Nerve graft immunogenicity as a factor determining axonal regeneration in the rat

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Acellular basal lamina grafts have recently been reported to support axonal regeneration and have been used in peripheral nerve repair. The present study was designed to determine the immunogenicity of such basal lamina allografts (grafts that are genetically different) and their potential as bridging material for nerve gap repair. Inbred strains of Fischer and Buffalo rats with known histocompatibility differences were used. Acellular grafts were prepared by repeated freezing and thawing nerve tissue predegenerated in situ for 6 weeks. Non-frozen predegenerated nerves were used as cellular grafts for comparison. Fischer rats were used as hosts and received cellular or acellular grafts obtained from Fischer (isograft, genetically identical) or Buffalo (allograft) donors. The grafts were evaluated morphologically at 1, 2, 4, and 12 weeks after transplantation. The cellular isografts supported axonal regeneration best. The cellular allografts were invariably rejected and were unsuccessful or only partially successful in supporting regeneration. In contrast, acellular allografts, in spite of their mild immunogenicity were successful in supporting regeneration, as were the acellular isografts. The rate of host axonal regeneration and recovery of target muscle was reduced in acellular allografts and isografts as compared to cellular isografts. It is concluded that acellular allografts are suitable for supporting axonal regeneration and may be used to bridge gaps in injured peripheral nerves.

KEY WORDS • nerve graft • immunogenicity • axonal regeneration • peripheral nerve • rat

The use of peripheral nerve allografts (grafts between genetically different members of the same species) for peripheral nerve repair has been explored by several investigators. Such grafts are successful in supporting host axonal regeneration only when the host animal is immunosuppressed to prevent immune rejection. The rejection response is evoked by the presence of transplantation antigens within the allografts that culminates in its destruction. Histocompatibility antigens responsible for rejection have been shown to be carried on the various cellular components of the nerve tissue (such as Schwann cells and endothelial cells). It is evident that, for an allograft to be successful, the immunogenicity of the allograft and/or the host immune response must be altered.

It has been proposed that the “endoneurial tubes,” comprised of Schwann cells and their basal lamina, guide the regenerating axons through the nerve grafts. Several recent studies have described the ability of endoneurial basal lamina, in the absence of viable Schwann cells, to support axonal regeneration through the graft material. Gulati compared the ability of cellular and acellular basal lamina grafts to support axonal regeneration. Although cellular grafts worked better (they supported regeneration over longer distance) than acellular basal lamina grafts, significant regeneration was observed through both types of grafts. As acellular grafts lack cells that are known to carry the histocompatibility antigens, it can be speculated that such grafts may be less immunogenic and better tolerated by an allogenic host. The present study compares the immunogenicity of cellular and acellular nerve allografts and the ability of each to support axonal regeneration. The results show that acellular allografts are less immunogenic, and are as effective as isografts (grafts between genetically similar members of the same species) in supporting host axonal regeneration.

Materials and Methods

Animals

Inbred strains of Fischer (FR) and Buffalo (BF) male rats, weighing 250 to 300 gm each, were used for these studies. These rat strains differ in major and minor loci-derived transplantation antigens that evoke a rapid and intense immune response after grafting. The FR rats obtained from Harlan Sprague-Dawley, Inc., Indianapolis, Indiana.
Nerve graft immunogenicity

Rats always served as hosts, and were grafted with nerve tissue obtained from BF and FR donors. The rats were anesthetized with intraperitoneal chloral hydrate (40 mg/100 gm body weight) during all of the procedures.

Nerve Graft Preparation

Fischer and BF rats were used as a source of donor isografts and allografts, respectively. After induction of anesthesia, the sciatic nerve in each leg was exposed and cut a few millimeters distal to the sciatic notch. The cut ends were tightly ligated with 4-0 silk suture, and allowed to undergo in situ degeneration for 6 weeks. After this time the donor rats were reanesthetized, and the entire length of degenerated peroneal nerve was removed. A 2-cm segment was cut and placed in alpha-minimum essential culture medium. These nerve segments were then used for transplantation without further preparation and served as the cellular basal lamina grafts.

Acellular basal lamina grafts were prepared as follows. Degenerated nerve segments were harvested as described above for the cellular grafts. The nerves were placed on a steel spatula, frozen by immersing the spatula in liquid nitrogen, and then thawed to room temperature. The nerves were frozen and thawed in this manner five times. This freeze-thaw procedure was similar to that used in our earlier study to prepare acellular basal lamina grafts. Treatment of nerves in this manner is known to kill all cell types, including Schwann cells, with no apparent effect on the basal laminae. These nerve segments, also 2 cm long, were placed in culture medium until transplantation and served as the acellular basal lamina grafts.

Experimental Groups and Nerve Grafting Procedure

Recipient FR rats were divided into two groups; one received only cellular grafts and the other received only acellular grafts. In both groups a nerve isograft (obtained from an FR rat) was placed in the left leg and a nerve allograft (obtained from a BF rat) was placed in the right leg. This paradigm resulted in four types of nerve graft allografts. After induction of anesthesia, the leg was opened to expose the peroneal nerve with the bridging graft. The proximal host nerve, the graft, and the distal host nerve were carefully removed and cut into pieces approximately 3 mm long throughout its entire length. Cut nerve segments from different regions were placed in slabs of skeletal muscle and frozen in liquid nitrogen. Longitudinal sections and cross sections, 8 and 24 μm thick, respectively, were cut in a cryostat set at −20°C and mounted on glass slides. The 8-μm sections were stained with periodic acid-Schiff-hematoxylin, whereas the 24-μm sections were stained by the cholinesterase-silver technique. All slides were examined for graft survival and the extent of host axonal regeneration through them. The extent and distribution of mononuclear immune cells in each graft type was determined and compared to determine graft immunogenicity. The presence of regenerated host axons in the graft as well as in the distal host nerve was also compared and used as an index for the ability of each graft type to aid in nerve repair.

The success of nerve graft repair was further determined by morphological analysis of the target muscle. In each rat, the extensor digitorum longus (EDL) muscle was removed at the time of nerve graft removal. Each muscle was weighed and frozen in liquid nitrogen, sectioned, and stained as described for nerve tissue. Muscle size, weight, and innervation status were used as an index of target muscle recovery after nerve repair with each of the four graft types.

Results

The immunological fate of each type of graft is summarized in Table 1. The recovery of target EDL muscle after each type of nerve repair and of normal EDL muscle is shown in Fig. 1. The individual weights of six normal muscles were 94, 87, 92, 92, 86, and 94 mg. A more detailed description of results for each graft type is given below.

Cellular Basal Lamina Isografts

As described in an earlier study from this laboratory, regeneration of host axons through cellular basal lamina grafts occurred rapidly with eventual innervation of
A. K. Gulati and G. P. Cole

**TABLE 1**

Relative presence of mononuclear immune cells in different regions of various types of nerve grafts*

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<th>Weeks After Transplantation</th>
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* Semiquantitative analysis: +++ extensive; ++ moderate; + minimal; - absent. Graft regions: P = perineurium; V = vascular; I = interior.

**FIG. 1.** The relative recovery of extensor digitorum longus (EDL) muscle after various types of nerve graft repair. CI = cellular isograft; CA = cellular allograft; AI = acellular isograft; and AA = acellular allograft. The mean wet weight (± standard error of the mean) is shown for normal muscles and muscles 12 weeks after nerve repair (six samples in each group). Individual muscle weights for each group are given in the Results section. Unpaired t-test revealed a significant difference in cellular allografts from all groups (p < 0.05).

Cellular Basal Lamina Allografts

The cellular allografts were invariably rejected as revealed by the extensive presence of mononuclear immune cells in different regions of the graft (Table 1). Limited regeneration of host axons was seen through such allografts. Mononuclear immune cells were present, especially in the region of perineurium and around blood vessels, in 1-week allografts. Immune cells increased at 2 weeks and were seen throughout the graft (Fig. 3A). The perineurium or columns of Schwann cells, observed clearly in cellular isografts were not identifiable in cellular allografts (compare Figs. 2B and 3A). At 4 weeks the entire graft was infiltrated with mononuclear immune cells (Table 1). At 12 weeks the cellular allografts were thin and in some cases composed of connective tissue matrix with no identifiable Schwann cells and regenerated axons (Fig. 3B). Some regenerated axons were seen. In most grafts they were restricted to the proximal 0.5-cm segment (Fig. 3C). Surprisingly, in two allografts some axons did regenerate through the entire graft in spite of the presence of immune cells and allograft rejection. However, these regenerated axons were much fewer than in cellular isografts. The reason for the success of these two allografts remains unknown and is examined in the Discussion section. The target EDL muscles were not innervated (Fig. 3D and E) and were much reduced in size and weight (Fig. 1). The individual muscle weights of the EDL muscles in this group were 29, 21, 30, 41, 36, and 25 mg. These weights were significantly different from the cellular isograft group.

Acellular Basal Lamina Isografts

Regeneration of host axons through acellular isografts and recovery of the target EDL muscle (Fig. 1) were observed. This response was somewhat limited as compared to cellular isografts but the difference was not statistically significant. The target EDL muscle weights in this group were 73, 61, 62, 35, 24, and 29 mg. One week after transplantation these frozen/thawed grafts appeared acellular because of the removal of dead cell debris. At 2 weeks, Schwann cells and other cells had migrated into these grafts from both the proximal and distal host nerves (Fig. 4A). Regeneration of
host axons from the proximal host nerve was not observed at this early stage. These grafts maintained their shape and the perineurium was also identifiable. Regenerating host axons were observed in the proximal portion of the graft at 4 weeks (Fig. 4B), and by 12 weeks many axons had traversed the entire graft (Fig. 4C and D) and had grown into the distal host nerve. The regenerating axons in the acellular basal lamina isografts were organized into minifascicles (Fig. 4C). The minifascicular appearance of axons in grafts was not observed in axons that had grown into the distal host. No mononuclear cells were observed in these grafts, showing that these grafts were nonimmunogenic. The regeneration pattern and fate of frozen/thawed acellular grafts was similar to that described in earlier studies.5,8

Acellular Basal Lamina Allografts

Successful regeneration of host axons was observed through acellular allografts in spite of the mild immune response to these grafts. In fact, recovery of target EDL
FIG. 3. Sections of cellular allografts at various time intervals after transplantation. PAS-hematoxylin (A, B, and D); cholinesterase-silver (C and E), x 115. A: Longitudinal section of a cellular allograft 2 weeks after placement which is undergoing immune rejection. Many mononuclear immune cells (arrows) are seen throughout the graft. The perineurium (P) and Schwann cell columns are not easily discernible (compare with Fig. 2B). B: Longitudinal section of a mid-graft region 12 weeks after grafting. Foci of mononuclear immune cells (arrow) are still present. The perineurium (P) is not clearly seen and the graft consists of connective tissue matrix (C) with no regenerated axons (compare to Fig. 2C). C: Cross section of a mid-graft region 12 weeks after implantation. Some dispersed and disorganized axons (arrows) are seen. D and E: Cross sections of the target extensor digitorum longus muscle 12 weeks after cellular allograft nerve repair. The muscle fibers are small and atrophic with no nerve fibers or motor end plates, indicating a state of denervation.

Muscle and axonal regeneration was better (not statistically significant) in this group than in nonimmunogenic acellular isografts (Fig. 1). The weights of the individual EDL muscles in this group were 42, 52, 74, 71, 32, and 61 mg. At 1 week acellular allografts resembled acellular isografts, but at 2 weeks a moderate presence of mononuclear immune cells was observed in acellular allografts (Table 1). The immune cells were primarily seen in the perineurial region of the acellular allografts (Fig. 5A). Schwann cells and regenerating axons were seen in the proximal region of 2- and 4-week acellular allografts (Fig. 5B). Regeneration progressed and by 12 weeks axons had traversed the entire graft and distal host nerve (Fig. 5C and D).
Nerve graft immunogenicity

FIG. 4. Sections of acellular isografts at various time intervals after transplantation. PAS-hematoxylin (A to C); cholinesterase-silver (D), ×115. A: Cross section of a proximal graft region 2 weeks after implantation. No regenerated axons are visible but various cells (including Schwann cells) have migrated into the graft. The perineurium (P) is also visible. B: Cross section of a mid-graft region 4 weeks after placement. The arrows point to regenerated host axons that are located to one side of the graft. The perineurium (P) is difficult to identify. C and D: Longitudinal sections of a mid-graft region 12 weeks after implantation. Many regenerated axons (A) are seen throughout the graft, with a majority of them forming minifascicles (arrows in C).

term allografts, the perineurium had reorganized around the regenerated axons, forming minifascicles. The axons in the distal host nerve did not have the minifascicular appearance (compare graft and host in Fig. 5C).

The target EDL muscle recovered (Fig. 1) and was innervated. Large myofibers of uniform size and motor end-plates were observed (Fig. 5E and F). Such recovery of muscle was not observed with cellular allografts (Fig. 3D and E).

Discussion

The immune destruction of peripheral nerve allografts by the host is a critical factor that precludes the eventual success of nerve graft repair. Host immunosuppression is effective in preventing allograft rejection, but long-term use of potentially dangerous drugs is unwarranted for repair of nerve lesions. An alternate approach for using nerve allografts involves attempting to reduce graft immunogenicity. Methods such as irradiation, fixation, and removal of myelin have proved unsuccessful in preventing rejection. The results of this study demonstrate that frozen/thawed pregenerated nerve allografts exhibit a limited immunogenicity that does not hinder the growth of host axons. Fawcett and Keynes have studied basal lamina grafts derived from the skeletal muscle of rabbits that were transplanted into rat hosts (xenografts). They reported successful regeneration through short (0.5-cm) basal lamina xenografts and concluded that such grafts exhibit no or little immunogenicity.Taken together, these results provide evidence that grafts composed of basal lamina are less immunogenic than the cellular grafts. Elimination of live cells and the associated transplantation antigens by the freeze/thaw procedure results in significant reduction of graft immunogenicity, rendering such allografts suitable for nerve gap repair.

The rate of host axonal regeneration and recovery of
Fig. 5. Sections of acellular allografts at various time intervals after transplantation. PAS-hematoxylin (A to C, and E); cholinesterase-silver (D and F), x 115. A: Cross section of a proximal graft region 2 weeks after implantation. A moderate presence of mononuclear cells (arrows) localized primarily in the perineurial region (P) is seen (compare with Fig. 3A). B: Longitudinal section of a mid-graft region 4 weeks after placement showing some regenerated myelinated axons (arrows). A few isolated mononuclear cells are also visible especially adjacent to the perineurium (P). C and D: Adjacent cross sections of a distal graft (G) and distal host (H) region 12 weeks after implantation. Many regenerated axons are seen throughout the graft and the distal host nerves. In C the regenerated axons form minifascicles in the graft (G) (arrows); such minifascicles are not seen in the distal host (H) nerve. The perineurial region (P) is not clearly observed. E and F: Cross sections of the target extensor digitorum longus muscle 12 weeks after acellular allograft nerve repair (compare with Fig. 3D and E). The muscle fibers are large and of uniform size. The presence of regenerated nerve fibers (arrowheads) and motor end-plates (arrows) demonstrates muscle reinnervation.
Nerve graft immunogenicity

the target muscle varied among the various experimental groups in the present study. Cellular isografts were the most effective; host axons had traversed the entire graft length by 4 weeks. In comparison, host axons occupied the entire length of acellular allografts and isografts only at the 12-week observation period. Earlier comparative studies have also described the slower rate of regeneration through acellular grafts. Furthermore, Gulati reported that the distance over which acellular grafts are effective in supporting axonal growth is shorter (maximum of 2 cm) than for cellular grafts. Therefore, both the rate of regeneration and distance of growth are reduced in acellular grafts. The presence of neuronotrophic and/or neurite promoting factors (such as nerve growth factor (NGF)) secreted by viable Schwann cells in cellular grafts as compared to acellular grafts may contribute to the difference in their ability to support axonal regeneration. These results imply that the growing axons exhibit a graded preference for growth rather than an absolute requirement as determined by the differential distance over which the axons can traverse (that is, the length of the graft). The number of regenerated axons in these various grafts is also likely to vary and is currently being investigated.

The pattern and organization of regenerating axons in these various graft types are also unique. In acellular grafts (both isografts and allografts) the regenerating axons form multiple minifascicles, each surrounded by a perineurial-like structure. This minifascicular pattern is not observed in the distal host nerves or in cellular isografts. Hall has related this type of compartmentalization to the severe disturbance in or a total loss of the perineurial function; this is possible during the nerve graft preparation. Formation of minifascicles has also been described in rejected allografts and irradiated grafts, but is not a feature of cellular isograft innervation. The regeneration of host axons seen in two of the six cellular allografts in spite of their rejection and the better (but not significantly different) target muscle recovery in acellular allografts than in cellular isografts were unexpected findings. One explanation for these results could be the release of other neuronotrophic factors by infiltrating mononuclear immune cells (primarily lymphocytes). In fact, a novel growth factor of this type, named "neuroleukin," has been purified which is also a lymphokine product of stimulated T cells. Thus, it would be interesting to compare neuroleukin levels in nerve allografts and isografts to determine the significance of this factor in nerve regeneration through grafts. Recent studies have shown a significant homology between neuroleukin and the enzyme glucose-6-phosphate isomerase. One proposed role of these compounds has been to serve as a binding substrate for the growing axon. Lindholm and colleagues have described the influence of interleukin, a macrophage product, on regulating the secretion of NGF and expression of NGF receptor on Schwann cells during nerve development and repair.

In summary, the present study compares the immunogenicity of cellular and acellular allografts and describes the efficacy of each in peripheral nerve gap repair. It is concluded that acellular allografts are less immunogenic and capable of supporting axonal regeneration. It is hoped that the immune response could be entirely circumvented by alternative methods for graft preparation, thereby enhancing the grafts' ability to support axonal regeneration.

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References


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