Observations on the blood and perineurial permeability barriers of surviving nerve allografts in immunodeficient and immunosuppressed rats

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The authors investigate whether there are any permeability changes in the endoneurial blood-nerve barrier and the perineurium-nerve barrier of surviving nerve allografts. In a normal nerve, the blood-nerve barrier regulates the passage of substances from endoneurial blood vessels into the endoneurium, whereas the perineurium-nerve barrier protects the endoneurium from agents that escape from permeable epineurial vessels and accumulate around the nerve. Nerves from ACI rats were transplanted into immunologically deficient nude rats or normal Fischer rats immunosuppressed with cyclosporin A. None of the nerve allografts was rejected. The blood-nerve barrier of nerve allografts at 2 and 6 weeks postoperatively was permeable to intravenously injected horseradish peroxidase, which spread into endoneurial tissue. Electron microscopy revealed that horseradish peroxidase escaped from endoneurial vessels through intercellular junctions between endothelial cells. At 24 weeks, the blood-nerve barrier of nerve allografts had recovered and the endoneurial vessels, like those in normal nerves, were impermeable to horseradish peroxidase. The perineurium-nerve barrier of nerve allografts remained impermeable to horseradish peroxidase at all times. Axons were grouped into numerous fascicles at nerve anastomosis zones at 24 weeks. Each nerve fascicle was surrounded by an impermeable perineurium. These results demonstrate that regenerated axons in long-term surviving nerve allografts and at anastomosis zones are protected by permeability barriers. It is concluded that permeability barriers of nerve allografts are not permanently altered by a foreign environment (grafts to nude rats) even when immunosuppression with cyclosporin A is required to prevent allograft rejection (grafts to Fischer rats).

KEY WORDS · nerve repair · nerve transplantation · immunosuppression · cyclosporin A · axonal regeneration · nerve anastomosis · perineurium · rat

The diffusion of agents such as protein into the endoneurial environment of nerve fibers of normal nerve is regulated by two distinct permeability barriers, the blood-nerve barrier and the perineurium-nerve barrier.10 The blood-nerve barrier is located in endoneurial blood vessels and regulates the movement of substances from inside these vessels into the endoneurium. The perineurium-nerve barrier is present in the perineurium and protects the endoneurial contents from agents that escape from the normally permeable epineurial blood vessels and accumulate around the nerve. The blood-nerve barrier and perineurium-nerve barrier exist because adjacent endothelial cells in the endoneurial blood vessels and adjacent perineurial cells in the perineurium develop impermeable intercellular junctions.10,12

In the present experiment, we studied rats to determine whether there were any acute or chronic permeability changes in the blood-nerve barrier and perineurium-nerve barrier of nerve allografts (that is, grafts exchanged between genetically different members of the same species). This determination is important since we can now prevent the rejection of nerve allografts with cyclosporin A in many species of animals.4,4

Indeed, with cyclosporin A therapy, it is possible to use a nerve allograft as a pathway for host axons to regenerate from a proximal nerve stump into a widely separated distal nerve stump. To our knowledge, there are no published studies on the permeability of surviving nerve allografts. Furthermore, we do not know whether cyclosporin A itself alters barrier permeability in grafts. Accordingly, we transplanted nerves into immunologically deficient nude rats.10 This experiment provided us the opportunity to study the blood-
Permeability of surviving nerve allografts

Nerve barrier and perineurium-nerve barrier of surviving nerve allografts in a foreign environment without the need for immunosuppression with cyclosporin A.

Materials and Methods

Experimental Animals

Inbred strains of immunologically normal male Fischer and American Cancer Institute (ACI) rats and an outbred strain of immunologically deficient Rowett nude rats, each weighing 250 to 275 gm, were used for the study. Fischer and ACI rats differ at their respective major and minor histocompatibility loci, and reject each other's nerves unless the recipient is immunosuppressed. Nude rats do not require immunosuppression to accept allografts. Nerve grafts from ACI rats were transplanted into nude rats or Fischer rats immunosuppressed with cyclosporin A.

Nerve Graft Procedure

All animals were anesthetized intraperitoneally with chloral hydrate (400 mg/kg body weight). Peroneal nerve grafts, 2 cm long, were taken from ACI rats and placed into standard culture medium (Dulbecco) at room temperature for no longer than 15 minutes, while the graft site of the recipient was being prepared. The peroneal nerve of the recipient was exposed in the thigh and a 1.5-cm piece of it removed. The ACI graft was then interposed and sutured to the cut ends of the host peroneal nerve with 10-0 nylon epineurial sutures, carefully placed, as viewed through an operating microscope. After nerve grafting, retracted muscle was returned to its anatomical position over the graft and joined with silk sutures while the overlying skin was closed with wound clips.

Preparation and Administration of Cyclosporin A

Cyclosporin A* was dissolved in olive oil by sonication. The first dose was injected into graft recipients 24 hours before surgery. Cyclosporin A was given intraperitoneally at 10 mg/kg for 7 days, at 5 mg/kg for the next 21 days, and thereafter at 5 mg three times a week until the animals were killed. A fresh batch of dissolved cyclosporin A was prepared weekly.

Evaluation of Grafts

Nerve grafts and host nerves (segments 6 to 8 mm proximal and distal to the graft) were removed from the recipient animals 2, 6, or 24 weeks postoperatively. Some nerve anastomosis zones were studied but only at 24 weeks. In order to evaluate nerve barriers, the animals were anesthetized with chloral hydrate and injected through the femoral vein with horseradish peroxidase,† a vascular permeability indicating agent. The tracer was dissolved in McIlwain's physiological salt solution and administered at a dose of 1 mg/5 gm body weight. Horseradish peroxidase was allowed to circulate for 20 minutes, at which time the animals were perfused through the heart with 200 ml of McIlwain's solution, followed by 400 ml of 4% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. The animals were left undisturbed for 2 hours to allow fixation to occur. The nerves described above were removed and postfixed overnight at 4°C, washed several times in buffer, and cut into 2-mm segments. Peroxidase activity in these segments was demonstrated by the histochemical method of Graham and Karnovsky, which yields a reaction product visible by light and electron microscopy. Unoperated animals were also injected with the tracer to determine the normal tissue localization of horseradish peroxidase. The specificity of the histochemical reaction was ascertained by omitting the substrate from the incubation medium. Finally, we examined nerves from normal (two from all rat strains) and nerve-grafted (one from each recipient at 2, 6, or 24 weeks) animals, all without horseradish peroxidase injection, to detect any endogenous horseradish peroxidase activity.

All histochemically reacted nerve segments were osmicated (1 hour in cacodylate buffer containing 1% osmium tetroxide), dehydrated through a graded series of ethanol and propylene oxide, and embedded in an Epon plastic mixture. The localization of horseradish peroxidase-reaction product was studied by light microscopy in unstained tissue sections, and routine tissue histology was evaluated in other sections stained with toluidine blue. A JEOL 1200 EX II electron microscope was used to detect reaction product in ultrathin sections stained with uranyl acetate and lead citrate.

Results

Normal Nerve

Cross sections of normal peroneal nerves from all rat strains revealed a large monofascicular nerve, divisible into endoneurial, perineurial, and epineurial portions (Fig. 1A). The endoneurium contained myelinated and unmyelinated axons and their associated Schwann cells. Blood vessels, fibroblasts, collagen fibers, and mast cells were also present. The perineurium was made up of four to seven layers of flattened cells (Fig. 2B) that encircled the endoneurial tissue. The layers of perineurium were separated from each other by a space containing some collagen fibers. The perineurial cells of each layer were covered by basement membranes on their outer and inner surfaces, and most of them contained cytoplasmic vesicles (Fig. 3C). In addition, intercellular junctions were present between adjacent perineurial cells in all layers (Fig. 3A and B). In certain regions of all nerves, the outer or inner perineurial layer was not circumferentially complete (Fig. 3C). The epineurium consisted of loose connective tissue, external to the perineurium, that supported blood vessels and the usual connective-tissue cells.

The permeability tracer horseradish peroxidase was detected in tissue by the presence of its histochemical reaction product which appeared as a brown precipitate by light microscopy and a granular, electron-dense material by electron microscopy. After an intravenous

* Cyclosporin A supplied by Dr. J. F. Borel, Pharmaceutical Division, Sandoz, Basel, Switzerland.
† Horseradish peroxidase type VI supplied by Sigma Chemical Company, St. Louis, Missouri.
FIG. 1. Photomicrographs illustrating the localization of horseradish peroxidase-reaction product (the black precipitate in the pictures) in cross sections of normal nerve (A–E; E is also stained with toluidine blue) and a 2-week nerve allograft (G); F is a control horseradish peroxidase section of normal nerve. A, F, and G, × 40; B, × 315; C–E, × 510. A: In normal nerve, horseradish peroxidase-reaction product is seen in the epineurium (EPI), perineurium (arrows), and lumen of an endoneurial blood vessel (ENDO). B: Although epineurial blood vessels are permeable to the horseradish peroxidase (A), the tracer spreads up to but not through the perineurium (short arrows). Note that horseradish peroxidase-reaction product is present on the luminal surface of the endothelial cells of endoneurial blood vessels (long arrows). C–E: Endoneurial vessels are not absolutely impermeable to horseradish peroxidase since horseradish peroxidase-reaction product is detectable in cells around blood vessels (C, arrow) or situated among the nerve fibers (D, arrows). The cell labeled in D can be seen after toluidine blue staining (E, arrows) to have the characteristics of a fibroblast (with an oval nucleus that extends one or more long cellular processes). F: No horseradish peroxidase-reaction product is detectable in this control nerve section from a non-horseradish peroxidase-injected rat that has been incubated in histochemical medium to detect horseradish peroxidase activity. G: Horseradish peroxidase-reaction product is present throughout the endoneurium of a 2-week degenerating nerve allograft.
Permeability of surviving nerve allografts

Fig. 2. Electron micrographs of normal nerve illustrating the localization of horseradish peroxidase in an epineurial blood vessel (A) and the perineurium (B). Epineurial blood vessels are normally permeable to intravascular horseradish peroxidase. Note the presence of horseradish peroxidase-reaction product (HRP-RP) in the junction between endothelial cells (J) and in the subendothelial basement membrane region (BM). Horseradish peroxidase-reaction product is also present in vesicles of endothelial cells (V). After its escape from epineurial blood vessels, horseradish peroxidase penetrates the outer but not inner layers of the perineurium, which consist here of several layers (labeled 1 to 7). The space between perineurial layers (labeled 1 to 7) is filled with HRP-RP. Note the absence of HRP-RP around the nerve and collagen fibers in the endoneurium (region bounded within arrows labeled "ENDO"). A, \( \times 54,500 \); B, \( \times 26,125 \).
FIG. 3. Electron micrographs of normal nerve. A and B: Localization of horseradish peroxidase in perineurial junctions. The junction in A (outlined by arrows) is clear whereas the junction in B (outlined by arrows) is filled with horseradish peroxidase-reaction product. Perineurial junctions containing horseradish peroxidase-reaction product were rarely observed. A, × 43,600; B, × 54,500. C: A perineurium is shown in which the innermost layer (arrows) was circumferentially incomplete. No horseradish peroxidase-reaction product is present in the space between the deep perineurial layers and the endoneurium (region bounded within arrows labeled "ENDO"). × 26,125.
Permeability of surviving nerve allografts

Fig. 4. Electron micrographs of normal nerve illustrating the localization of horseradish peroxidase in an impermeable endoneurial blood vessel. Horseradish peroxidase-reaction product is present on the luminal surface of endothelial cells (arrows). Note that no horseradish peroxidase-reaction product is detectable in the junction between endothelial cells (J), the endothelial basement membrane of the blood vessel (BM), or the endoneurium (region bounded within arrows labeled “ENDO”). A, × 32,700; B, × 54,500.
injection of horseradish peroxidase into normal rats, horseradish peroxidase-reaction product was seen by light microscopy inside and outside the epineurial blood vessels (Fig. 1A). Electron microscopy demonstrated that horseradish peroxidase-reaction product lined the luminal surface of these vessels and was present in some cytoplasmic vesicles of the endothelial cells; it also filled the junctions and subendothelial basement membrane region of endothelial cells (Fig. 2A). Outside epineurial vessels, the horseradish peroxidase-reaction product extended up to but not through the perineurium (Fig. 1B). Electron microscopy revealed the reaction product in the space between the superficial but not the deeper layers of the perineurium (Fig. 2B); it was also found in vesicles in the cytoplasm of perineural cells in the superficial layers. Despite an intense search, we were only able to find horseradish peroxidase-reaction product in an occasional junction between these perineurial cells (Fig. 3A and B); in contrast to epineural blood vessels, none was seen in the junctions or basement membrane region of endothelial cells of the endoneurial blood vessels (Fig. 4). However, the tracer was detectable on the luminal surface and in cytoplasmic vesicles of endothelial cells. We consistently found horseradish peroxidase-reaction product in cells around endoneurial blood vessels (Fig. 1C) and in the vicinity of nerve fibers (Fig. 1D). These cells had an oval nucleus and long cellular processes that were filled with the reaction product (Fig. 1D and E).

We have previously published an electron microscopic description of these cells, demonstrating that they are fibroblasts. It is noteworthy that fibroblasts in the epineurium also contain horseradish peroxidase-reaction product. In an attempt to resolve how horseradish peroxidase reached the endoneurial fibroblasts, we bathed four normal nerves in situ with horseradish peroxidase for 1 hour. In these nerves, horseradish peroxidase-reaction product was confined to the epineurium and perineurium, and none was seen in endoneurial fibroblasts. Furthermore, we noted the lack of horseradish peroxidase activity in epineurial and endoneurial fibroblasts (from two normal nerves) 7 days after the intravenous administration of horseradish peroxidase. These results indicated that blood was the source of horseradish peroxidase activity in endoneurial fibroblasts and that the tracer was metabolized. No horseradish peroxidase activity was detected in nerves of horseradish peroxidase-injected animals when the substrate was omitted from the histochemical incubation medium. Similarly, no horseradish peroxidase-reaction product was found in nerves from normal animals not injected with horseradish peroxidase (Fig. 1F).

Nerve Allografts

Three ACI nerve allografts in nude rats and three in cyclosporin A-treated Fischer rats were examined 2 weeks postoperatively, and a similar number of grafts were studied at 6 weeks. At 24 weeks, eight grafts obtained from each type of recipient were analyzed. No allografts were rejected; all of them contained Schwann cells, a normal number and distribution of blood vessels, and an intact perineurium. The allografts presented a histological picture of degeneration and regeneration that would be expected at the time intervals studied. At 2 weeks postoperatively, photomicrographs demonstrated the presence of Schwann cells and degenerating myelin (Fig. 5D). At 6 weeks, host axons had regenerated through the graft and some entered the distal peroneal nerve of the host. These regenerated axons were in various stages of myelination (Fig. 5F). Finally, at 24 weeks, numerous myelinated and unmyelinated axons were seen throughout the nerve grafts and distal host nerves. The final degree of myelination of the nerve fibers varied and was clearly less than that seen in normal nerve (compare Fig. 7C and D). The regenerated axons reinnervated muscles in the leg that had been denervated at the time of graft insertion. Animals also felt pain (prick from the tip of a needle) in lateral foot and toe regions corresponding to the distribution of sensory nerve fibers of a normal peroneal nerve.

Horseradish peroxidase was present in the endoneurium of all nerve allografts transplanted for 2 weeks into nude and cyclosporin A-treated Fischer rats. The localization of the tracer in grafts was variable, even in different segments of the same nerve. In general, horseradish peroxidase-reaction product was found perivascularly, with only focal accumulation in adjacent endoneurium (Fig. 5A), or it was present throughout the endoneurium (Figs. 1G, 5B, and 5C). Figure 5B is unusual in that intense endoneurial horseradish peroxidase-reaction product was seen extending to but not beyond the inner boundary of the perineurium. The perineurium maintained its impermeability to horseradish peroxidase 2 weeks postoperatively. This was most evident in nerve segments with focal endoneurial horseradish peroxidase extravasation. In these portions of nerves, large regions of the endoneurium extending from permeable blood vessels to the perineurium were free of horseradish peroxidase-reaction product (Fig. 5A). Electron microscopy revealed that, in contrast to normal nerve, horseradish peroxidase-reaction product was present in the junctions and basement membrane region of endothelial cells of endoneurial blood vessels. These permeable junctions were similar to that illustrated in Fig. 2A. The endothelial cells appeared normal and had horseradish peroxidase-reaction product on their luminal surface and in cytoplasmic vesicles. No evidence of transendocytosis of horseradish peroxidase (that is, the passage of horseradish peroxidase through tubules from one surface of the endothelial cell to the other) was observed. Furthermore, no fenestrae (pores in the endothelial cell membrane) were seen in the endothelial cells. Horseradish peroxidase-reaction product in the endoneurium was located around the Schwann cells and degenerating myelin. Some endoneurial fibroblasts and macrophages exhibited horseradish peroxidase activity.

The endoneurium of all nerve allografts at 6 weeks postoperatively contained horseradish peroxidase-reaction product (Fig. 5E). The tracer had the same variable localization as that described in 2-week grafts. The perineurium of 6-week grafts was impermeable to horseradish peroxidase, whereas endoneurial blood vessels leaked horseradish peroxidase through endothelial
Permeability of surviving nerve allografts

Fig. 5. Photomicrographs illustrating the localization of horseradish peroxidase-reaction product in a 2-week (A–D) and a 6-week (E and F) nerve allograft. D and F are also stained with toluidine blue. x 316. Endoneurial blood vessels (arrows) of 2- and 6-week grafts are now permeable to horseradish peroxidase. In some regions of nerve, horseradish peroxidase-reaction product is present focally outside permeable vessels (A) whereas in other regions the tracer spreads throughout the endoneurium (B, C, and E). Note that the perineurium in A prevents the inward movement of epineurial horseradish peroxidase, while in B the perineurium inhibits the exit of endoneurial horseradish peroxidase. Toluidine blue staining reveals that the endoneurium of a 2-week graft (D) is filled with degenerating myelin, whereas the endoneurium of a 6-week graft (F) contains numerous regenerated but thinly myelinated nerve fibers (some indicated by M in F).

The major difference between 2- and 6-week nerve allografts was the presence of regenerated axons in the latter. Electron micrographs showed that horseradish peroxidase-reaction product in the endoneurium was present around the surface of the Schwann cells that ensheathed each axon (Fig. 6A). More endoneurial fibroblasts than expected contained horseradish peroxidase-reaction product, whereas macrophages, although less numerous than at 2 weeks, still expressed horseradish peroxidase activity.

All nerve allografts studied at 24 weeks displayed the permeability characteristics of normal nerves. In these grafts, horseradish peroxidase was found lining the lumen of endoneurial blood vessels and in the superficial
Nerve Anastomosis Zones

The host nerve-graft nerve anastomosis zones from two nude and three cyclosporin A-treated rats were examined at 24 weeks, and similar findings were noted in all. In both the proximal and distal nerve anastomosis zones, the regenerated nerve fibers (myelinated and unmyelinated) were intermixed and grouped into small and large minifascicles (Fig. 8A). Interestingly, each minifascicle of nerve fibers was surrounded by one to four layers of cells that were morphologically similar to perineurial cells of normal nerve. Blood vessels were located outside rather than inside the perineurium of the minifascicles (Fig. 8A), and their endothelial cell junctions were permeable to horseradish peroxidase. The extravasated horseradish peroxidase did not penetrate the outer layer of the perineurium that encircled the minifascicles of nerve fibers (Fig. 8). Indeed, only one layer of perineural cells was sufficient to act as a permeability barrier (Fig. 8B). In essence, these findings indicated that a perineurial but not a vascular endoneurial permeability barrier had developed at nerve anastomosis zones.

Discussion

The major finding of our study was that host nerve fibers that regenerated into long-term surviving nerve allografts were protected by normal permeability barriers. The perineurium and endoneurial vasculature of the donor had to be the source of the perineurium-nerve barrier and blood-nerve barrier because there was no evidence of rejection in any of the nerve allografts. The perineurium-nerve barrier of grafts appeared to maintain its impermeability throughout the 2- to 24-week period of study. However, the blood-nerve barrier became markedly permeable to horseradish peroxidase at 2 and 6 weeks postoperatively, but it recovered by 24 weeks. By recovery, we mean physiological recovery, since horseradish peroxidase was detected in endoneurial fibroblasts of grafts as it was in normal nerve. Horseradish peroxidase-reaction product was present in the junctions between many of the endothelial cells of the endoneurial blood vessels at 2 and 6 weeks; this localization was similar to that observed in permeable epineurial blood vessels of normal nerve. Accordingly, one portal of horseradish peroxidase entry into the endoneurium of grafts was through endothelial junctions. We do not know if there was any pinocytotic transport of horseradish peroxidase from graft vessels into the endoneurium since the histochemical procedure used cannot distinguish horseradish peroxidase entry into nerve that is derived from junctional or vesicular transport.

It remains to be determined how horseradish peroxidase reaches endoneurial fibroblasts in normal nerve. The findings that topical horseradish peroxidase did not enter the endoneurium and that horseradish peroxidase was metabolized by fibroblasts imply but do not prove an endoneurial pinocytotic mechanism of horseradish peroxidase transport. It is likewise uncertain how horseradish peroxidase enters the superficial layers of the perineurium. Kлемm suggested that the external lay-
Permeability of surviving nerve allografts

Fig. 7. Photomicrographs illustrating the localization of horseradish peroxidase-reaction product in 24-week nerve allografts. C and D are also stained with toluidine blue. × 315. In A, a nerve allograft from a nude rat is presented, while in B and C nerve allografts from different cyclosporin A-treated rats are illustrated: in D, a normal nerve is shown. The localization of horseradish peroxidase-reaction product is similar in nerve allografts from nude rats (A) and cyclosporin A-treated rats (B) and is comparable to that seen in normal nerve (see Fig. 1B). Note that axons in a 24-week allograft are less myelinated (C) than axons in a normal nerve (D).

ers of the perineurium were metabolically very active since they faced a different environment than the deep layers. He stated that perineurial cells in the superficial layers had more cytoplasmic vesicles and might therefore be engaged in greater pinocytotic activity and transport of agents to deeper layers. It is interesting that neither we nor Klemm could detect horseradish peroxidase in perineurial junctions comparable to the frequency of that seen in leaky epineurial vessels or endoneurial vessels of 2- and 6-week grafts. Investigators should be aware that the most superficial and deepest perineurial layers may not be circumferentially complete (Fig. 3A) at all regions of the nerve.32 These observations explain why horseradish peroxidase, given intravenously or intraneurally, can be found beyond these layers without having to pass through intercellular junctions.

There is only one other study that deals with the permeability of surviving nerve grafts. Ahmed and Weller1 studied an autograft of sural nerve in rats and reported no changes in either the perineurium-nerve barrier or blood-nerve barrier of the grafts. While our and their data agree regarding the perineurium-nerve barrier, results concerning the blood-nerve barrier differ. We found horseradish peroxidase-reaction product in endothelial junctions of endoneurial blood vessels of 2- and 6-week grafts, whereas they did not. Their finding is puzzling since many other investigators have reported a prolonged increase in blood-nerve barrier permeability in degenerating distal nerve stumps after nerve crush of cut.4,18,24,26 Indeed, the present data show that our grafts degenerated, and that this process was accompanied by increased blood-nerve barrier permeability.

Of great interest is the observation by Ahmed and Weller1 that nerve fibers at anastomosis zones were ensheathed by variable layers of perineurial cells, dividing the zone into compartments or minifascicles. Furthermore, these authors observed, as did we, that blood vessels were located outside the compartments and were permeable to horseradish peroxidase. Ahmed and
FIG. 8. Electron micrographs illustrating the localization of horseradish peroxidase in a typical anastomosis zone between host and graft nerves at 24 weeks. A: Four minifascicles of nerve fibers, each surrounded by a different perineurium, are shown (arrows outline each perineurium). Note that the blood vessel is located outside the perineuria and is permeable to horseradish peroxidase. Horseradish peroxidase-reaction product (HRP-RP) in the perivascular space is prevented from reaching nearby minifascicles of nerve fibers by the outermost cellular layer of each perineurium. $\times$ 5450. B: The presence of HRP-RP is seen in the perivascular space of a blood vessel found outside a perineurium. Note that the perineurium consists of only one perineurial cell layer (arrows) which has HRP-RP on its outer but not inner surface. $\times$ 54,500.
Weller reported that horseradish peroxidase passed through the perineurial layers at anastomosis zones. In contrast, our results demonstrated that the perineurium of minifascicles were impermeable to horseradish peroxidase. It is worth mentioning that Ahmed and Weller did not actually suture their nerves together. In their experiment, nerve ends were held in alignment by Gelfoam soaked in thrombin and further treated with fibrinogen. Since nerves joined by a clot could pull apart, it might be that tissue healing was more prolonged after their nerve-clot attachment than our nervesuture anastomosis procedure. Indeed, Ahmed and Weller stated that at 24 weeks the central areas of the anastomosis sites were free of compartments and showed no penetration by horseradish peroxidase. It is noteworthy that, in a previous study,13 we found that the perineuria surrounding minifascicles of nerve fibers in cables formed inside silicone chambers were impermeable to horseradish peroxidase.

Our data are significant because they show for the first time that neither a foreign environment nor drug immunosuppression permanently alters the permeability of surviving nerve allografts. We predict that similar results should occur, with immunosuppression, in surviving human nerve allografts. It is important to point out that nerve allografts are rejected in animals after cyclosporin A therapy is stopped.12,18,26-30 which indicates that continuous immunosuppression is needed to maintain allogeneic Schwann, perineurial, and vascular cells in grafts. Furthermore, it must be realized that, while a perineurium-nerve barrier has been demonstrated in normal human nerve, there are no data on the anatomical permeability of a blood-nerve barrier.25 The latter fact is important since Olsson27 has reported considerable species variability regarding the permeability of the blood-nerve barrier. Accordingly, it might be that endoneurial blood vessels of human nerve are more physiologically permeable than those of the rat. If this is true, effective immunosuppressive therapy should preserve the cellular mechanism(s) that prevents endoneurial pressure elevation, a condition that is known to adversely affect normal nerve function.17

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