Experience with Nerve Allograft Transplantation

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ABSTRACT

Nerve allograft transplantation should be used for the repair of devastating peripheral nerve injuries that cannot be reconstructed through traditional means such as autologous nerve grafting or nerve transfer procedures. The risks of required systemic immunosuppression, although only temporary for nerve allograft recipients, preclude widespread use of this treatment modality. Translational research has led to several advancements in this field including the use of preoperative allograft cold preservation in University of Wisconsin organ preservation solution and inclusion of tacrolimus as part of the immunosuppressive regimen. Investigation of how to further diminish nerve allograft immunogenicity, speed neuroregeneration by use of agents such as tacrolimus, and promote preferential motor regeneration will further advance this field with the goal of restoring optimal function while minimizing patient morbidity.

KEYWORDS: Peripheral nerve, allograft, transplantation

Treatment of peripheral nerve injuries runs the gamut from simple observation and patient reassurance to nerve allograft transplantation. Other options that may be appropriate include nerve decompression, primary repair of simple transection injuries, repair with interposed autologous graft, and nerve transfers. Use of allograft nerve should be reserved for unique patients with otherwise irreparable extensive peripheral nerve injuries. This is because, although allotransplantation is appealing due to ability to repair significant segmental injuries and because it dispenses with nerve graft donor site problems, the potential risks of systemic immunosuppression must be balanced against treatment of a non-life-threatening injury.

The morbidity of systemic immunosuppression is well described.¹ For example, a specific medication used for immunosuppression in nerve allograft recipients, tacrolimus, can cause nephrotoxicity, neurotoxicity, and gastrointestinal disturbances among other problems.^{2,3}

Recently, however, there has been an acceptance of these risks for other non-life-sustaining tissue transplantation. There is increased demand to improve not just length but also quality of life. Transplantation of kidneys, pancreas, and small bowel are well accepted. Hand⁴ and face^{5,6} transplantation have been touted as the next frontier in the field of human transplant medicine.

One reason for increasing acceptance for this has been more recent advances in immunology especially as it relates to transplant medicine. There is some work to suggest that a state of immune hyporesponsiveness can develop and allow for limiting or even ceasing immunosuppressive therapy.⁷ For nerve allotransplantation, in particular, there is fairly clear evidence that systemic immunosuppression can be stopped once nerve regeneration through the allograft has occurred.⁸ This requirement for only temporary immunosuppression does make recommendation of nerve allograft reconstruction of otherwise unreconstructable injuries acceptable.

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In the following, we will discuss general nerve regeneration physiology, nerve allograft immunology, reported clinical cases of human nerve allograft transplantation, and what the future holds.

THE REGENERATION OF PERIPHERAL NERVES

Peripheral nerve cell bodies are in the spinal cord (motor and presynaptic sympathetic nerve fibers) or dorsal root ganglion immediately adjacent to the spinal cord (sensory nerve fibers). The peripheral nerves housing these nerve fibers stretch for millimeters to meters to carry input to and from the central nervous system. Each peripheral nerve is composed of thousands of these fibers, which can potentially regenerate, reach their target tissue, and restore function after being injured.⁹ In some species, nerves have been shown to regenerate for significant distances without an interposed conduit.¹⁰ By contrast, segmental human nerve injury requires some interposed material that supports regeneration. This material must provide a scaffold, support cells, growth factors, and an extracellular matrix.¹¹

Traditionally, the optimal choice for repair of segmental peripheral nerve injury has been to use interposed autologous nerve graft. In most cases, nonessential sensory nerves such as the sural or medial antebrachial cutaneous nerve are reversed and placed as cable grafts to bridge the defect.¹² The sural and medial antebrachial cutaneous nerves in an adult will provide 30 cm and 20 cm of graft material, respectively.¹³ Another viable option for the repair of short (< 3 cm) gaps in small sensory nerves is use of synthetic nerve conduits. Resorbable conduits of polyglycolic acid or polylactide-caprolactone have been shown to promote reasonable regeneration under these restricted indications in human studies.¹⁴ Considerable efforts are being made to further develop, through tissue engineering, constructs that allow successful regeneration of mixed nerve types over longer distances. Successively more detailed synthetic three-dimensional constructs are being created with additives such as cultured Schwann cells,^{15–18,65,66} stem cells¹⁹ or neurotrophic factors,^{20,21} but this work has yet to translate to successful clinical results.

Reported clinical use of nerve allografts to repair significant peripheral nerve injuries date to the 1800s.²² Until systemic immunosuppression was available, these were largely unsuccessful, and as one author commented “. . . I am not yet convinced that these homografts contribute. I certainly do not wish to advocate their further use at this stage” (p. 83).²³ Subsequent use of recipient immunosuppression combined with allograft freeze-drying and radiation led to reported moderate success in restoring sensation.²⁴ In 1992, successful use of a fresh peripheral nerve allograft to restore sensation in a patient who received systemic immunosuppression

was reported and corroborated by several independent medical examiners.²⁵ Since then, there have been reports of occasional use of nerve allograft for repair of otherwise irreparable injuries.^{26–29}

NERVE ALLOGRAFT IMMUNOLOGY

Significant research has been done that explores the immunology of nerve allograft transplantation. This body of work has been primarily aimed at exploiting the unique attributes of peripheral nerve allografts toward the end of allowing acceptable regeneration but minimizing immunosuppression for this allogeneic material.

Rejection of transplanted organs occurs through hyperacute, acute, and chronic rejection. Hyperacute rejection, due to the presence of donor-specific preformed antibodies, can be largely avoided by cross-matching prior to transplantation.³⁰ Acute rejection is mediated by recipient T cells that react to donor-derived peptides or directly to donor major histocompatibility complex.³¹ This T cell-activated inflammatory response is primarily mediated by the action of cytokines. Chronic rejection is mainly thought to be secondary to neointimal hyperplasia that may be mediated by alloantibodies.³²

Nerve transplantation between genetically different animals will produce both a humoral and cellular mediated immune response.^{33–35} Early research on nerve allograft transplantation in rodent models showed that minor or major histo-incompatibility led to allograft rejection.³⁶ Additional work revealed that Schwann cells are the main immunogenic material within the nerve allograft^{37,38} and that these likely display major histocompatibility complex type II on their cell surface.³⁹ Others found that both major histocompatibility complexes I and II are important and cellular elements such as vascular endothelial cells and Schwann cells within the graft combine to trigger the immune response.⁴⁰ Recipient cells that are responsible for mounting the antigraft response include helper T cells,⁴¹ cytotoxic T cells, and, to a lesser extent, macrophages.⁴²

SUCCESSFUL CLINICAL CASES OF NERVE ALLOGRAFT TRANSPLANTATION IN THE MODERN ERA

With the underlying premise that a nerve allograft would serve only as a “temporary scaffold,” thereby eliminating the need for lifelong systemic immunosuppression, successful clinical use has been reported. Over time, by combining knowledge gained from other fields of organ transplantation as well as from laboratory- and animal-based research, the implementation has been refined. In the following, we will discuss the major recent case reports and case series as reported in the

English literature. There is additional work reported in the non-English literature as well, which is not discussed.⁴³

In 1988, the senior author (S.E.M.) performed nerve allograft transplantation on an 8-year-old boy with an otherwise unreconstructable sciatic nerve defect. Ten cables of 23-cm length of nerve allograft were harvested from a matching blood type, negative cross-match donor and immediately placed across the recipient defect. The patient was treated with a regimen of oral cyclosporin A (goal serum level of 50 to 60 ng/mL) and 10 mg of daily prednisone beginning on the day of transplantation and continued for a total of 26 months at which time sensory nerve regeneration had been completed. Good protective sensation was recovered, and this allowed the patient to return to walking to and attending school. Of note, motor recovery was not anticipated as the patient was already 4 months postinjury at the time of transplantation, and the length of regeneration required did not allow for arrival at motor end-plates in a timely enough fashion.⁴⁴

In 1993, a second nerve allograft procedure was performed. Changes in the clinical regimen were based on significant research performed during the time interval since the previous procedure. This 12-year-old boy also had a significant lower-extremity injury for which the goal was restoration of protective sensation. In this case, the donor nerves were stored in University of Washington organ preservation solution for 1 week,

and the recipient was started on systemic cyclosporin A 4 days prior to transplantation. The 20-cm-long gap was reconstructed with eight cable grafts and the recipient maintained on the cyclosporin A as well as prednisone systemic immunosuppression for 19 more months. The patient was able to achieve return of sensation graded at 7/10 when compared with the contralateral 10/10 extremity and resumed normal age-appropriate activities including sports. The fundamental difference in this case report was the pretransplantation 1-week period of allograft preservation, which did not downgrade results, but did allow for preoperative testing, initiation of recipient immunosuppression, and performance of the exacting surgical procedure on a semielective basis.⁴⁵

In the 10-year period between 1988 and 1998, seven additional patients underwent nerve allograft transplantation. In these patients, additional changes in the immunosuppression included use of tacrolimus instead of cyclosporin A in the latter two patients, addition of azathioprine, and a limited (1- to 2-month) course of prednisone. Both upper- and lower-extremity injuries were treated, and sural nerve autografts were also employed if available. In this series of patients, one developed rejection due to sub-immunosuppressive levels of cyclosporin A; the other six patients gained sensation and three of them also had evidence of motor regeneration. In those three, due to use of a combination of autograft and allograft material, it is only possible to

Table 1 Nerve Allotransplantation Patient Protocol

Preoperative Preparation	Intraoperative Details	Postoperative Care
Assess patient suitability	Resect to healthy proximal and distal stumps	
Medical comorbidities		Induction immunotherapy (dose 2)
Malignancy/infection	Use patient autografts also	Basiliximab 20 mg intravenously
Psychosocial fitness		(postoperative day 4)
Laboratory tests	Insert extra allograft subcutaneously for	Standard immunotherapy
HIV/hepatitis tests	rejection monitoring	Continue tacrolimus
ABO type		(goal level 5–8 ng/mL)
Cross-match		Azathioprine 1–1.5 mg kg ⁻¹ day ⁻¹
Nerve allografts		Antibiotics
Harvest and cold preserve		Sulfamethoxazole-trimethoprim
(7 days, 4°C, UW solution)		3 times per week
Immunotherapy loading dose		For rejection Steroid therapy
Tacrolimus at 1–2 mg by mouth b.i.d.		
(start 4 days preoperatively)		
Induction immunotherapy (dose 1)		Stop immunotherapy
Basiliximab 20 mg intravenously		6 months after Tinel's sign crosses
(immediately preoperatively)		distal repair site

This table includes our current recommendations for patients who are to receive nerve allografts.

definitively attribute motor regeneration to that across the allograft cables in two of the patients. Nevertheless, this represents a significant achievement—successful motor regeneration across transplanted nerve allograft cables in otherwise irreparable-injury patterns. Another interesting finding, paralleling results seen in animal nerve allograft transplantation models, was improved regeneration in recipients who received tacrolimus. These patients also had immunosuppression withdrawn 6 months after evidence of nerve regeneration was seen distal to the interposed graft again pointing to this unique attribute of nerve transplantation—the need for temporary immunosuppression only.⁴⁶

Finally, since the last reported case series (above), two additional patients underwent nerve transplantation (S.E.M., unpublished observations). Both patients had living donor (maternal) nerve allograft transplantation in combination with autologous nerve grafting. One of the two patients required additional cadaveric nerve allograft. Both were treated with induction basiliximab then tacrolimus and azathioprine immunosuppression. See Table 1 for details regarding the current peri-operative treatment regimens for nerve transplant recipients. One patient has been lost to follow-up, and the second is in the regenerative period but does show evidence of early motor recovery. Because of the combined use of allograft and autograft, it is difficult to precisely delineate the graft responsible for functional recovery, but it is clear that there is no evidence of rejection, and regeneration is proceeding as predicted.

One independent case report describes somewhat less satisfying results obtained in an infant with brachial plexus palsy⁴⁷; however, this patient underwent a complex reconstruction using contralateral seventh cervical nerve root and contralateral ulnar nerve transfers to the affected ulnar, median, and radial nerves via allograft cables tunneled across the chest. This procedure was a secondary procedure performed at 9 months of age, and the findings of some positive motor and sensory function return is actually quite commendable in this challenging setting. A recent abstract presenting a case series of eight suggested use of living donor nerve transplantation but fails to elaborate on long-term result for both the transplant recipients as well as adverse effects in the live donors.²⁷

MEANS TO MINIMIZE IMMUNOSUPPRESSION IN NERVE ALLOGRAFT RECIPIENTS

Significant research, spurred in part by the intense interest in upper-extremity and face composite tissue as well as nerve allograft transplantation, has been done to determine means to diminish the requirement for systemic, potentially quite morbid, immunosuppression. There are three fronts upon which inves-

tigation has proceeded. This includes manipulation of the nerve allograft to render it less immunogenic, addition of pro-neuroregenerative treatment to speed regeneration and limit the total time of systemic regeneration, and recipient pretreatment to achieve graft tolerance. This last method is less appealing in the case of nerve transplantation where only temporary immunosuppression is required but remains quite attractive for solid organ or composite tissue transplantation.

Graft pretreatment has already been implemented in the previously reported clinical cases where nerve allografts were preserved for 7 days in University of Wisconsin organ preservation solution.^{48,49} Initially, preservation of nerve grafts was developed to provide means to create a nerve graft bank full of appropriate size and shape nerves that could be assessed for donor and recipient cross-match whenever the need arose.^{50,51} Work to establish the optimal period of preservation was focused on identifying the time at which Schwann cell viability would drop off thereby rendering the graft ineffective in promoting nerve regeneration.^{52,53} Along the way, it was discovered that prolonging the period of preservation seemed to reduce the immunogenicity of the allograft.^{54–57}

In fact, 7 weeks of cold preservation seems to decrease the presence of intercellular adhesion molecule I and major histocompatibility complex II expression on rodent nerve allograft—these molecules have been linked to human allograft rejection.⁵⁸ Also, in a rodent model, these 7-week-preserved allograft nerves when transplanted resulted in an absence of response by some immunologic tests but supported regeneration.⁵⁹ Other means to pretreat nerve grafts by lyophilization⁶⁰ and detergent denaturing^{61–63} met with less success.

In rodent, short gap models, the absence of Schwann cells in pretreated nerves did not seem to preclude regeneration^{64,65}; however, Schwann cells are necessary for successful nerve regeneration over longer nerve defects.^{66,67} Work to acellularize nerve allograft then repopulate with autologous Schwann cells has become popular as success with extraction and in vitro expansion of human Schwann cells was reported.⁶⁸ This aims to create a nonimmunogenic scaffold to which cells, neurotrophic factors, and other elements may be added that will allow subsequent regeneration. Additional work must be completed before clinical implementation.

Another important aspect to minimizing nerve allograft immunogenicity is to use multiple nonvascularized cables instead of one large-caliber vascularized graft. Although use of large-caliber vascularized nerve grafts has been advocated in a variety of situations,^{69–71} use of a vascularized *allograft* would require inclusion of some length of highly immunogenic allograft vascular pedicle that would require lifelong immunosuppression to maintain. On the other hand, animal research shows

that the volume of transplanted tissue does not alter the resultant immune response.⁷² These cable allografts will revascularize via longitudinal inosculation^{73,74} and, as in traditional autologous cable grafts, will support regeneration.⁷⁵

An observation made after nerve transplantation and immunosuppression with tacrolimus was that regeneration seemed to be more rapid than that normally observed.⁷⁶ This has led impetus to the goal of accelerating regeneration, which normally occurs at a rate of 1 mm/day. Studies have shown that tacrolimus makes in vitro neurite outgrowth more rapid^{77,78} and promotes nerve regeneration in a variety of peripheral nerve injury models.^{79–89} This action appears to be separate from the immunosuppressive mechanism, which acts through FK506 binding protein 12.⁹⁰ In cases of nerve allograft transplantation, it makes sense to employ tacrolimus as part of the treatment regimen as it may, in fact, speed recovery and limit total immunosuppressive therapy time.

Animal research has been performed to create a state of immune tolerance to nerve allograft. Recipient pretreatment with donor-specific erythrocytes or lymphocytes met with only temporary success.⁹¹ Treatment with monoclonal antibodies directed at various molecules important in mounting a rejection response,^{92–94} portal venous administration of irradiated alloantigen,^{95,96} or a combination of these therapies was more successful.⁹⁷ At present, however, these treatments seem equal to or more morbid than the temporary immunosuppression required for allograft transplantation.

FUTURE DIRECTIONS IN NERVE ALLOGRAFT TRANSPLANTATION

Work in animal models has shown that transected motor nerve fibers preferentially reinnervate via motor pathways.^{98–100} In fact, motor nerve-derived cables supported superior regeneration distal to the interposed graft when compared with sensory nerve-derived cables.^{101,102} Use of primarily motor or mixed nerve fiber allografts may improve motor regeneration, ultimately improving functional outcomes. This hypothesis should be investigated more closely to determine feasibility regarding length of clinically available small-caliber motor cables.

CONCLUSION

We believe that at present, nerve allotransplantation should be reserved only for cases in which other options have been exhausted and failure to intervene would otherwise result in a completely nonfunctional limb (i.e., essentially for the salvage of limb function). In addition, the patient must have a clear understanding of the risks of immunosuppression that are currently

required for successful transplantation and must not have contraindications.

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