Nerve Regeneration through Allogenic Nerve Grafts in Mice

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Received January 27, 1986

Summary. The purpose of this study was to examine whether the basal laminae of Schwann cells in allografts could survive immunological rejection and serve as a conduit for regenerating nerves, as in the case of autogenic nerve grafts. Allografts of nerves were carried out using sciatic nerves of mice after the grafts had been repetitively frozen to kill their Schwann cells. Two mouse strains, C57BL/6N and C3H/HeN, were used, as they are known to differ in major histocompatibility complex. The mid-portion of the grafted nerve segments was examined by electron microscopy. In addition, the toe pad skin and lumbrical muscles were examined for determining whether regenerating nerves reinnervate sensory end organs and motor endplates.

The process of nerve regeneration in the allograft was the same as that seen in the autograft. Cells in the graft disintegrated into cell debris and were phagocytized by macrophages, whereas the basal laminae of Schwann cells were not removed by macrophages, remaining in the form of tubes or scaffolds. Regenerating nerve fibers grew out through such basal lamina scaffolds, keeping in contact with the inner surface. Digital sensory corpuscles and motor endplates of the operated side were well reinnervated. The results indicate that the basal laminae of Schwann cells of the allograft may survive and serve as a conduit for regenerating axons in the same way as in the case of an autograft.

Recently it has been demonstrated that regenerating nerves do not need the living Schwann cell columns, but require only the basal lamina scaffolds of Schwann cells as their pathways over long distances. Such an observation has been made in autogenic grafts of mouse sciatic nerves in which Schwann cells had been killed, immediately before grafting, by repetitive freezing and thawing (IDE, 1983; IDE et al., 1983). In these grafts, dead Schwann cells were removed by macrophages, while their basal laminae were left intact in the form of tubes of the original nerve, through which regenerating nerves grew. For the survival of the basal laminae, no immunological rejection would take place in the autograft. In an allograft, however, it is still debated as to what kinds of changes the basal laminae of Schwann cells might undergo. In the present study, reciprocal grafting of sciatic nerves which were treated by repetitive freezing and thawing was conducted between two mouse strains (C57BL/6N and C3H/HeN) which have been known to differ from each other in their major histocompatibility complex (H-2). Cells can not survive between these two strains, resulting in the degeneration of the tissue grafts. Non-cellular components of connective tissue, however,
have been claimed to have only slight antigenicity and are able to survive the immunological rejection for a certain period of time (Elvis, 1983). Therefore, it would seem a biologically and clinically important problem as to whether the basal laminae deprived of Schwann cells might remain in the allograft for a period long enough to serve as a conduit for regenerating nerves, as in the case of an autograft. The present study will give some data on the antigenicity of the basal lamina in animals with different major histocompatibility complexes on one hand and, on the other, on the possible clinical utility of allogenic human nerve grafts obtained from other subjects.

MATERIALS AND METHODS

Thirty male adult mice of two different strains (C57BL/6N and C3H/HeN) were used. It has been known that these two strains of mice differ in their major histocompatibility complex (H-2). Nerve segments 5-7 mm long were excised from the left sciatic nerve of one strain, and treated five times by repetitive freezing and thawing to kill the Schwann cells. The nerve segments thus treated were grafted to the corresponding site of the sciatic nerve of the other strain. The grafted nerve segments were sutured on both the proximal and distal ends of the host sciatic nerve with each stitch using 9-0 silk thread. Animals were killed at 4, 7, 14, 20, 30, 70 and 100 days after grafting. They were anesthetized with Nembutal (sodium pentobarbital, 30 mg/kg) and fixed by perfusion through the heart with a fixative containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). The grafted nerve segments were excised together with small pieces of the host nerve at the proximal and distal ends. Toe pads and lumbrical muscles of the left hindlimb (operated side) were also obtained in order to examine whether regenerating nerve fibers reached the sensory as well as the motor end organs. All these specimens were stored in the same fixative for 24 hrs at room temperature, postfixed at 4°C in 0.1 osmium tetroxide solution adjusted to pH 7.4 with 0.1 M cacodylate buffer, then dehydrated through a graded series of ethanol and embedded in Epon 812. Thin sections were cut mainly from the middle part of the graft with a diamond knife on an LKB Ulrotome and double-stained with uranyl acetate and lead citrate. Sections were observed with a Hitachi H-700 electron microscope.

RESULTS

The findings in the nerves grafted from C57BL/6N to C3H/HeN and vice versa were the same.

Four days after grafting, all the dead Schwann cells of myelinated as well as unmyelinated nerve fibers had disintegrated into cell debris. Schwann cell basal laminae were left apparently intact in the form of tubes containing degenerated nerve elements. These remaining basal laminae of the Schwann cells were called lamina tubes or scaffolds. Macrophages were found invading some of these basal lamina scaffolds to phagocytized cell debris and myelin fragments. Endoneurial collagen fibrils in the grafts were present between the scaffolds. No regenerating axons were found in the graft at this stage (Fig. 1).

Seven days after grafting, cell debris in the basal lamina scaffolds was observed being vigorously removed by macrophages. Macrophages were also found outside the

70 T. Osawa, C. Ide and K. Toyama:
basal lamina, i.e., in the connective tissue compartment, and they were considered to have migrated from the basal lamina scaffolds after having phagocytized cell debris within the scaffolds. These findings were the same as those observed in the autogenic nerve grafts. Some basal lamina scaffolds contained none or only a small amount of

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Fig. 1. Four days after grafting (C3H/HeN to C57BL/6N). Schwann cells (S) and myeline sheaths have disintegrated, while basal laminae (BL) of myelinated and unmyelinated fibers remain intact. Presumable macrophages (M) are seen in the endoneurial space. ×9,000
cell debris, probably due to phagocytosis by macrophages and/or autolysis of degraded cells. The basal laminae were apparently intact, being similar in their ultrastructure to those seen in the cases of autograft. It should be noted that some regenerating axons were found inside the basal lamina scaffolds at this stage (Fig. 2); they were thin, measuring 0.2–0.5 μm in diameter, and attached themselves to the inner or cellular

Fig. 2. Seven days after grafting (C57BL/6N to C3H/HeN). Regenerating axons (A) are seen in contact with the inner surface of the basal lamina scaffolds (BL). A small amount of cell debris is seen inside the basal lamina scaffolds. ×38,000
side (the side originally facing the Schwann cells) of the basal lamina scaffolds. Neurotubules, occasional mitochondria and a small amount of smooth endoplasmic reticulum were found in the axoplasm of such regenerating axons. Some non-neuronal cells, which were presumed to be developing Schwann cells, were associated with these regenerating axons. They were believed to have migrated from the proximal stumps of the host sciatic nerve (Fig. 3).

Fourteen days after grafting, basal lamina scaffolds were left apparently unchanged from their previous stage (Fig. 6a). The majority of the basal lamina scaffolds

![Image of a basal lamina scaffold with regenerating axons](image.png)

**Fig. 3.** Seven days after grafting (C3H/HeN to C57BL/6N). The cytoplasm of a presumable Schwann cell (S) surrounds several regenerating axons (A) in a bundle within a basal lamina scaffold (BL). × 29,000
Fourteen days after grafting (C57BL/6N to C3H/HeN). Myelination begins in some axons (long arrows). Schwann cells have their own basal laminae at this stage (arrowheads). Old basal laminae still remain, though partly interrupted (short arrows). A small number of collagen fibrils are seen within this old basal lamina scaffold, which are believed to be newly produced by the developing Schwann cells. The thin cytoplasmic processes of fibroblast-like cells (F) begin to surround the basal lamina scaffolds to form the endoneurial compartment. x 21,000

Twenty days after grafting (C57BL/6N to C3H/HeN). Regenerating nerves are divided into nerve bundles which are surrounded by two or three layers of flattened developing perineurial cells (P). Each nerve bundle contains both large myelinated (A) and small unmyelinated (a) axons. x 7,000. b. High-power micrograph showing endoneurial collagen fibrils, developing perineurial cells (P) and Schwann cells (S). Endoneurial collagen fibrils (e) are thinner than those seen outside the perineurial sheath (c). Perineurial cells exhibit patches of basal laminae (arrowhead) on the surface, indicating the initiation of basal lamina formation as seen in matured perineurial cells. x 43,000
Fig. 5. Legend on the opposite page.
contained many regenerating axons, while the rest—which had collapsed—did not. Regenerating axons in the basal lamina scaffolds were already being sorted out by the Schwann cells: large-diameter axons (1–2 μm) were separated from each other, being individually surrounded with different Schwann cells, while small-diameter axons (less than 0.5 μm) were enveloped in a bundle with a common Schwann cell. It was noted at this stage that some Schwann cells accompanying the regenerating axons began to sporadically exhibit their own basal laminae on the plasmalemma, while at the same time fine collagen fibrils began to appear between these Schwann cells, indicating that the regenerating axons and Schwann cells were becoming mature. Furthermore, the basal lamina scaffolds containing regenerating axons, as a whole, tended to be surrounded by a single layer of unidentifiable fibroblast-like cells, which evidently were destined to become perineurial cells in the later stages. Schwann cells associated with large-diameter axons began to form myelin sheaths. These findings were also the same as in the case of autogenic nerve grafts (Fig. 4).

Twenty days after grafting, the myelinated fibers increased in number, and at the same time their myelin sheaths became thicker (Fig. 5a). Each Schwann cell surrounding the myelinated and unmyelinated fibers had its own basal laminae. On the other hand, the basal lamina scaffolds which had contained the early regenerating fibers became obscure (Fig. 6b), and eventually disappeared. Instead, the fibroblast-like cells mentioned in the previous stage completely surrounded the regenerating fiber bundles with their thin cytoplasmic processes. They extended additional cell processes, ultimately dividing these nerve bundles into smaller ones. There were basal laminae on their surface, suggesting that these cells were newly developing perineurial cells. Thus, the old endoneurial collagen fibrils adjacent to the remaining basal laminae were compartmentalized by the developing perineurial cells. This process was considered to define the new endoneurial space for the regenerating nerve. In fact, collagen fibrils in the endoneurial space were thinner than those of ordinary connective tissues outside the perineurial sheath (Fig. 5b), as seen in the normal peripheral nerve bundle (Thomass, 1963; Osawa and Ide, 1986).

At 30 and 50 days after grafting, the regenerating axons and accompanying Schwann cells were morphologically fully matured and the developing perineurial cells gave rise to many cytoplasmic processes which divided the endoneurial space into smaller compartments of variable sizes. In keeping with such compartmentalization by the perineurial cells, the regenerating nerves in the grafts were divided into groups of smaller bundles consisting of myelinated and unmyelinated fibers. A few fibroblasts were occasionally seen in the areas between such compartments.

Regenerating sensory axons began to reach the level of digital corpuscles in the pad skin at the toe tip about 30 days after grafting (Fig. 7a). The lamellar cells of digital corpuscles had been in an atrophic state due to denervation. However, once such atrophic lamellar cells received regenerating axons, they attained their original vigorous state and, by 70 days after grafting, formed fully-regenerated digital corpuscles. On the other hand, regenerating motor nerves were first seen in the endplates of lumbrical muscles 50 days after grafting. Later, reinnervated endplates gradually increased in number, almost fully maturing by 100 days after grafting (Fig. 7b).

The above results show that regenerating axons can extend through the allogenic nerve grafts as far as the end organs in the same way as was noted in the case of nerve regeneration following an autogenic graft of simple denervation due to nerve transection.
Fig. 6.  

a. Fourteen days after grafting (C57BL/6N to C3H/HeN). At this stage, both the old basal lamina (arrow) and newly-formed basal lamina (arrowheads) are clearly seen. They may be locally fused (star). × 55,000  
b. Twenty days after grafting (C3H/HeN to C57BL/6N). The old basal lamina (arrow) becomes obscure, while the newly-formed one (arrowheads) is definite. × 55,000
Fig. 7. 

a. Thirty days after grafting (C3H/HeN to C57BL/6J). One small regenerating axon (A) has reached the remaining Meissner-like digital corpuscle, being in contact with lamellar cell processes (L) of the corpuscle in the toe pad. BV blood vessel, E epidermis. \( \times 12,000 \).

b. One hundred days after grafting (C3H/HeN to C57BL/6J). An example of a regenerated motor endplate seen in the lumbrical muscle. This regenerated endplate has almost a normal morphology: deep primary trough with well developed subsynaptic folds (SF) receiving the axon terminal which contains synaptic vesicles (SV) and mitochondria (Mi). S Schwann cell. \( \times 32,000 \)
DISCUSSION

The present study has demonstrated that the basal laminae of Schwann cells remain in the form of scaffolds in the allograft and that regenerating sciatic nerves can grow out through such basal lamina scaffolds. These regenerating nerves reached as far as the end organs, both sensory and motor, as seen in the ordinary nerve regeneration following simple denervation due to the cutting or crushing of nerves. The process of nerve regeneration in the allograft was almost the same as in the autograft (Ide, 1983; Ide et al., 1983).

The two strains of mice, C57BL/6N and C3H/HeN, used in this study differ from each other in their histocompatibility complex (H-2) (Iványi, 1970; Snell and Stimpfling, 1975). Ordinary reciprocal transplants between these two strains will encounter an immunological reaction, leading to the rejection of the grafts. In the present study, however, the cells in the grafts were killed by repetitive freezing and thawing; after grafting they were phagocytized by macrophages, which is the usual biological process for dead cell removal. It should be noted that the Schwann cell basal laminae in the allografts remained intact for at least 20 days after grafting without being attacked by macrophages, the same as in the autografts. This finding shows that Schwann cell basal laminae were not so very antigenic between the strains of C57BL/6N and C3H/HeN as to be removed immediately after grafting. Pollard and Fitzpatrick (1973a) reported the same finding in allografts of sciatic nerves from D.A. rats to Wister. Although there is no evidence as to whether or not the constituents of the basal laminae might be different in molecular moiety between these two strains, it can at least be said that Schwann cell basal laminae of allografts can survive immunological rejection and serve as pathways for regenerating nerves in the same way as in the autograft. Gulati and Zalewski (1985) have reported that basal laminae persisted after the Schwann cells were rejected in allografts containing viable Schwann cells, when using strains of rats which were not immunologically compatible with each other. Gulati et al. (1984) reported similar results in the transplantation of skeletal muscles. They reported that the laminin of muscle basal laminae remained after the muscle fibers were removed. These studies indicate that basal laminae cause no immunological reaction when grafted into mice or rats of different strains. However, Watson et al. (1954) found that antibodies against type I collagen can be induced in rabbits, though low on titer, using acetic acid-solubilized collagen obtained from rat-tail tendon. Steffen et al. (1970) also induced anti-type I collagen antiserum in rabbits using acid-solubilized human collagen. On the other hand, Elvis (1983) has mentioned that the cartilage, when it is grafted clinically, is not rejected but very slowly resorbed. He has stated that the collagen components of the cartilage matrix have only a slight antigenicity. Generally, it can be said that the collagen components from type I through type IV, when they are solubilized, have only slight antigenicity, if any, between strains of the same animal. However, if collagen components are not solubilized, i.e., remain in their solid structure as in the case of grafting, they have almost no antigenicity, resulting in successful grafting without immunological rejection among the different strains.

The finding of the present study is also of great interest from the clinical point of view, since it suggests that Schwann cell basal laminae in allogenic nerve grafts can be effective pathways for regenerating nerves. In an experiment using rabbit sciatic nerve, it was reported that nerve regeneration can occur to some extent in allografts (Hirasawa et al., 1984). Clinically various methods have been used in an attempt to
facilitate nerve regeneration in an allograft. Irradiation, freezing, lyophilization of the graft, or a combination of these methods were adopted to reduce the immunological rejective reaction using rat sciatic nerves (MARMOR, 1964; DAS GUPTA, 1967; POLLARD and FITZPATRICK, 1973b; BUCKO and STEINMULLER, 1974), radial or peroneal nerves of beagle dogs and chimpanzees (DUCKER and HAYES, 1970) and peroneal nerves of mongrel dogs (MARMOR, 1964). Immunosuppressives, such as antilymphocyte serum, azathioprine, hydrocortisone or cyclosporine A were used, sometimes in combination with the above mentioned treatments (POLLARD et al., 1971; POLLARD and FITZPATRICK, 1973b; ZALEWSKI and GULATI, 1981; 1984). The results of these studies give evidence that not all attempts are as effective as expected for allografts to survive for a relatively long period of time, but that they are eventually eliminated due to the immunological rejection. The present study indicates that allogenic nerve grafts are as effective as autogenic nerve grafts when Schwann cells in the allografts have been previously killed. This result supports the possibility that nerve allografts can be clinically applied, utilizing the corresponding segments of damaged nerves, which may be made continuously available from a “nerve bank.” Nerve segments taken from cadavers, in which cells are no longer alive in the process of autolysis, could be grafted into nerve defects in patients, provided the basal laminae of the Schwann cells have not degraded yet. The dead Schwann cell debris in such grafted nerve segments would be removed by macrophages, leaving basal lamina tubes, which work as the effective conduit for regenerating nerves for a certain period of time. Basal lamina scaffolds, whether autogenic or allogenic, survive for only 3–4 weeks, probably due to the gradual disintegration of basal laminae without any additional synthesis of their components. This implies that basal lamina scaffolds can work only for 3–4 weeks after grafting. Regenerating nerves can extend at the average rate of 1 mm per day, meaning that, at most, the effective graft length might be 3 cm. It is unlikely that regenerated nerves may extend further through the graft after the basal laminae have disintegrated and disappeared. Therefore a nerve graft to fill a defect larger than 3 cm in length would not be successful. To conquer this difficulty, repeated surgical operations are advised in which a new graft is added to the distal end of the old one 3–4 weeks after the previous grafting.

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