Injury to peripheral nerve initiates a degenerative process that converts the denervated nerve from a suppressive environment to one that promotes axonal regeneration. We investigated the role of matrix metalloproteinases (MMPs) in this degenerative process and whether effective predegenerated nerve grafts could be produced in vitro. Rat peripheral nerve explants were cultured for 1–7 d in various media, and their neurite-promoting activity was assessed by cryoculture assay, in which neurons are grown directly on nerve sections. The neurite-promoting activity of cultured nerves increased rapidly and, compared with uncultured nerve, a maximum increase of 72% resulted by 2 d of culture in the presence of serum. Remarkably, the neurite-promoting activity of short-term cultured nerves was also significantly better than nerves degenerated in vivo. We examined whether in vitro degeneration is MMP dependent and found that the MMP inhibitor N-[[2R]-2(hydroxamidocarbonylmethyl)-4-methylpantanoyl]-L-tryptophan methylamide primarily blocked the degenerative increase in neurite-promoting activity. In the absence of hematogenic macrophages, MMP-9 was trivial, whereas elevated MMP-2 expression and activation paralleled the increase in neurite-promoting activity. MMP-2 immunoreactivity localized to Schwann cells, and the endoneurium and colocalized with gelatinolytic activity as demonstrated by in situ zymography. Finally, in vitro predegenerated nerves were tested as acellular grafts and, compared with normal acellular nerve grafts, axonal ingress in vivo was approximately doubled. We conclude that Schwann cell expression of MMP-2 plays a principal role in the degenerative process that enhances the regeneration-promoting properties of denervated nerve. Combined with their low immunogenicity, acellular nerve grafts activated by in vitro predegeneration may be a significant advancement for clinical nerve allografting.

Key words: Wallerian degeneration; acellular nerve graft; MMP-2; Schwann cell; cryoculture; basal lamina; metalloproteinase; chondroitin sulfate proteoglycan; axon regeneration; rat sciatic nerve
(Osawa et al., 1990; Ochi et al., 1994; Danielsen et al., 1995). Nonetheless, *in vivo* predegeneration of nerve is not feasible for clinical allografting.

In this study, we used nerve explant cultures to examine the role of MMPs in the degenerative process in the absence of hematogenic macrophages. Cultured nerves were also tested for their ability to support nerve regeneration in *vivo* to determine whether effective predegenerated acellular nerve allografts can be produced in *vivo*.

**MATERIALS AND METHODS**

*Nerve explant culture.* Adult (180–200 gm) female Sprague Dawley rats (Harlan, Indianapolis, IN) were used as nerve donors and graft recipients. This project was reviewed and approved by the Institutional Animal Care and Use Committee. Donor rats were deeply anesthetized with isoflurane and decapitated. Sciatic nerves were exposed through a gluteal muscle-splitting incision and isolated free of underlying fascia. A 15 mm nerve segment was excised rostral to the bifurcation into common peroneal and tibial nerves. The segments were rinsed with sterile Ringer’s solution and stabilized by pinning the ends to a thin plastic support. The nerve explants were cultured for 1, 2, 4, and 7 d in DMEM containing N2 supplements (DMEM/N2) or DMEM supplemented with 2 or 10% fetal bovine serum (FBS) (Atlanta Biologicals, Atlanta, GA). As specified, some explants were cultured in the presence of the MMP inhibitor N-[2(hydroxamidocarbonylmethyl)-4-methylpantanoyl]-L-tryptophan methyl ester (2 uM) (Galardy et al., 1994). The cultured nerves were washed thoroughly in DMEM and then transferred to sealed tubes. The –20° C samples were agitated for 30 min at 4° C on 10% SDS-polyacrylamide gels containing 50 uM intramolecularly quenched, fluorescein-labeled gelatin substrate (Molecular Probes Inc., Eugene, OR) (Oh et al., 1999). After incubation for 24 hr at 37° C, the sections were rinsed with PBS and fixed with 4% paraformaldehyde in phosphate buffer. The sections were rinsed with water and mounted using Citifluor. Fluorescein–gelatin peptides generated by gelatinolytic activity in the tissue sections were observed and photographed by epifluorescence microscopy.

*Interpositional nerve grafting.* Six rats were given bilateral acellular nerve grafts, one normal (uncultured) and one predegenerated in *vivo* (cultured for 2 d in 2% serum). Host rats were deeply anesthetized using xylazine (15 mg/kg, i.m.) and ketamine (110 mg/kg, i.p.). The sciatic nerve was exposed and supported by a plastic insert placed between the nerve and underlying tissue. The region of the nerve halfway between the sciatic notch and bifurcation was first coated with fibrin glue. A 2.5 mm segment of the host nerve was excised using serrated scissors. The graft was thawed and freshly trimmed to 10 mm with a scalpel blade. The graft was coapted to the host nerve stumps by epineural neurorrhaphy using 8-0 Ethilon sutures at each end. Fibrin glue was then applied to stabilize the coaptations that, in combination with the initial fibrin coating applied to the host nerve, reduced protrusion of nerve elements (endoneurial mushrooming) (Menovsky and Bartels, 1999). The muscle was closed with 4-0 sutures, and the skin was closed with wound clips. After recovery from the anesthetic, animals were returned to standard housing. At 8 d after grafting, the host rats were deeply anesthetized and decapitated. The graft and 3 mm of proximal and distal host nerve were removed and immersed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, overnight at 4° C. The specimens were equilibrated with PBS and immersed in 30% sucrose in phosphate buffer for 2 d at 4° C. The specimens were embedded and cryosectioned on the transverse plane in a recorded measure. Regenerating axons within the grafts were labeled by GAP-43 immunofluorescence (see below). Epifluorescent photomicrographs were acquired, and GAP-43-positive axon profiles (pixel counts) were scored using Image-Pro Plus software.

*Immunofluorescent labeling.* Fixed tissue sections were treated with 0.5% Triton X-100 in PBS for 10 min. Nonspecific antibody binding was blocked by 1% Triton X-100 containing 10% normal serum (blocking buffer). Primary antibodies were diluted in blocking buffer and applied overnight at 4° C. Bound primary antibodies were labeled with swine anti-rabbit IgG (Dako, Carpenteria, CA) or goat anti-mouse IgG (Sigma, St. Louis, MO) conjugated with fluorescein or rhodamine for 1 hr at room temperature in darkness. The anti-mouse secondary antibody was preadsorbed with rat serum before use. Neurite length (cryoculture) and axonal regeneration (grafting) were assessed by immunolabeling with polyclonal anti-GAP-43 IgG (2 uM/ml) (Ferguson and Muir, 2000) (NB300-143; Novus Biological, Littleton, CO). Other primary antibodies included: polyclonal anti-MMP-2 IgG (4 uM/ml) (MMP-2/475) (Muir, 1995); polyclonal anti-MMP-9 IgG (4 uM/ml) (AB91047; Chemicon, Temecula, CA); polyclonal anti-S-100 antisemur (1:500; Dako); polyclonal OA42 antisemur (1:500; Serotek, Raleigh, NC); and monoclonal anti-neurofilament IgG (4 uM/ml) (NAP4) (Harris et al., 1993). In some instances, epifluorescent photomicrographs were inverted and contrast-enhanced for printing in Photoshop (Adobe Systems, San Jose, CA).

**RESULTS**

The neurite-promoting activity of cultured nerve segments

Freshly excised (cellar) rat sciatic nerve segments were cultured for up to 7 d in medium containing 0, 2, and 10% FBS. Control (uncultured) and cultured nerves were cryosectioned, and their neurite-promoting activity was assessed by cryoculture assay. Re-
results are shown in Figure 1A. Embryonic chick DRG neurons grown on sections of control nerves had an average neurite length of 118 μm. Neuritic growth on sections of nerve explants cultured for 1–4 d was significantly greater than that in the control condition (p < 0.001). For nerves cultured in defined medium (0% serum), neurite-promoting activity reached a maximum at 2 d in vitro, representing a 43% increase compared with control nerves. There was more than a 70% increase in the neurite-promoting activity for nerve explants cultured for 1 or 2 d in medium containing 2% serum. Nerve explants cultured in 10% serum reached a similar maximum at 2 d in vitro as well. The neurite-promoting activity of nerve explants declined after longer culture periods and fell below the level of the control condition at 7 d. These data indicate that the neurite-promoting activity of nerve explants increased markedly when cultured for short periods in vitro with and without the addition of serum to the culture medium. Nerve explants were prevented from adhering to the culture vessel, and no cell outgrowth was observed. However, cell viability in all conditions was confirmed in separate experiments in which robust cell migration was observed from nerve explants that were minced and pressed to the culture surface.

Comparison of in vitro and in vivo predegeneration
Several laboratories, including our own, have found that peripheral nerves predegenerated in vitro are more capable of supporting neurite growth than normal nerve in cryoculture assays (Bedi et al., 1992; Agius and Cochard, 1998; Ferguson and Muir, 2000). Here, using the cryoculture assay, we compared the neurite-promoting activity of rat sciatic nerve explants predegenerated in vitro to those predegenerated in vivo. As described above (Fig. 1), neuritic growth of DRG neurons on nerve explants cultured for 2 d in 2% serum (in vitro predegeneration) was 70% greater than control nerves (not predegenerated). Also, nerve explant culture for longer periods (4 and 7 d) resulted in progressively less neurite-promoting activity. Nerves cultured for 7 d had 37% less activity than the control condition. In comparison, the neurite-promoting activity of nerves predegenerated in vivo was much lower that that seen for nerves predegenerated in vitro. Neuritic growth on nerves predegenerated in vivo for 2 d was 35.8 μm, which corresponds to 72% less activity than the control condition (126.5 μm) (p < 0.001). However, this inhibition was reversed over time, and in vitro predegeneration for 7 d resulted in neuritic growth that was 12% greater than the control condition (p = 0.06). These data show that in vitro predegeneration increased the neurite-promoting activity of nerve segments to a greater extent than that achieved by in vivo predegeneration. Although longer time points were not included in the present study, other cryoculture and grafting studies indicate that the maximal positive effects of in vivo predegeneration are achieved by 7–8 d (Bedi et al., 1992; Danielsen et al., 1994; Ochi et al., 1994; Keilhoff et al., 1999).

In vitro degeneration is MMP dependent
Our previous studies indicate a role for MMPs in the degenerative process (Zuo et al., 1998b; Ferguson and Muir, 2000). In the in vitro nerve degeneration model, which excludes the contribution of hematogenic macrophages, we tested the hypothesis that the elevation of neurite-promoting activity observed for cultured nerve explants was dependent on MMP activity. Nerve segments were cultured for 2 d in medium containing 2% serum with and without the addition of the MMP inhibitor GM6001. The neurite-promoting activity of the cultured nerves was assessed by cryoculture assay. Results are shown in Figure 1B. Similar to that shown in Figure 1A, the mean neurite length of DRG neurons grown on cultured nerves (2 d; 2% serum) was 210 μm, representing a 68% increase over that of (uncultured) control nerves (p < 0.001). However, this increase was reduced to only 14% for nerves cultured in the presence of GM6001 (p < 0.001). Dissociation culture (squash preparations) of the nerve segments in each condition showed profuse cell outgrowth, indicating no loss of cell viability. In addition, treatment of isolated Schwann cell cultures with GM6001 confirmed the very low toxicity of this hydroxamate-based dipeptide (data not shown). We have reported previously that GM6001 is not toxic to DRG neurons (Zuo et al., 1998b). At the concentration used in these experiments (50 μM), GM6001 is a potent inhibitor of the gelatinases (MMP-2 and MMP-9) as well as many other MMPs (Galardy et al., 1994). These results strongly implicate MMP activity in a degenerative process that increases the neurite-promoting activity of cultured nerve explants.

MMP expression in the cultured nerve segments: zymographic gel analysis
MMP-2 and MMP-9 are the main extracellular proteinases capable of degrading gelatin (cleaved collagen), and their major substrate is collagen type IV of the basal lamina. MMP-2 is constitutively expressed by Schwann cells in vivo and is upregulated after nerve injury in the rat. In contrast, MMP-9 is undetectable in normal nerve and is present after injury in association with invading granulocytes and macrophages (Yamada et al., 1995; La Fleur et al., 1996; Kherif et al., 1998; Ferguson and Muir, 2000). Our examination of in vitro nerve degeneration provides a unique opportunity to determine the role of MMP expression by resident nerve cells with a minimal contribution by hematogenic cells. MMP levels in cultured nerve explants were first examined by gelatin substrate-overlay gel electrophoresis (zymography). Gelatin zymography is very sensitive in the detection of MMP-2 and MMP-9 and has the added advantage of revealing both latent and activated forms. Nerve segments were
MMP-2 was observed (the proform of human recombinant MMP-2. A trace of activated ples was negligible. The identi- al., 2002), signifying that the MMP-9 content in the nerve sam- fold more sensitive in detecting MMP-9 than MMP-2 (Ladwig et in vitro study. The activity of MMPs is regulated by gene transcription, by proenzyme activation, and by the action of tissue inhibitors of MMP activity in the cultured nerve segments: proenzymes are activated and upregulated in nerve degeneration in vitro. It is notable that gelatin zymography is several-fold more sensitive in detecting MMP-9 than MMP-2 (Ladwig et al., 2002), signifying that the MMP-9 content in the nerve samples was negligible. The identification of gelatinolytic bands as pro-MMP-9 and activated MMP-9 in nerve was confirmed previously by Western immunoblotting (Ferguson and Muir, 2000).

MMP activity in the cultured nerve segments: in situ zymography
The activity of MMPs is regulated by gene transcription, by proenzyme activation, and by the action of tissue inhibitors of metalloproteinases (TIMPs). We examined the net gelatinolytic activity in nerve segments by zymography. Tissue sections were overlaid with quenched, fluorescent gelatin, which is converted to fluorescent peptides by gelatinolytic activity within tissues. Constitutive gelatinolytic activity was detected in normal nerve, (A) which, at higher magnification (B), was associated with Schwann cells. Gelatinolytic activity was more intense and diffuse throughout the endoneurium in the cultured nerves (C, D). Gelatinolytic activity in nerves cultured in the presence of GM6001 was markedly decreased (E, F). All epifluorescent images were obtained using the same exposure parameters, and image enhancements were applied equally. Scale bars: (in A) A, C, E, 100 μm; (in B) B, D, F, 25 μm.

Figure 2. Zymographic analysis of nerve explant cultures. Nerve explants were cultured for 0 (C, control), 1, 2, 4, and 7 d in DMEM/N2 containing 2% serum. The nerves were then extracted and analyzed by gelatin-overlay electrophoresis. Zymography reveals both proform and activated gelatinases that appear as clear bands within the stained gel. Control nerve contained predominantly pro-MMP-2 and trace amounts of activated MMP-2. There was a progressive increase in MMP-2 content and a rapid conversion to the activated form within the nerve explants cultured for ≥2 d. MMP-9 was negligible in the control and early explants, whereas a trace amount was detected at 4 and 7 d. The molecular masses indicate that MMP-9 (92 kDa), activated MMP-9 (84 kDa), pro-MMP-2 (72 kDa), and activated MMP-2 (66 kDa).

Figure 3. Localization of net gelatinolytic activity in nerve segments by zymography. Tissue sections of control nerve (A, B) and cultured nerve explants (2 d; 2% serum) (C, D) were overlaid with quenched, fluorescent-labeled gelatin, which is converted to fluorescent peptides by gelatinolytic activity within tissues. Constitutive gelatinolytic activity was detected in normal nerve, (A) which, at higher magnification (B), was associated with Schwann cells. Gelatinolytic activity was more intense and diffuse throughout the endoneurium in the cultured nerves (C, D). Gelatinolytic activity in nerves cultured in the presence of GM6001 was markedly decreased (E, F). All epifluorescent images were obtained using the same exposure parameters, and image enhancements were applied equally. Scale bars: (in A) A, C, E, 100 μm; (in B) B, D, F, 25 μm.

MMP localization in the cultured nerve segments: immunofluorescent labeling
We confirmed previous findings by Kherif et al. (1998) that immunolabeling for MMP-2 in normal nerve is localized in Schwann cells, whereas MMP-9 is undetectable (data not shown). We also examined the distributions of MMP-2 and MMP-9 in nerve explants cultured for 2 d by immunofluorescence microscopy. MMP-2 immunolabeling of culture nerves was intense within Schwann cells and the surrounding basal laminas (Fig. 4A). Schwann cell staining with S-100 indicated that the most intense MMP-2 immunolabeling was associated with migrating Schwann cells (Fig. 4B; and see below). Also, MMP-2 immuno-
expression was very similar to the pattern of gelatinolytic activity obtained by in situ zymography. In contrast, MMP-9 immunolabeling was virtually absent within the nerve fascicles, except for rare cellular profiles. Some cellular immunexpression of MMP-9 was seen in the surrounding epineurium (Fig. 4C). OX42 labeling was used to identify macrophages that were scattered throughout the epineurium and rarely within the nerve fascicles of cultured nerves (Fig. 4D). The compartmental distributions of MMP-9 and OX42 labeling suggested that macrophages were the main source of MMP-9. In addition, Schwann cells, and perhaps perineurial fibroblasts, expressed MMP-2, and MMP-2 immunoreactivity was also observed diffusely in the surrounding ECM.

**Cell distributions and axonal degeneration in the cultured nerve segments**

After nerve injury, Schwann cells become activated, dissociate their myelin, and migrate extensively. S-100 immunolabeling of the cultured nerve explants showed that many Schwann cells had lost their elongated morphology and close association with axons, typical of the injury response (Fig. 4B). As expected when disconnected from the circulatory system, the number of macrophages in the nerve explants was much lower than that observed in nerve degeneration in vivo. Moreover, very few macrophages were found within the nerve fascicles, and nearly all OX42-labeled cells were confined to the epineurium (Fig. 4D). It was clear that the macrophages present in the epineurial compartment at the time of nerve excision did not invade the inner nerve compartments during culture. Accordingly, the nerve explants in vitro represent a model of nerve degeneration in which the contribution of Schwann cells may be assessed independently from those of invading macrophages.

The degradation of axons was examined in cultured nerve explants by immunolabeling of neurofilaments. Results are shown in Figure 5. Unlike the contiguous neurofilament staining observed in normal nerve (Fig. 5A), the neurofilament profiles in nerve segments cultured for 2 d were fragmented and irregular (Fig. 5B). Similar to axonal degeneration in vivo, the cultured nerves contained both annular and condensed neurofilament profiles, indicative of cytoskeleton disintegration and axonal degeneration. The degeneration of axons was especially obvious in semithin sections stained with toluidine blue. Degenerative processes resulting in additional myelin degeneration (collapse and condensation) and phagocytic removal were not observed in the 2 d cultured nerve segments (D, inset). Scale bars: (in A) A, B, D, 25 μm; C, 100 μm. Insets in C and D are magnified 4×.

**Cultured nerve as acellular interpositional grafts**

Previous studies show that peripheral nerves predegenerated in vivo are better acellular nerve grafts than are normal nerves. We
tested the hypothesis that predegeneration in vitro improves nerve regeneration through acellular nerve allografts. Host rats received bilateral, acellular nerve grafts, one control (not predegenerated) and one predegenerated in vitro (cultured for 2 d in 2% serum). Axonal regeneration was assessed after 8 d by scoring GAP-43-immunopositive profiles (total pixel count) in transverse sections. Axonal growth was observed in all grafts and was centrally distributed, indicating good alignment and coaptation of proximal host nerve and graft (Fig. 6). In six of six animals, the number of axons (inferred by GAP-43-immunopositive pixels) that crossed the proximal nerve–graft coaptation and entered the graft was greater in the in vitro predegenerated graft than in the contralateral control graft. On average the score of axons within the in vitro predegenerated grafts was twofold greater, and there was a significant difference in the mean axon scores at each distance within the grafts (p < 0.05) (Fig. 6B). This indicates that in vitro predegenerated grafts improved regeneration by decreasing the initial delay of axonal growth. Moreover, the greatest difference was found in the number of axons observed in the initial 1–2 mm of the grafts (p < 0.01), suggesting that the relative rate of axonal ingress into the in vitro predegenerated grafts continued to increase throughout the 8 d period. In both graft conditions, axonal growth occurred within basal lamina tubes and was accompanied by host derived Schwann cells. These findings show that axonal regeneration into acellular nerve grafts is enhanced and accelerated by in vitro predegeneration.

**DISCUSSION**

Sciatic nerve explant cultures are a valuable model to examine cellular and molecular aspects of Wallerian degeneration in the absence of hematogenic macrophages. Our observations confirm previous reports that significant degenerative changes occur rapidly in cultured nerve explants that lead to Schwann cell proliferation, fragmentation and liberation of myelin debris, and axonal disintegration (Crang and Blakemore, 1986; Perry and Brown, 1992; Reichert et al., 1994; Brück et al., 1995; Shen et al., 1999).

Peripheral nerve degeneration in vivo results in an increased turnover of several ECM molecules that depends on the release and activation of proteolytic enzymes by neurons, Schwann cells, and invading macrophages. Modulation of MMP activities after injury implicates MMP-2 and MMP-9 in remodeling of the ECM during nerve degeneration and regeneration (K:selected references). MMP-2 is expressed constitutively by Schwann cells in normal peripheral nerve (Yamada et al., 1995). Several days after injury, MMP-2 expression is upregulated and latent enzyme is substantially converted to its active form (Ferguson and Muir, 2000). MMP-9 is expressed in the peripheral nerve immediately after injury and primarily at the site of injury. MMP-9 expression correlates with the breakdown of the blood–nerve barrier, the accumulation of granulocytes, and the invasion of macrophages (Shubayev and Myers, 2000; Siebert et al., 2001). Most evidence suggests that hematogenic cells contribute significantly to the elevation of MMP-9 activity (Taskinen and Röyttä, 1997). In contrast, MMP-2 is expressed constitutively by Schwann cells in normal peripheral nerve (Yamada et al., 1995). Several days after injury, MMP-2 expression is upregulated and latent enzyme is substantially converted to its active form (Ferguson and Muir, 2000). In the present study of peripheral nerve degeneration in vitro, we find that MMP-9 is present in trace amounts primarily associated with a minor population of cells restricted to the epineurial sheath. Immunolabeling for MMP-9 is essentially absent in the endoneurium of cultured nerves. Taken together with immunolocalization and in situ zymography data, we conclude that MMP-2 is expressed by Schwann cells and that active enzyme is released into the surrounding endoneurium during in vitro nerve degeneration. MMP-2 is activated at the cell surface through a unique multistep pathway involving membrane-type MMPs (MT-MMPs) and the tissue inhibitor of MMPs, TIMP-2 (Sternlicht and Werb, 2001). Although little is known about MT-MMP expression in peripheral nerve, activated Schwann cells upregulate MMP-2 and TIMP-2 and appear capable of autonomous activation of MMP-2, which occurs in isolated Schwann cell culture (Muir, 1995; Huang et al., 2000).

In vitro degeneration results in a substantial increase in the neurite-promoting activity of nerve explants. This increase is blocked by the addition of MMP inhibitor, as is the coincidental
increase in the growth-promoting ability of acellular nerve grafts that was readily demonstrated in our cryoculture and grafting models. Acellular nerve grafting is associated with a substantial latency in the onset of axonal regeneration (Danielsen et al., 1995). Importantly, in vitro predegeneration markedly accelerates the ingress of axons into acellular nerve grafts and thus overcomes a major shortcoming associated with freeze-killed nerve grafts. Moreover, our evidence indicates that the neurite growth-promoting effects achieved by in vitro predegeneration are superior to those resulting from predegeneration in vivo. Also, because in vivo predegeneration of human donor nerve is impractical, in vitro predegeneration may greatly expand the clinical potential for acellular grafts. Additional study is required to determine whether in vitro predegeneration alone or in combination with other enhancement strategies can overcome other shortcomings of acellular grafts such as limits on graft length.

Many of the research on nerve explant culture and nerve graft preservation has focused on the cold storage of nerve segments. Cold-storage methods aim to preserve the nerve structure using minimal and ischemic conditions that suppress cellular and proteolytic activities. Cold storage greatly decreases the viability of antigen-presenting cells and therefore reduces the concerns of allograft immunorejection (Levi et al., 1994). Immunorejection of cellular allografts negates their regenerative potential. For this reason, prolonged cold storage of allografts results in better regeneration than fresh allografts (Evans et al., 1998, 1999). However, Lassner et al. (1995) reported that culture medium (DMEM rather than Cold Storage Solution) has a positive effect on maintaining Schwann cell viability and sustaining Wallerian degeneration and improves the regenerative potential of nerve grafts stored in cold, ischemic conditions. However, the concerns of immunorejection increase with more cellular allografts. More than cold preservation, the complete destruction of antigen-presenting cells in nerve grafts by freeze-killing virtually eliminates the concerns of graft immunorejection (Evans et al., 1998). As stated above, predegeneration enhances the growth-promoting properties of nerve grafts. Importantly, the combination of in vitro predegeneration and freeze-killing is more effective for obtaining a growth-promoting allograft not hampered by cellular immunorejection (Osawa et al., 1990). We find that culture of nerve grafts using conditions to support cell viability and cell-mediated degeneration significantly enhances the regenerative potential of nerve allografts. Once optimal degeneration/remodeling in vitro is achieved, the nerve explants are then freeze-killed and stored frozen for later use as interpositional nerve grafts. These findings support the assertion that predegenerated acellular nerve grafts have a greater potential for clinical applications than do cellular nerve grafts in allografting without immunosuppression.

In conclusion, it is evident that degeneration/remodeling of denervated nerve plays an important role in the regenerative capacity of peripheral nerves. All nervous tissues contain a preponderance of growth-inhibiting signals, but it is a robust degenerative competence that enables the regenerative capacity of the peripheral nervous system. We provide strong evidence that MMP-2 plays a principal role in establishing the growth-promoting properties of the basal lamina, which then retains the ability to stimulate nerve regeneration after the cellular elements have been killed. In vitro predegeneration results in a substantial
logical studies are required to assess the full potential of this graft preparation to improve recovery of function.

REFERENCES


