Published in final edited form as:

J Neurosurg. 2011 January ; 114(1): 256–262. doi:10.3171/2010.5.JNS10111.

Effect of cold nerve allograft preservation on antigen presentation and rejection:

Laboratory investigation

Wilson Z. Ray, M.D.¹, Santosh S. Kale, M.D., M.B.A.², Rahul Kasukurthi, M.D.³, Esther M. Papp, B.S.³, Philip J. Johnson, Ph.D.², Katherine B. Santosa, B.A.³, Ying Yan, M.D., Ph.D.², Daniel A. Hunter, R.A.², Susan E. Mackinnon, M.D.², and Thomas H. Tung, M.D.²

¹ Department of Neurological Surgery, Washington University School of Medicine, St. Louis, Missouri

² Division of Plastic and Reconstructive Surgery, Washington University School of Medicine, St. Louis, Missouri

³ Washington University School of Medicine, St. Louis, Missouri

Abstract

Object—Nerve allotransplantation provides a temporary scaffold for host nerve regeneration and allows for the reconstruction of significant segmental nerve injuries. The need for systemic the current clinical utilization of nerve allografts, although this need is reduced by the practice of cold nerve allograft preservation. Activation of T cells in response to alloantigen presentation occurs in the context of donor antigen presenting cells (direct pathway) or host antigen-presenting cells (indirect pathway). The relative role of each pathway in eliciting an alloimmune response and its potential for rejection of the nerve allograft model has not previously been investigated. The objective of this investigation was to study the effect of progressive periods of cold nerve allograft preservation on antigen presentation and the alloimmune response.

Methods—The authors used wild type C57Bl/6 (B6), BALB/c, and major histocompatibility Class II-deficient (MHC^{−/−}) C57Bl/6 mice as both nerve allograft recipients and donors. A nonvascularized nerve allograft was used to reconstruct a 1-cm sciatic nerve gap. Progressive cold preservation of donor nerve allografts was used. Quantitative assessment was made after 3 weeks using nerve histomorphometry.

Results—The donor-recipient combination lacking a functional direct pathway (BALB/c host with MHC^{−/−} graft) rejected nerve allografts as vigorously as wild-type animals. Without an intact indirect pathway (MHC^{−/−} host with BALB/c graft), axonal regeneration was improved ($p < 0.052$). One week of cold allograft preservation did not improve regeneration to any significant degree in any of the donor-recipient preservation did improve regeneration significantly ($p < 0.05$) for all combinations compared with wild-type animals without pretreatment. However, only in the

Address correspondence to: Susan E. Mackinnon, M.D., Division of Plastic and Reconstructive Surgery, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, Missouri 63110. mackinnon@wudosis.wustl.edu.

Disclosure

This work was supported by NIH grant No. 2R01NS033406-13A1 and a CNS/AANS Spine and Peripheral Nerve Section Kline Award.

Author contributions to the study and manuscript preparation include the following. Conception and design: Ray, Mackinnon, Tung. Acquisition of data: Ray, Kasukurthi, Papp, Santosa, Yan. Analysis and interpretation of data: Ray, Kasukurthi, Papp, Johnson, Yan, Hunter. Drafting the article: Ray, Kale. Critically revising the article: Kale, Johnson, Tung. Reviewed final version of the manuscript and approved it for submission: Mackinnon, Tung. Statistical analysis: Hunter. Study supervision: Tung.

presence of an intact indirect pathway (no direct pathway) did 4 weeks of cold preservation improve regeneration significantly compared with 1 week and no preservation in the same donor-recipient combination.

Conclusions—The indirect pathway may be the predominant route of antigen presentation in the unmodified host response to the nerve allograft. Prolonged duration of cold nerve allograft preservation is required to significantly attenuate the rejection response. Cold preservation for 4 weeks improves nerve regeneration with significant effect on indirect allorecognition.

Keywords

allograft; peripheral nerve; immunosuppression; cold preservation; costimulatory blockade; major histocompatibility complex

The repair of significant or segmental nerve injuries is often limited by the supply of suitable autogenous donor nerves. Nerve allotransplantation associated donor site morbidity. The clinical use of nerve allografts continues to be limited by the need for a period of systemic immunosuppression.¹⁷ Several studies have demonstrated other methods (such as cold preservation, irradiation, and lyophilization) to reduce allograft alloantigenicity,^{2,6,8,12,21,22} yet none have proven effective at maintaining viable SCs while rendering the graft nonantigenic. The pretreatment of donor nerve allografts with cold preservation has been shown to decrease the expression of MHC Class II molecules² and decrease immunosuppression requirements,^{5,6,8,20} and has been used in the clinical setting to reduce nerve allograft rejection.^{4,16,17}

In the direct pathway of allorecognition, intact MHC molecules on the surface of donor APCs of the fresh peripheral nerve allograft are recognized directly by the host immune cells. Schwann cells are known to act as facultative APCs and represent the main target in nerve allograft rejection (Fig. 1).^{9,10,13,15,18,24,26} Host APCs also present processed donor allopeptides to activate T cells, constituting the indirect pathway of allorecognition (Fig. 1). Although the importance of direct recognition in acute allograft rejection is well established, a significant role for indirect recognition is now being recognized.^{3,7,19} The relative contribution of each of these pathways in the preservation is not well defined. Characterization of the immunological mechanisms involved in nerve allograft rejection will provide a basis for designing strategies to manipulate the host immune system or modify nerve allografts to broaden the indications for nerve allotransplantation. To this end, we have used MHC Class II molecule knockout (MHC^{-/-}) mice as donors and recipients of nerve allografts in a peripheral nerve injury model to study the effects of cold allograft preservation. We used MHC^{-/-} allografts placed in wild-type recipients to isolate the indirect immune pathway, and wild-type allografts placed into MHC^{-/-} mice to isolate the direct immune pathway.²⁵ Using this framework, our study examined the relative contribution of each of these pathways in nerve allograft cold preservation and rejection.

Methods

Animal Preparation and Care

The study used male MHC^{-/-} knockout mice with a C57Bl/6 background and wild type C57Bl/6 (B6) and BALB/c mice (Jackson Laboratory). The animals were housed in a central animal care facility with access to water and standard rodent feed ad libitum. All housing, care, and surgical procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and the specific protocol met with the approval of the Washington University Animal Studies Committee.

Nerve Reagents

All cold-preserved nerve segments were harvested, rinsed in 0.9% saline, and immediately placed in sterile airtight containers containing freshly made University of Wisconsin cold storage solution, supplemented with 100 U/ml penicillin, 40 U/L insulin, and 16 mg/L dexamethasone.⁵ Nerves were then stored at 4°C, and preservation solution was changed every 7 days.

Surgical Procedure

All surgeries were performed on anesthetized 8-week-old mice, with the right hindquarter shaved and depilated (Nair lotion). A skin incision was made parallel to the femur and the biceps femoris split. Under a range of magnification (16–40), the sciatic nerve was exposed with microinstruments to include the sciatic notch proximally and its trifurcation to tibial, peroneal, and sural nerves distally. The sciatic nerve was transected 5-mm proximal to its trifurcation, and a peripheral nerve allograft was reversed in orientation and interposed between the transected ends and secured with 11-0 microsutures under a magnification of 40. Muscle was closed using 8-0 Vicryl sutures and skin with 6-0 nylon suture (Ethicon), and mice were recovered with injections of atipamezole hydrochloride (Novartis) on a warming pad. Nerve grafts were obtained from isogenic strains (isografts) or dysgenic strains (allografts) that were harvested using the same anesthetic and surgical approach as above. Nerves destined for cold preservation were harvested bilaterally to minimize animal use, and oriented with an 11-0 microsuture proximally, after which donor animals were immediately killed. The harvested, nonvascularized, 1-cm sciatic nerve allograft was transplanted in reverse orientation into the recipient. As detailed in Table 1, MHC^{−/−} deficient mice were used as both donor and recipient with a fully allogenic wild-type BALB/c recipient or donor. Wild type controls with C57Bl/6 (B6) and BALB/c mice were used as donors and recipients, respectively, in isograft and allograft control groups (Groups 1, 2, 5, and 8). Recipient animals in Groups 5–7 received nerves stored for 1 week in University of Wisconsin cold storage solution. Groups 8–10 were recipients of donor nerve allografts stored for 4 weeks in University of Wisconsin cold storage solution.

Histomorphometric Analysis

Sciatic nerve segments were harvested en bloc after 3 weeks postoperatively. Labeled nerve specimens from distinct regions along the graft itself and proximal and distal to the graft were preserved in glutaraldehyde, post-fixed in osmium tetroxide, and embedded in Araldite 502 adhesive (Huntsman Advanced Materials), and 1- μ m cross-sections were obtained with an LKB III ultramicrotome. Nerves were qualitatively assessed for the preservation of nerve architecture, quality and quantity of regenerated nerve fibers, extent of myelination, and the presence of ongoing Wallerian degeneration. The system utilizes a series of custom algorithms and 8-biplane digital pseudo-coloring to distinguish axons, myelin, nerve sheath, and debris from each other, and results are double-checked by our histomorphometrist.¹¹ Processed cross-sections were digitized and assessed for total fascicular area and total fiber number.

Statistical Analysis

The mean $\pm$ SD was used to present all data in this study. A 2-tailed ANOVA was performed to determine the differences between individual groups. Histomorphometric calculations were performed using Statistica statistical software (StatSoft, Inc.). If significant, a Student-Newman-Keuls test was performed to compare groups. Statistical significance was established at $p < 0.05$.

Results

Unmodified Nerve Allograft Rejection and the Indirect Pathway of Antigen Presentation

The redundancy in the immune system facilitates host rejection in other allotransplantation models in the absence of a normal direct or indirect pathway.²⁵ All histomorphometric data are summarized in Table 1 with superior regeneration noted in the untreated isograft group and minimal regeneration in the allograft control group (Fig. 2). We found that untreated wild-type BALB/c mice (Group 3) that received fully allogenic MHC^{-/-} donor nerve grafts, and therefore had an intact indirect pathway but no functional direct pathway, demonstrated poor histomorphometric evidence of distal graft axonal regeneration, similar to that observed in the allograft controls (Group 2; total number of distal fibers 37 ± 87 and 135 ± 300 , respectively; Fig. 3). However, untreated MHC^{-/-} mice that received fully allogenic BALB/c nerve allografts (Group 4), and therefore had an intact direct pathway but no functional indirect pathway, demonstrated increased axonal regeneration (total number of distal fibers 699 ± 440 ; Fig. 2), which was very close to a statistically significant difference compared with allograft controls ($p < 0.052$) but significantly less ($p < 0.009$) than isograft controls (total number of fibers 1570 ± 440). Thus, although a significant rejection response still occurred, eliminating the indirect pathway appeared to diminish the host response and improve axonal regeneration, while eliminating the direct pathway showed no difference in comparison with wild-type controls. These findings suggest that the indirect pathway of antigen presentation may be predominant in the unmodified host response to the nerve allograft.

Nerve Regeneration After Cold Allograft Preservation

Cold preservation is a well-established method to reduce nerve allograft antigenicity and has been shown to affect the direct pathway via diminished expression of MHC Class II molecules by donor SCs. With 1 week of cold nerve allograft preservation, no significant differences in total number of myelinated fibers were noted between any of the allograft control groups or those utilizing MHC Class II deficient donors or recipients (Fig. 4). Histomorphometric parameters using wild-type mice were no better after 1 week of cold preservation than fresh allografts (272 ± 280 total fibers [Group 5] vs 135 ± 300 total fibers [Group 2]). Wild-type mice with 1-week cold-preserved MHC^{-/-} grafts (Group 6, no direct pathway) and MHC^{-/-} hosts with 1-week cold-preserved wild-type grafts (Group 7, no indirect pathway) were not significantly different from each other or the controls (total number of fibers 274 ± 380 [Group 6] vs 679 ± 518 [Group 7] vs 272 ± 280 [Group 5]). However, it is interesting to note that there is a similar pattern of findings, as the groups that received fresh allografts showed better regeneration when the indirect pathway was not functional but no difference when the indirect pathway was intact. Therefore, 1 week of cold allograft preservation was not enough to provide any improvement in regeneration without additional treatment. The findings were similar to those groups receiving fresh allografts with a trend toward better regeneration when the indirect pathway was eliminated, suggesting a persistent predominance over the direct pathway in this model.

Increasing cold preservation to 4 weeks substantially improved neural regeneration (Fig. 5). The allograft control group using mismatched wild-type donors and hosts had significantly better axonal regeneration with cold allograft preservation for 4 weeks than fresh allografts (total number of fibers 870 ± 718 [Group 8] vs 135 ± 300 [Group 2]; $p < 0.05$). Wild-type BALB/c mice (Group 9) that received MHC^{-/-} donor nerve grafts and therefore did not have an intact direct pathway demonstrated significantly increased distal fiber counts (1175 ± 654 ; Fig. 5). Improvements in neural regeneration were also observed in the absence of the indirect pathway; MHC^{-/-} mice (Group 10) that received BALB/c nerve allografts had total distal fiber counts of 1401 ± 529 , which was not statistically different ($p < 0.19$) than

allograft controls (Group 8) or BALB/c hosts with MHC^{-/-} grafts (Group 9) that also received 4 weeks of cold preservation (870 ± 718). However all groups (8–10) that received 4 weeks of cold preservation showed statistically better results than untreated allograft controls (Group 2).

Cold Nerve Allograft Preservation and the Indirect Pathway of Antigen Presentation

Evaluation of the groups using Class II deficient mice provided further information on the effect of cold preservation on the method of antigen presentation. One week of cold allograft preservation provided no significant effect on nerve regeneration in wild-type hosts receiving Class II deficient donor nerve allografts (Group 6, no direct pathway; total fibers 274 ± 380) compared with no cold preservation (Group 3; 37 ± 87 total fibers). However, 4 weeks of cold preservation significantly increased regeneration in the same donor-recipient combination (Group 9, 1175 ± 654 total fibers) compared with 1 week of cold preservation and no cold preservation (Fig. 6). These findings suggest that longer periods of cold allograft preservation significantly diminished the indirect pathway because this remained the only functional pathway for antigen presentation in this donor-recipient combination. When the direct pathway was the only functional method of antigen presentation (Fig. 7), 4 weeks of cold preservation (Group 10, 1401 ± 529 total fibers) was not significantly better than 1 week of cold preservation (Group 7, 679 ± 518 total fibers) or no cold preservation (Group 4, 699 ± 440 total fibers), although it provided for significantly better regeneration than the wild-type allograft control group combination (Group 2, 135 ± 300 total fibers). Essentially, when the direct pathway was the only functional manner of antigen presentation, 1 week of cold allograft preservation showed no difference compared with no cold preservation; 4 weeks of cold preservation showed better regeneration but it did not quite reach statistical significance. Thus, there appeared to be some but limited effect by cold preservation on direct antigen presentation. When only the indirect pathway was intact, 1 week of cold preservation improved regeneration modestly but not significantly, but 4 weeks of cold preservation provided a very significant improvement by histomorphometric parameters. As such, longer cold allograft preservation (4 weeks) has a greater impact on the indirect pathway of antigen presentation.

Discussion

Cold preservation of nerve allografts has evolved into a clinically applicable practice, decreasing nerve antigenicity and reducing required doses of systemic immunosuppression with only 7 days of allograft pretreatment. We know that after only 2 weeks of cold preservation, SCs decrease their expression of MHC Class II molecules,² and it has been demonstrated in numerous studies that SCs behave as facultative APCs, representing the primary target of host allograft rejection.^{9,10,13,15,18,24,26} Although prolonged cold preservation has proven useful in a short gap model, the viable SCs that are required for regeneration across longer distances are ultimately lost with progressive cold preservation.^{5,6,14,23} Despite the inherent limitations of cold preservation, it remains a useful adjunct in nerve allotransplantation and when used with immunosuppression allows for the use of cadaveric nerves as a source of graft material.

In this study we investigated the effect of cold nerve allograft preservation on the unmodified rejection response and antigen presentation. The importance of direct recognition in acute allograft rejection is well established, but a significant role for indirect recognition is now understood.^{3,26} However, the relative contribution of each pathway and the role of T-cell subsets in mediating rejection remains unclear. These factors may vary with the cellular makeup of the allograft and may contribute to differential tissue resistance to immunomodulation. The parenchymal component of a peripheral nerve allograft is much smaller than an organ allograft and the immune response is directed at cellular components

including SCs, endothelial cells, and perivascular macrophages. The use of transgenic mice lacking Class II MHC molecules allowed us to eliminate either the direct or indirect pathways of antigen recognition to evaluate their relative roles with and without cold allograft preservation.

In the unmodified rejection response groups without cold preservation, the strength of the rejection response when the direct pathway was not functional was just as strong as when both pathways were intact. However, when the indirect pathway was eliminated, total axonal regeneration was much improved and very close to reaching statistical significance. The indirect pathway would therefore appear to play a greater role in the unmodified rejection response to the nerve allograft than expected. Perhaps the nonvascularized nature and a relative lack of APCs on the nerve allograft could account for a weaker direct component. Professional APCs include dendritic cells, macrophages and other mononuclear phagocytes, and B-lymphocytes, whereas nonprofessional APCs include epithelial and mesenchymal cells. The physiological significance of nonprofessional APCs in antigen presentation remains unclear, and it is unlikely that they play a major role in the initiation of most T-cell responses.¹ The nerve allograft is much more homogeneous in tissue type and quantitatively smaller than a vascularized organ allograft, which also contains sizable blood vessels for vascular anastomoses. In humans, microvascular endothelial cells may also present antigens and may be particularly important during allograft rejection. Nonvascularized nerve grafts do contain microvessels in the epi- and perineurium, but they are extremely small and not directly exposed to host blood initially. As such, they are more like a nonvascularized skin allograft, except that donor skin also contains Langerhans cells, which like dendritic cells are professional APCs. Because SCs are only facultative APCs, the relatively smaller quantity of donor professional APCs may provide an explanation for a strong indirect component to the host response in the nerve allograft model.

As we have previously shown, longer periods of cold nerve allograft preservation resulted in significant attenuation of the host immune response and improvement in nerve regeneration.⁶ Based on our current knowledge of the effects of cold preservation, we expected to see a greater degree of nerve regeneration when the indirect pathway was not functional, leaving only an intact direct pathway that would be diminished by the decrease in donor Class II MHC expression during cold preservation. If direct antigen recognition were eliminated leaving only a functional indirect pathway, progressive periods of cold preservation would be expected to have little effect and regeneration should remain poor. However our findings demonstrated the opposite trend. When the indirect pathway was eliminated and therefore only direct recognition was intact, treatment with 1 week of cold allograft preservation in these recipients showed no improvement in regeneration compared with no allograft pretreatment. Although 4 weeks of cold preservation did show some improvement, it did not quite reach statistical significance. On the other hand, when the direct pathway was not functional and therefore only indirect recognition was intact, 4 weeks of cold preservation improved regeneration significantly, even though the improvement after 1 week of cold preservation was modest and not statistically significant. We have previously shown that 1 week of cold allograft preservation decreases donor Class II MHC expression,² and that SC viability is maintained for up to 3 weeks of cold preservation.^{4,5} However, up to 7 weeks of preservation is required before all donor allograft antigenicity is diminished,⁶ indicating the degeneration and loss of donor peptides capable of eliciting a host immune response. It would follow, then, that after 4 weeks of cold preservation in this model, most of the donor cells are no longer viable and there has also been significant loss of antigenic donor peptides. Therefore, indirect recognition by host APCs of donor antigens is decreased by a diminishing pool of allograft peptides. Direct recognition by donor APCs is also decreased by the loss of donor cell viability, but likely to a lesser degree because of the smaller

contribution by the direct pathway in the unmodified nerve allograft rejection response as discussed above.

Conclusions

This study demonstrates unexpected findings in the alloimmune response to a nerve allograft that may be related to unique characteristics, including its nonvascularized nature, tissue homogeneity, and lack of a significant amount of supporting tissue such as vascular and perivascular structures that provide a source of professional APCs. Indirect allorecognition may play a more significant role in the rejection of the nerve allograft than in the response to a vascularized organ or heterogeneous tissue transplant. Cold allograft preservation undoubtedly affects both the direct and indirect pathways of antigen presentation, but its effect on the indirect pathway in the nerve allograft model may be more significant. A better understanding of how these pathways are differentially affected by donor pretreatment and host treatment may allow a more complementary combination of treatment modalities to improve functional outcomes.

Abbreviations used in this paper

APC	antigen-presenting cells
MHC	major histocompatibility complex
SC	Schwann cell

References

1. Abbas, AK.; Lichtman, AH.; Pober, JS. Cellular and Molecular Immunology. Philadelphia: WB Saunders; 1997.
2. Atchabahian A, Mackinnon SE, Hunter DA. Cold preservation of nerve grafts decreases expression of ICAM-1 and class II MHC antigens. *J Reconstr Microsurg* 1999;15:307–311. [PubMed: 10363556]
3. Auchincloss H Jr, Sultan H. Antigen processing and presentation in transplantation. *Curr Opin Immunol* 1996;8:681–687. [PubMed: 8902394]
4. Calvert GT, Doolabh VB, Grand AG, Hunter DA, Mackinnon SE. Rat-strain differences in recovery following peripheral-nerve allotransplantation. *J Reconstr Microsurg* 2001;17:185–191. [PubMed: 11336150]
5. Evans PJ, Mackinnon SE, Levi AD, Wade JA, Hunter DA, Nakao Y, et al. Cold preserved nerve allografts: changes in basement membrane, viability, immunogenicity, and regeneration. *Muscle Nerve* 1998;21:1507–1522. [PubMed: 9771677]
6. Evans PJ, MacKinnon SE, Midha R, Wade JA, Hunter DA, Nakao Y, et al. Regeneration across cold preserved peripheral nerve allografts. *Microsurgery* 1999;19:115–127. [PubMed: 10231120]
7. Gould DS, Auchincloss H Jr. Direct and indirect recognition: the role of MHC antigens in graft rejection. *Immunol Today* 1999;20:77–82. [PubMed: 10098326]
8. Grand AG, Myckatyn TM, Mackinnon SE, Hunter DA. Axonal regeneration after cold preservation of nerve allografts and immunosuppression with tacrolimus in mice. *J Neurosurg* 2002;96:924–932. [PubMed: 12005401]
9. Gulati AK. Immune response and neurotrophic factor interactions in peripheral nerve transplants. *Acta Haematol* 1998;99:171–174. [PubMed: 9587399]
10. Gulati AK, Cole GP. Nerve graft immunogenicity as a factor determining axonal regeneration in the rat. *J Neurosurg* 1990;72:114–122. [PubMed: 2294170]
11. Hunter DA, Moradzadeh A, Whitlock EL, Brenner MJ, Myckatyn TM, Wei CH, et al. Binary imaging analysis for comprehensive quantitative histomorphometry of peripheral nerve. *J Neurosci Methods* 2007;166:116–124. [PubMed: 17675163]

12. Kamei T, Callery MP, Flye MW. Pretransplant portal venous administration of donor antigen and portal venous allograft drainage synergistically prolong rat cardiac allograft survival. *Surgery* 1990;108:415–422. [PubMed: 2382235]
13. Lassner F, Schaller E, Steinhoff G, Wonigeit K, Walter GF, Berger A. Cellular mechanisms of rejection and regeneration in peripheral nerve allografts. *Transplantation* 1989;48:386–392. [PubMed: 2781604]
14. Levi AD, Evans PJ, Mackinnon SE, Bunge RP. Cold storage of peripheral nerves: an in vitro assay of cell viability and function. *Glia* 1994;10:121–131. [PubMed: 7513298]
15. Mackinnon S, Hudson A, Falk R, Bilbao J, Kline D, Hunter D. Nerve allograft response: a quantitative immunological study. *Neurosurgery* 1982;10:61–69. [PubMed: 7035993]
16. Mackinnon SE. Nerve allotransplantation following severe tibial nerve injury. Case report. *J Neurosurg* 1996;84:671–676. [PubMed: 8613862]
17. Mackinnon SE, Hudson AR. Clinical application of peripheral nerve transplantation. *Plast Reconstr Surg* 1992;90:695–699. [PubMed: 1410009]
18. Pollard JD, Gye RS, McLeod JG. An assessment of immunosuppressive agents in experimental peripheral nerve transplantation. *Surg Gynecol Obstet* 1971;132:839–845. [PubMed: 4995335]
19. Shoskes DA, Wood KJ. Indirect presentation of MHC antigens in transplantation. *Immunol Today* 1994;15:32–38. [PubMed: 8136009]
20. Siemionow M, Bozkurt M, Kulahci Y. Current status of composite tissue allotransplantation. *Handchir Mikročir Plast Chir* 2007;39:145–155. [PubMed: 17602375]
21. Simon JC, Tigelaar RE, Bergstresser PR, Edelbaum D, Cruz PD Jr. Ultraviolet B radiation converts Langerhans cells from immunogenic to tolerogenic antigen-presenting cells. Induction of specific clonal anergy in CD4+ T helper 1 cells. *J Immunol* 1991;146:485–491. [PubMed: 1670944]
22. Singh R, Lange SA. Experience with homologous lyophilised nerve grafts in the treatment of peripheral nerve injuries. *Acta Neurochir (Wien)* 1975;32:125–130. [PubMed: 1163312]
23. Song JW, Yang LJ, Russell SM. Peripheral nerve: what's new in basic science laboratories. *Neurosurg Clin N Am* 2009;20:121–131. viii. [PubMed: 19064185]
24. Trumble TE, Shon FG. The physiology of nerve transplantation. *Hand Clin* 2000;16:105–122. [PubMed: 10696580]
25. Yamada A, Chandraker A, Laufer TM, Gerth AJ, Sayegh MH, Auchincloss H Jr. Recipient MHC class II expression is required to achieve long-term survival of murine cardiac allografts after costimulatory blockade. *J Immunol* 2001;167:5522–5526. [PubMed: 11698419]
26. Yu LT, Rostami A, Silvers WK, Larossa D, Hickey WF. Expression of major histocompatibility complex antigens on inflammatory peripheral nerve lesions. *J Neuroimmunol* 1990;30:121–128. [PubMed: 2172305]

Fig. 1. Direct and indirect pathways of alloantigen presentation by donor and host (recipient) antigen-presenting cells, respectively, for T-cell activation.

Fig. 2.

Photomicrographs of histological specimens of nerve graft or distal to nerve graft showing excellent regeneration in the untreated isograft group (**A**), poor regeneration in the allograft group (**B**), and improved regeneration with 4 weeks of cold allograft preservation (**C**). Toluidine blue. Original magnification $\times 400$.

Fig. 3.

Graph showing the total number of myelinated fibers in the isograft and allograft control groups and in knockout mice without cold preservation (CP). Superior regeneration was observed in the isografts and improved regeneration in the absence of the indirect pathway (MHC-/- with BALB/c allograft).

Fig. 4.

Graph showing the total number of myelinated fibers after 1 week of cold allograft preservation with no significant difference between groups or improvement compared with no cold preservation.

Fig. 5.

Graph showing the total number of myelinated fibers after 4 weeks of cold allograft preservation with all groups demonstrating significantly improved regeneration.

Fig. 6. Graph showing that when only the indirect pathway was intact, the total number of myelinated fibers was significantly improved after 4 weeks compared with 1 week or no cold preservation.

Fig. 7. Graph showing that when only the direct pathway was intact, improvement in nerve regeneration after 4 weeks was not statistically different compared with regeneration after 1 week or no cold preservation.

TABLE 1

Morphometric analysis of all experimental groups

Group No.	Treatment	No. of Animals	Total No. Fibers	Fiber Width (μm)	Fiber Density ($\mu\text{m}/\text{mm}^2$)	% Regenerating Nerve Fiber of Total Volume	% Debris of Total Volume
1	isograft control	7	1570 $\pm$ 440	3.07 $\pm$ 0.28	12294.00 $\pm$ 4904.19	12.74 $\pm$ 8.16	11.75 $\pm$ 8.05
2	allograft control	6	135 $\pm$ 300*	1.15 $\pm$ 1.27	652.03 $\pm$ 1370.28	0.42 $\pm$ 0.92	21.67 $\pm$ 8.73
3	BALB/c w/MHC-/- allograft	6	37 $\pm$ 87 [†]	0.87 $\pm$ 1.35	292.58 $\pm$ 681.27	0.19 $\pm$ 0.45	36.07 $\pm$ 6.48
4	MHC-/- w/BALB/c allograft	5	699 $\pm$ 440 [‡]	3.05 $\pm$ 0.31	3600.34 $\pm$ 1891.22	3.40 $\pm$ 1.46	14.65 $\pm$ 6.89
5	allograft control & 1 wk CP	4	272 $\pm$ 280	2.64 $\pm$ 0.31	1217.16 $\pm$ 1166.41	0.88 $\pm$ 0.74	23.58 $\pm$ 3.99
6	BALB/c w/MHC-/- allograft & 1 wk CP	5	274 $\pm$ 380 [‡]	1.65 $\pm$ 1.54	1964.68 $\pm$ 2986.78	2.00 $\pm$ 3.52	25.06 $\pm$ 9.72
7	MHC-/- w/BALB/c allograft & 1 wk CP	8	679 $\pm$ 518	2.76 $\pm$ 0.28	4564.47 $\pm$ 3465.64	4.00 $\pm$ 3.05	16.73 $\pm$ 7.03
8	allograft control & 4 wks CP	6	870 $\pm$ 718*	2.71 $\pm$ 0.26	4951.58 $\pm$ 4347.29	3.98 $\pm$ 3.49	14.02 $\pm$ 10.30
9	BALB/c w/MHC-/- allograft & 4 wks CP	6	1175 $\pm$ 654 ^{‡*}	2.82 $\pm$ 0.14	7958.52 $\pm$ 4031.51	6.74 $\pm$ 3.31	20.18 $\pm$ 7.59
10	MHC-/- w/BALB/c allograft & 4 wks CP	7	1401 $\pm$ 529 ^{‡*}	2.92 $\pm$ 0.22	11611.30 $\pm$ 4903.36	11.01 $\pm$ 5.72	12.45 $\pm$ 8.90

* All groups with 4 weeks cold preservation (8–10) significantly different from allograft controls (Group 2; $p < 0.05$, Student-Newman-Keuls test). CP = cold preservation.

[†] Group 9 significantly different from Groups 3 and 6 ($p < 0.05$, Student-Newman-Keuls).

[‡] Group 10 significantly different from Group 4 ($p < 0.05$, Student-Newman-Keuls test).