PNS myelin. This is in contrast with PSA-NCAM overexpressing SC, which after migration contributed to only 1% of the newly formed PNS myelin, whereas normal SC were unable to do so [20]. Thus, not only did BC progeny migrate toward the lesion, but YFP+ cells migrated and differentiated in such a manner that they out-competed endogenous SC for remyelination. In addition, BC remyelination led to the re-establishment of nodes of Ranvier, a process known to correlate with improved neural conduction [33, 34]. The participation of BC progeny to remyelination was also confirmed when the negative YFP fraction was grafted away from the lesion. The YFP+ cells, representing most likely a more immature neural crest-like phenotype, were indeed much less efficient to participate to CNS repair. Cells of the negative fraction could have contributed to BC migration and remyelination potential. However, their delayed migration and differentiation in myelin forming SC undermine a possible influence of the negative fraction on the total fraction.

**CNS Versus PNS Fate: Trans-differentiation or Reprogramming?**

The boundary between central and peripheral myelin-forming cells is first obvious when, during development, the two cell populations derive from different neural compartments, with oligodendrocytes arising directly from the neural tube, and SC from the neural crest. During that time, SC and oligodendrocyte lineages are under the influence of different morphogenic factors with bone morphogenetic protein (BMP) and Sonic hedgehog driving SC and oligodendroglial lineages, respectively. Several transplantation studies in the adult CNS highlighted the possibility that CNS precursors could be reprogrammed to differentiate along the neural crest lineage in response to certain environmental signals of CNS lesions [35]. While the precise molecular mechanisms involved in such trans-differentiation remains to be determined, recent data identified a role for BMP in instructing CNS precursors into the SC fate [36]. Previous studies suggested the possibility that neural crest-derived stem cells such as bone marrow [37] or epidermal NCC [38] can give rise to oligodendrocytes in the spinal cord. The present data provide solid proof both at the immunohistochemical and ultrastructural levels that immature neural crest derivatives can give rise in vivo to both CNS and PNS myelinating cells, thus indicating the possibility of trans-differentiation of CNS precursors into CNS cells.
However, in the absence of demonstration of co-expression of PNS and CNS markers in the same YFP+ cells, the possibility of dedifferentiation and reprogramming should not be disregarded. Interestingly, BC-derived oligodendrocytes were observed only in astrocyte-rich areas, suggesting that astrocyte-derived factors possibly involved in BMP signaling may have accounted for this process.

**CONCLUSION**

The clear incompatibility between exogenous SC and the CNS environment has remained a major obstacle to their use for CNS remyelination. This study highlights that BC, due to their stem-like features, present several advantages over SC and BC precursors when grafted in the demyelinated spinal cord. Further developments should clarify the molecular mechanisms involved in their successful interactions with the CNS and highlight their potential presence in adult peripheral tissues. Indeed, recent data indicate the presence of NCC stem cells in the adult DRG, suggesting the persistence of BC in the adult [39, 40]. Alternatively, finding ways to reprogram committed cells to such immature phenotype may open the gate to their therapeutic exploitation for CNS demyelinating diseases.

**ACKNOWLEDGMENTS**

We thank members of the Baron-Van Evercooren team for critical reading of the manuscript and C. Javallet and R. Panic for their technical assistance. We thank E. Peles for Caspr antibody, and D. Langui of the Imaging and Cell-Sorting Platforms at the Salpétrière Hospital for technical assistance. This work was supported by a grant from the United States National Multiple Sclerosis Society (NMSS) and the NRJ Prize. V.Z. was supported by a fellowship from the French multiple sclerosis associations, and J.T. was supported by a fellowship from the NMSS and ARSEP (Association pour la recherche sur la sclérose en plaque). A.B.-V.E. is a recipient of a Contract d’Interface AP-HP/Fédération de Neurologie, Hôpital Pitie-Salpêtrière.

**REFERENCES**

1. Zujovic V, Bachelin C, Baron-Van Evercooren A. Remyelination of the central nervous system: a valuable contribution from the periphery. Neuroscientist 2007;13:383–391.
2. Lavdas AA, Papastefanaki F, Thomaidou D et al. Schwann cell transplanation for CNS repair. Curr Med Chem 2008;15:151–160.
3. Kocsis JD, Waxman SG. Schwann cells and their precursors for repair of central nervous system myelin. Brain 2007;130:1978–1980.
4. Fairless R, Frame MC, Barnett SC. N-cadherin differentially determines Schwann cell and olfactory ensheathing cell adhesion and migration responses upon contact with astrocytes. Mol Cell Neurosci 2005;28:253–263.
5. Franklin RJ, Crang AJ, Blakemore WF. Transplanted type-1 astrocytes facilitate repair of demyelinating lesions by host oligodendrocytes in adult rat spinal cord. J Neurocytol 1991;20:420–430.
6. Franklin RJ, Crang AJ, Blakemore WF. The reconstruction of an astrocytic environment in glia-deficient areas of white matter. J Neurocytol 1993;22:382–396.
7. Lakatos A, Barnett SC, Franklin RJ. Olfactory ensheathing cells induce less host astrocyte response and chondroitin sulphate proteoglycan expression than Schwann cells following transplantation into adult CNS white matter. Exp Neurol 2003;184:237–246.
8. Blakemore WF, Crang AJ, Curtis R. The interaction of Schwann cells with CNS axons in regions containing normal astrocytes. Acta Neuro pathol 1986;71:295–300.
9. Oudega M, Xu XM. Schwann cell transplantation for repair of the adult spinal cord. J Neurotrauma 2006;23:453–467.
10. Baron-Van Evercooren A, Gansmuller A, Duhamel E et al. Repair of a myelin lesion by Schwann cells transplanted in the adult mouse spinal cord. J Neuroimmunol 1992;40:235–242.
11. Woodhoo A, Sahni V, Gibson J et al. Schwann cell precursors: a favourable cell for myelin repair in the central nervous system. Brain 2007;130:2175–2185.
12. Maro GS, Vermeren M, Vooiclescu O et al. Neural crest boundary cap cells constitute a source of neuronal and glial cells of the PNS. Nat Neurosci 2004;7:930–938.
13. Vermeren M, Maro GS, Bron R et al. Integrity of developing spinal motor columns is regulated by neural crest derivatives at motor exit points. Neuron 2003;37:403–415.
14. Topilko P, Schneider-Maunoury S, Levi G et al. Krox-20 controls myelinogenesis in the peripheral nervous system. Nature 1994;371:796–799.
15. Hjerling-Leffler J, Marmigere F, Heglind M et al. The boundary cap: a source of neural crest stem cells that generate multiple sensory neuron subtypes. Development 2005;132:2623–2632.
16. Aquino JB, Hjerling-Leffler J, Koltzenburg M et al. In vitro and in vivo differentiation of boundary cap neural crest stem cells into mature Schwann cells. Exp Neurol 2006;198:438–449.
17. Vooiclescu O, Charnay P, Schneider-Maunoury S. Expression pattern of a Krox-20Cre knock-in allele in the developing hindbrain, bones, and peripheral nervous system. Genesis 2000;26:123–126.
18. Srinivas S, Watanabe T, Lin CS et al. Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. BMC Dev Biol 2001;1:4.
19. Coulpier F, Le Crom S, Maro GS et al. Novel features of boundary cap cells revealed by the analysis of newly identified molecular markers. Glia 2009;57:1450–1457.
20. Bachelin C, Zujovic V, Buchet D et al. Ectopic expression of polysialylated neural cell adhesion molecule in adult macaque Schwann cells promotes their migration and remyelination potential in the central nervous system. Brain 2009 [Epub ahead of print].
21. Papastefanaki F, Chen J, Lavdas AA et al. Grafts of Schwann cells engineered to express PSA-NCAM promote functional recovery after spinal cord injury. Brain 2007;130:2150–2174.
22. Peters A, Palay S, Webster H de. F. The neurons and supporting cells. In: Saunders W, ed. The fine structure of the nervous system. Philadelphia: Oxford University Press, 1976:232–263.
23. Penzer O, Greigore A. Electron microscopic features of multiple sclerosis lesions. Brain 1965;88:937–952.
24. Aldskogius H, Berens C, Kanaykina N et al. Regulation of boundary cap neural crest stem cell differentiation after transplantation. Stem Cells 2009;27:1592–1603.
25. Santos-Silva A, Fairless R, Frame MC et al. FGFr2heparin differentially regulates Schwann cell and olfactory ensheathing cell interactions with astrocytes: a role in astrocytosis. J Neurosci 2007;27:7154–7167.
26. Lavdas AA, Franceschini I, Dubois-Dalcq M et al. Schwann cells genetically engineered to express PSA show enhanced migratory potential without impairment of their myelinating ability in vitro. Glia 2006;53:868–878.
27. Bithell A, Williams BP. Neural stem cells and cell replacement therapy: making the right cells. Clin Sci (Lond) 2005;108:13–22.
28. Brederlau A, Correia AS, Anisimov SV et al. Transplantation of human embryonic stem cell-derived cells to a rat model of Parkinson’s disease: effect of in vitro differentiation on graft survival and teratoma formation. Stem Cells 2006;24:1433–1440.
29. Martino G, Pluchino S. The therapeutic potential of neural stem cells. Nat Rev Neurosci. 2006;7:395–406.
30. Real C, Glavieux-Pardauda C, Vaigot P et al. The instability of the neural crest phenotypes: Schwann cells can differentiate into myofibroblasts. Int J Dev Biol 2005;49:151–159.
31. Dupin E, Calloni G, Real C et al. Neural crest progenitors and stem cells. C R Biol 2007;330:521–529.
32. Aguado M, Woodhoo A, Webber D et al. Schwann cell precursors transplanted into the injured spinal cord multiply, integrate and are permissive for axon growth. Glia 2008;56:1263–1270.
33. Black JA, Waxman SG, Smith KJ. Remyelination of dorsal column axons by endogenous Schwann cells restores the normal pattern of Nav1.6 and Kv1.2 at nodal Ranvier. Brain 2006;129:1319–1329.
34. Sasaki M, Honmou O, Akiyama Y et al. Transplantation of an acutely isolated bone marrow fraction repairs demyelinated adult rat spinal cord axons. Glia 2001;35:26–34.
35. Keirstead HS, Ben-Hur T, Rogister B et al. Polysialylated neural cell adhesion molecule-positive CNS precursors generate both
oligodendrocytes and Schwann cells to remyelinate the CNS after transplantation. 

J Neurosci 1999;19:7529–7536.

36 Talbott JF, Cao Q, Enzmann GU et al. Schwann cell-like differentiation by adult oligodendrocyte precursor cells following engraftment into the demyelinated spinal cord is BMP-dependent. Glia 2006;54:147–159.

37 Akiyama Y, Radtke C, Honmou O et al. Remyelination of the spinal cord following intravenous delivery of bone marrow cells. Glia 2002;39:229–236.

38 Sieber-Blum M, Schnell L, Grim M et al. Characterization of epidermal neural crest stem cell (EPI-NCSC) grafts in the lesioned spinal cord. Mol Cell Neurosci 2006;32:67–81.

39 Li HY, Say EH, Zhou XF. Isolation and characterization of neural crest progenitors from adult dorsal root ganglia. Stem Cells 2007;25:2053–2065.

40 Nagoshi N, Shibata S, Kubota Y et al. Ontogeny and multipotency of neural crest-derived stem cells in mouse bone marrow, dorsal root ganglia, and whisker pad. Cell Stem Cell 2008;2:392–403.

See www.StemCells.com for supporting information available online.