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Remyelination of the Spinal Cord Following Intravenous Delivery of Bone Marrow Cells

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Abstract

Bone marrow contains a population of pluripotent cells that can differentiate into a variety of cell lineages, including neural cells. When injected directly into the demyelinated spinal cord they can elicit remyelination. Recent work has shown that following systemic delivery of bone marrow cells functional improvement occurs in contusive spinal cord injury and stroke models in rat. We report here that secondary to intravenous introduction of an acutely isolated bone marrow cell fraction (mononuclear fraction) from adult rat femoral bones separated on a density gradient, ultrastructurally defined remyelination occurs throughout a focal demyelinated spinal cord lesion. The anatomical pattern of remyelination was characteristic of both oligodendrocyte and Schwann cell myelination; conduction velocity improved in the remyelinated axons. When the injected bone marrow cells were transfected to express LacZ, β -galactosidase reaction product was observed in some myelin-forming cells in the spinal cord. Intravenous injection of other myelin-forming cells (Schwann cells and olfactory ensheathing cells) or the residual cell fraction of the gradient did not result in remyelination, suggesting that remyelination was specific to the delivery of the mononuclear fraction. While the precise mechanism of the repair, myelination by the bone marrow cells or facilitation of an endogenous repair process, cannot be fully determined, the results demonstrate an unprecedented level of myelin repair by systemic delivery of the mononuclear cells.

Keywords

Schwann cell; central myelin; peripheral myelin

INTRODUCTION

Bone marrow stromal cells can differentiate into astrocytes when transplanted into rodent brain (Azizi et al., 1998; Kopen et al., 1999), into neurons in vitro under appropriate cell culture conditions (Woodbury et al., 2000), and myocytes when injected into the heart following acute infarction (Orlic et al., 2001a). Systemic injection of bone marrow cells into lethally X-irradiated mice leads to differentiation of neuronal cells in brain (Brazelton et al., 2000; Mezey et al., 2000). Moreover, systemic delivery of bone marrow cells has been shown to enhance functional recovery in rodents following contusive spinal cord injury (Chopp et al., 2000) and middle cerebral artery infarction (Chen et al., 2001). When directly injected into the

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demyelinated rat spinal cord, isolated marrow cells derived from the mononuclear layer remyelinate these axons (Sasaki et al., 2001). These studies raise the intriguing possibility that bone marrow cells could be delivered intravenously to achieve repair of demyelinated axons. There are two critical determinants for appropriate differentiation of bone marrow cells in a number of tissues: tissue damage and a high number of circulating stem cells (Bjornson et al., 1999; Brazelton et al., 2000; Lagasse et al., 2000; Mezey et al., 2000; Orlic et al., 2001b). Based on this, we reasoned that intravenous injection of a large number of isolated bone marrow cells derived from the mononuclear layer that contains marrow stem cells into an acute experimental demyelinating lesion would provide an optimal model to study the remyelinating potential of systemic bone marrow cell delivery. A problem inherent in such studies is to clearly separate endogenous repair mechanisms from repair associated with other aspects of the cellular injections.

To study the remyelination potential of systemic bone marrow cell delivery, we first made a demyelinating lesion in the spinal cord by focal X-irradiation of the spinal cord, to delay endogenous repair, followed by direct microinjection of ethidium bromide into the spinal cord to kill all glial cells in our target lesion zone (Blakemore and Crang, 1984; Honmou et al., 1996). This experimental lesion model results in virtually complete demyelination and loss of astrocytes and oligodendrocytes in the central region of the dorsal funiculus over 6–8 mm of longitudinal length. However, demyelination with axonal preservation and loss of cells within the dorsal funiculus is stable and virtually complete for 6–8 weeks postlesion induction. At this time, remyelination commences from endogenous repair processes and progresses with time. The lesion is further associated with an inflammatory response in that phagocytic cells are present, which contain myelin and cellular debris.

We intravenously injected large numbers of bone marrow cells derived from the mononuclear cell layer, Schwann cells, olfactory ensheathing cells, or the residual debris fraction of the Ficoll gradient used to prepare mononuclear cells into the lesioned rats. The spinal cords were studied at 3-week postlesion induction, a time well before endogenous repair of myelin begins. We found that only after the intravenous delivery of the bone marrow cells and not the other cell injections did remyelination result. While in some experiments using LacZ-transfected bone marrow cells we found a small number of remyelinating cells associated with β -galactosidase reaction product, the majority of remyelinated cells were not. Given issues related to low efficiency of LacZ transfection, and the possibility of the gene being turned off as the cells progress to a myelinating phenotype, we cannot distinguish whether the remyelination was from the injected cells or whether the injection procedure facilitated an endogenous repair mechanism. It is important in this regard that injection of other cell types and fragments isolated from the Ficoll gradient did not result in remyelination. While we cannot fully distinguish bone marrow cell repair from facilitation of an endogenous repair mechanism, we report extensive reestablishment of myelin with improvement in electrophysiological function following intravenous delivery of the isolated bone marrow cells. These results indicate that systemic delivery of mononuclear layer bone marrow cells results in an unprecedented level of myelin repair that warrants further investigation to decipher the precise mechanism.

MATERIALS AND METHODS

Animal Preparation

Experiments were performed on 12-week-old Wistar rats. A focal demyelinated lesion was created in the dorsal funiculus of the spinal cord using X-irradiation and ethidium bromide injection (Blakemore and Crang, 1984; Honmou et al., 1996). Briefly, rats were anesthetized with ketamine (75 mg/kg) and xylazine (10 mg/kg) i.p. and a 40 Grays surface dose of X-irradiation was delivered to the spinal cord caudal to the tenth thoracic level (T-10) using a Siemens Stabilipan radiotherapy machine (Honmou et al., 1996). Three days after irradiation,

rats were anesthetized and a laminectomy was performed at T-11. The demyelinating lesion was induced by the direct injection of ethidium bromide (EB) into the dorsal column via a drawn glass micropipette. Injections of 0.5 μ l of 0.3 mg/ml EB in saline were made at depths of 0.7 and 0.4 mm.

Isolation of Bone Marrow, Schwann, and Olfactory Ensheathing Cells

Bone marrow (10 μ l) was obtained from femoral bones of adult rats. The samples were diluted in 5 ml α -minimum essential medium (α -MEM) containing 5 ml Ficoll. The cells were collected from the mononuclear cell layer following centrifugation (2,000 rpm, 15 min), and resuspended in 5 ml α -MEM. Following a second centrifugation (2,000 rpm, 15 min), the cells were collected in saline solution. Schwann cells and olfactory ensheathing cells were prepared from adult rats as previously described by Honmou et al. (1996) and Imaizumi et al. (1998), respectively.

Cell Injection Procedures

A suspension of the cells isolated from the mononuclear layer of bone marrow or from Schwann cell or olfactory ensheathing cell cultures (0.5 ml, 2×10^7 cells/ml) was slowly injected into the femoral vein. Additionally, a suspension of the residual cell fraction of Ficoll gradient (bottom layer) was also injected. This latter procedure was to control for a possible effect of the Ficoll on myelination. All transplants were studied at 3 weeks after cell injection.

Histological Examination

The rats were deeply anesthetized (60 mg/kg sodium pentobarbital) and perfused through the heart with heparinized phosphate-buffer solution (PBS) followed by fixative solution containing 2% glutaraldehyde and 2% paraformaldehyde in 0.14 M Sorensen's phosphate buffer, pH 7.4. Following in situ fixation for 10 min, the spinal cord was excised, placed into fresh fixative, and stored overnight. Then tissue was cut into 2 mm segments, postfixated with 1% OsO₄ for 4 h, dehydrated in graded ethanol solutions, and embedded in Epox-812 (Ernest Fullam, Latham, NY). The tissue was sectioned on an ultramicrotome. Finally, the sections were counterstained with 0.5% methylene blue/0.5% azure II in 0.5% borax. Sections were examined with a Nikon Eclipse 800 microscope at 10 $\times$ and 100 $\times$ and photographed with a Spot RT Color CCD camera. Thin sections were counterstained with uranyl and lead salts and examined with a Zeiss EM902A electron microscope operating at 80 kV.

LacZ Gene Transfection and Detection of β -Galactosidase Reaction Products

Isolated bone marrow cells were transfected by pcDNA3.1/His/LacZ (Invitrogen) constructed by cloning the β -galactosidase gene into the pcDNA vector. Lipofectamine (4 μ l/ml in Opti-MEM) was used to transfect the expression vector pcDNA3.1/His/LacZ (2 μ g/ml in Opti-MEM) to bone marrow cells. Rats were perfused through the heart with fixative (4% paraformaldehyde in phosphate buffer). After removal of the spinal cords, they were left in fixative for 1 h. Sections were cut and β -galactosidase-expressing myelin-forming cells were detected by incubating the sections at 37°C overnight with X-Gal in a final concentration of 1 mg/ml in X-Gal developer (35 mM K₃Fe(CN)₆/35 mM K₄Fe(CN)₆ · 3H₂O/2 mM MgCl₂ in PBS) to form a blue color within the cell. The slices were then fixed for overnight in 2% paraformaldehyde and 2% glutaraldehyde in phosphate buffer (0.14 M) and then embedded as described above. The sections were counterstained with 0.15% basic fuchsin and were examined by light microscopy for the presence of a blue reaction product (β -galactosidase reaction product).

Field Potential Recording

After induction of deep anesthesia (60 mg/kg sodium pentobarbital), spinal cords of control ($n = 15$), demyelinated ($n = 15$), and transplanted rats ($n = 15$) were quickly removed and maintained in an in vitro submersion-type recording chamber with a modified Krebs' solution containing (in mM) 124 NaCl, 26 NaHCO₃, 3.0 KCl, 1.3 NaH₂PO₄, 2.0 MgCl₂, 10 dextrose, 2.0 CaCl₂, saturated with 95% O₂ and 5% CO₂. Field potential recordings of compound action potentials were obtained at 36°C with glass microelectrodes (1–5 MΩ; 1 M NaCl) positioned in the dorsal columns, and signals were amplified with high-input impedance amplifier. The axons were activated by electrical stimulation of the dorsal columns with bipolar Teflon-coated stainless steel electrodes cut flush and placed lightly on the dorsal surface of the spinal cord. Constant current stimulation pulses were delivered through stimulus isolation units and the timing device. The recorded field potentials were positive-negative-positive waves corresponding to source-sink-source currents associated with propagating axonal action potentials; the negativity represents inward current associated with the depolarizing phase of the action potential. All variances represent standard error $\pm$ SEM. Differences among groups were assessed by unpaired two-tailed *t*-test to identify individual group differences. Differences were deemed statistically significant at $P < 0.05$.

RESULTS

Focal demyelinating lesions were induced by X-irradiation and ethidium bromide (X-EB) injection in the spinal cord of adult rats (Blakemore and Crang, 1984; Honmou et al., 1996). This lesion kills glia-including oligodendrocytes from the toxic action of the EB on cellular DNA and the X-irradiation delays endogenous remyelination for up to 8 weeks (Honmou et al., 1996). Normal dorsal funiculus is shown in Figure 1A and B at low and high power, respectively, and is replete with myelinated axons (Fig. 1B). In the lesion zone, virtually all axons were demyelinated (Fig. 1C). The lesions covered nearly the entire dorso-ventral extent of the dorsal funiculus and extended between 5 and 8 mm longitudinally. The lesions were characterized by regions of densely packed demyelinated axons separated by islands of cellular debris and macrophages (Fig. 1C). In this lesion model, virtually no myelination is observed in the lesion zone for up to 8 weeks, upon which time some myelination is observed, beginning at the peripheral boundaries of the lesion, continuing toward the lesion interior with time. Three days after lesion induction in the spinal cord, bone marrow cells from the femoral bones of other adult rats were separated on a density gradient to isolate the mononuclear cell fraction (central band in the gradient) from cellular residues (bottom band) of the bone marrow. No immunosuppression was used with these allogeneic cell injections.

Myelination was not observed in sham control (intravenous saline injections) rats (0/9), rats injected with the cellular residue fraction (0/9), Schwann cells (0/5), or olfactory ensheathing cells (0/4). Figure 1D shows a field of demyelinated dorsal funiculus with no remyelination in a rat where intravenous delivery of Schwann cells was performed. Myelinated axons were observed throughout the lesion zone in rats that received the intravenous bone marrow cell injections (8/15) isolated from the density interface. Using a one-way ANOVA for comparison of the control injection groups ($n = 27$) with the bone marrow injection group ($n = 15$), significance was at $P < 0.01$. Figure 2A shows a dorsal funiculus after lesion induction and intravenous bone marrow cell delivery. The light photomicrograph in Figure 2B, obtained in the area marked "b" in Figure 2A, illustrates a field of remyelinated axons in the lesion zone; nearly all axons in this field were remyelinated. Myelination was found throughout the dorso-ventral and rostro-caudal margins of the lesion zone. Figure 2C was taken from a region in the lateral margin of the lesion zone ("c" in Fig. 2A). Note the remyelinated axon profiles are thinner than control and abut against the normal white matter (left). Myelination was not uniform at the 3-week postlesion time point. This is evident in Figure 2D, where a set of nearly

complete myelinated axons is present on the left, but promyelinating axons are observed on the right.

Electron microscopic observations indicate that the myelinated axons show structural characteristics of either peripheral Schwann cell-like or central oligodendrocyte-like myelination. Normal dorsal funiculus and an area containing both myelinated and demyelinated axons following intravenous delivery of the bone marrow cells are shown in Figure 3A and B, respectively. Both demyelinated (Fig. 3B, left) and remyelinated (right) axons were observed in the lesion zone. An example of a myelinated axon with Schwann cell-like characteristics is shown in Figure 3C. The myelinated axon is surrounded by a large cytoplasmic and nuclear domain and a basement membrane (inset, arrowheads) that is not observed in oligodendrocyte myelinated axons. These morphological features are characteristic of peripheral myelin and are similar to those observed in the same model system following intraspinal injection of Schwann cells (Blakemore and Crang, 1984; Honmou et al., 1996; Kohama et al., 2001). Other myelinated axons did not have cell bodies surrounding them, nor did they have a basement membrane suggesting that the myelin was formed by oligodendrocytes (Fig. 3D). Many of the myelinated axons in Figure 3D surround a central cell body and nucleus that is reminiscent of oligodendrocyte myelination. In this regard, cell transplantation studies using neural precursor cells derived from brain and directly injected into demyelinating lesions report that both central and peripheral myelin can form from the transplanted cells (Keirstead et al., 1999; Akiyama et al., 2001).

In the EB-X lesion model, endogenous remyelination does not begin for at least 6–8 weeks; our studies were carried out at just over 3 weeks after lesion induction so it is unlikely that endogenous remyelination occurred. Cells transfected with the LacZ reporter gene prior to intravenous injection were also delivered intravenously ($n = 4$). The majority of cells in culture were β -galactosidase-positive, but there was considerable variation in intensity of reaction with the majority of the cells showing weak β -galactosidase reaction. Figure 4A shows an intense blue β -galactosidase reaction in the lesion zone from a 2 mm whole mount section. Microscopic examination of the tissue indicates that many macrophages as well as some cellular debris within the lesion zone were β -galactosidase-positive. We suspect that the intense blue reaction in the whole mounts was primarily the result of these cellular elements. However, some cellular profiles associated with myelination contain β -galactosidase reaction products. Figure 4B shows cells with reaction product that have the morphological appearance of peripheral myelin-forming cells. The higher-power micrograph in Figure 4C shows two Schwann-like cells with β -galactosidase reaction product. The cell with reaction product in Figure 4D has morphological similarity to an oligodendrocyte. About 9% of myelin-forming cells showed β -galactosidase reaction products. In white matter regions outside of the lesion zone, we did not observe cells associated with β -galactosidase reaction products (Fig. 4E). We also marked cells in culture with the fluorescent cell marker (PKH26) prior to injection. While we could not distinguish cell morphology of the fluorescent labeled cells in vivo, fluorescence was detected only in the lesion zone (Fig. 4F). This suggests that the blood-brain barrier was compromised in the lesion zone and that delivered cells or cellular debris entered the lesion zone.

Electrophysiological studies were carried out in vitro to examine the conduction properties of the axons. Figure 5A shows field potential recordings of compound action potentials recorded from the dorsal columns of control, demyelinated, and bone marrow injected rats, respectively, at sequential longitudinal distances. The demyelinated axons displayed considerable conduction slowing (0.83 ± 0.05 m/s; $n = 15$) as compared to control (10.88 ± 1.21 m/s; $n = 15$). In the bone marrow injection group, multicomponent negativities were observed indicative of axons with various conduction velocities. The early negativity (fastest-conducting fiber group) had a conduction velocity of 6.21 ± 0.82 m/s ($n = 15$) that was significantly faster than

the demyelinated axons (Fig. 5B). These data indicate that a subpopulation of remyelinated axons in the bone marrow-injected rats showed increased conduction velocity. The intermediate-conducting group and the slow group suggest that at the time studied, axons with partial or no myelination were also present as indicated by the histology. It will be important to determine if conduction shows greater improvement with longer recovery times.

DISCUSSION

The present study shows that following intravenous delivery of bone marrow cells prepared from adult rats leads to repair of demyelinated axons in the X-EB lesion model of the spinal cord. The bone marrow cells were prepared acutely by centrifugation through a density gradient to isolate the mononuclear cell layer and not treated with trophic factors. We found that intravenous delivery of this cellular bone marrow fraction was sufficient to elicit relatively extensive remyelination, without obvious ectopic cell differentiation of neurons or other cell types within the lesion. Intravenous injection of Schwann cells, olfactory ensheathing cells, or a residual cell fraction on the density gradient did not result in remyelination. We are uncertain as to which of the numerous cell types within the bone marrow cell fraction is responsible for the *in vivo* differentiation of myelin-forming cells. Moreover, we cannot rule out the possibility of facilitation of an endogenous repair process by the intravenous delivery of the cells. Although it is uncertain as to which cell type from bone marrow may develop a myelinating phenotype, or if the cell delivery leads to an enhancement of endogenous repair mechanisms, it is clear that intravenous injection of this bone marrow cell fraction is sufficient to elicit myelin repair in our lesion model.

Although a relatively large number of axons were remyelinated subsequent to transplantation of LacZ-transfected bone marrow cells, a small percentage (9%) of myelin-forming cells were associated with β -galactosidase reaction product. However, we are cautious in interpreting these data. First, macrophages in the lesion zone were β -galactosidase-positive; this likely accounted for the macroscopic blue observed in whole mount sections. Moreover, the β -galactosidase reaction products associated with myelin-forming cells presented as granules and not diffuse staining as reported in other transplantation studies of myelin-forming cells (Franklin et al., 1996). It is possible that endogenous β -galactosidase reaction was present in the macrophages and that the granular particles in myelin-forming cells were not specific for LacZ expression in those cells. However, we are certain that the blood-brain barrier is compromised in the lesion zone, leaving open the possibility of cell entry, because when intravenously injected with fluorescent-labeled cells, fluorescence was only observed in the lesion zone. Unfortunately, we were unable to obtain a clear structural correlate of fluorescent-labeled cells with myelinating profiles. Quantitative cell identification using appropriate markers clearly presents a technical difficulty in such studies, and future work in this regard will be very important.

An alternative hypothesis to account for the remyelination is that the injected bone marrow cells induced or accelerated endogenous repair by resident cells. While the X-EB model blocks endogenous repair for about 8 weeks because of the X-irradiation (Honmou et al., 1996), it is possible that the cell injection procedure resulted in recruitment or induction of endogenous cells to form myelin. The lack of remyelination by injection of the residual cell fraction on the density gradient and other myelin-forming cell types suggests that a nonspecific immune response from the cell injection procedure, including exposure to Ficoll or contamination of the bone marrow with Schwann cells, could not account for the remyelination. There are a number of phagocytic cells within the lesion and whether these or the injected cells themselves release cytokines or trophic substances that recruit endogenous repair is yet to be determined.

While we have not as yet quantitated the relative proportion of central (oligodendrocyte-like) and peripheral (Schwann cell-like) myelin, our ultrastructural analysis indicates that indeed both CNS-like and peripheral nervous system (PNS)-like myelin are present within the lesion zone after intravenous bone marrow cells injection. Many myelin profiles had thick myelin, a basement membrane surrounding large nuclear and cytoplasmic domains with extracellular collagen deposition: hallmark features of peripheral myelin (Blakemore and Crang, 1984; Honmou et al., 1996; Kohama et al., 2001). In this regard, direct intraspinal injection of neonatal rodent (Keirstead et al., 1999) and adult human (Akiyama et al., 2001) neural precursor cells derived from the subventricular zone into the X-EB lesion can give rise to similar peripheral-like myelin. These observations indicate that both CNS- and PNS-derived stem-like cells from the adult can form peripheral-like myelin after reintroduction into the CNS. A notable difference between our intravenous delivery experiments and direct injection experiments is that remyelination was more disperse following systemic injection. The remyelination observed following direct microinjection of cells in the spinal cord is more intense near the transplantation site. While the dispersion of remyelination with systemic delivery does not prove that the injected mononuclear cells were in fact the myelin-forming cells, it does suggest that the effect of the injection protocol was for more global repair.

We are careful not to generalize and assume that intravenous bone marrow cells will be effective in the repair of other demyelinating lesion models or in demyelinating disease. Blood-brain barrier status and differences in the astrocytic environment in other lesion models may not permit the degree of remyelination we observed in the X-EB model. Moreover, we did not get repair in all animals that we injected. Whether this was the result of imperfect cell injection or differences in the host's reaction to the delivered cells will be important to determine in future work.

The demonstration of extensive anatomical repair and some recovery of electrophysiological function by systemic delivery of bone marrow cells suggests the possibility that autologous bone marrow delivery could facilitate remyelination in CNS demyelinating diseases. An important experimental challenge differentiating between bone marrow cells as progenitors for myelin-forming cells or as possibly providing trophic support to enhance endogenous repair is yet to be fully understood. However, this model system provides an interesting experimental environment to study the biology of this potentially clinically useful approach to elicit remyelination by systemic bone marrow cell delivery.

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Fig. 1. Morphology of dorsal funiculus from control, demyelinated, and sham cell transplanted rat spinal cord. Normal dorsal funiculus (arrowheads) of the spinal cord is shown in **A** and **B** at low and high power, respectively. **C**: High-power field of demyelinated axons in control lesion with no cell injections. No myelination was observed following intravenous delivery of Schwann cells (**D**). DF, dorsal funiculus; DH, dorsal horn. Scale bar, 100 μ m in **A**; 10 μ m in **B**–**D**.

Fig. 2.

Dorsal funiculus following intravenous delivery of mononuclear cells. (A) Low power micrograph of dorsal funiculus lesion zone three weeks after intravenous bone marrow delivery. Remyelinated axons in the center (B) and lateral edge (C) of the lesion. Remyelination was observed throughout the lesion zone. However, some areas had various degrees of remyelination. This is shown in D, where an area of intense myelination (left) is adjacent to an area with promyelinating profiles. Scale bar, 100 μm in A; 15 μm in B and C; 6 μm in D.

Fig. 3. Electron micrographs of remyelinated axons in the dorsal funiculus of the spinal cord after bone marrow transplantation. **A:** Normal dorsal funiculus axons. **B:** A field of axons showing some with myelin and others without. Some myelin-forming cells were similar to peripheral myelin-forming cells, characterized by large cytoplasmic and nuclear domains (**C**) and a basement membrane (arrowheads in inset). **D:** Other myelinated axons did not have the Schwann cell-like morphology and were similar to axons remyelinated by oligodendrocytes. Scale bar, 5 μm in A and B; 1.5 μm in C; 3 μm in D.

Fig. 4.

A: Two mm whole mount of coronally cut spinal cord from a rat that was injected intravenously with LacZ-transfected rat bone marrow cells. Note the intense blue β -galactosidase reaction in the lesion zone only. Numerous cells with β -galactosidase blue reaction product are observed in the remyelinated dorsal funiculus 3 weeks after intravenous delivery of LacZ-transfected rat bone marrow cells (**B**). **C:** Higher-power photomicrograph showing a Schwann-like cell with blue β -galactosidase reaction product in the cytoplasm. β -galactosidase reaction product is seen in cell bodies reminiscent of oligodendrocytes (**D**). **E:** Micrograph of normal white matter from same animal as that for B–D. Note the paucity of β -galactosidase reaction product in the nonlesion white matter. The sections in B–E were counterstained with 0.15% basic fuchsin. **F:** Micrograph showing PKH26 fluorescence in the dorsal funiculus lesion zone after intravenous delivery of labeled bone marrow cells. Dashed line indicates dorsal funiculus. Scale bar, 1 mm in A and F; 8 μ m in B; 4 μ m in C; 6 μ m in D; 30 μ m in E.

Fig. 5.

A: Compound action potentials recorded at 1.0 mm increments longitudinally along the dorsal columns in normal, demyelinated (EB-X lesion) and after bone marrow cells were delivered intravenously in an EB-X lesioned rat. Note that multicomponent waves were observed in the spinal cord from the bone marrow-injected rat. The dashed lines link the peak negativities of three primary negativities. Histograms of conduction velocity (error bars indicate SEM) of dorsal column axons obtained from normal, demyelinated, and the fastest component (dashed line with 1) of recordings obtained from the bone marrow injected rats are shown in **B**. At 36°C, conduction velocity of the early negativity in control, demyelinated, and bone marrow delivered groups were 10.88 ± 1.21 m/s ($n = 15$), 0.83 ± 0.05 m/s ($n = 15$), and 6.21 ± 0.82 m/s ($n = 15$), respectively. Double asterisk: $P < 0.001$, control vs. demyelinated; asterisk: $P < 0.01$, demyelinated vs. transplanted.