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Minireview

Laryngeal Transplantation in 2005: A Review

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There is no good surgical, medical or prosthetic solution to the problems faced by those with a larynx whose function is irreversibly damaged by tumor or trauma. Over the past 10 years, the pace of research designed to establish laryngeal transplantation as a therapeutic option for these persons has increased steadily. The biggest milestone in this field was the world's first true laryngeal transplant performed in Cleveland, Ohio in 1998. The recipient's graft continues to function well, in many respects, even after 7 years. However, it has also highlighted the remaining barriers to full-scale clinical trials. Stimulated by these observations, several groups have accumulated data which point to answers to some of the outstanding questions surrounding functional reinnervation and immunomodulation. This review seeks to outline the progress achieved in this field by 2005 and to point the way forward for laryngeal transplantation research in the 21st century.

Key words: Cancer, larynx, reinnervation, speech, transplantation

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Introduction

It has been apparent for many years now that there is no practical way of substituting for all the functions of the larynx using tissue transfer or prosthetic means. In short, nothing is likely to replace a larynx as well as another larynx. Progressive advances in tissue engineering, materials science and nanotechnology have done nothing to change this view at the time of writing. It is for this reason that the possibility of laryngeal transplantation remains an enticing Nirvana for head and neck surgeon-scientists as they search for a solution to the considerable quality-of-life diminution faced by persons with irreversible disease of the larynx.

While conventional methods of voice and swallowing replacement after total laryngectomy remain disappointingly sub-optimal, the striking vocal, deglutition and quality of life achievements of the one human laryngeal transplant recipient (1) show what may be just round the corner given some well-targeted research.

This paper is the result of the world's first symposium on head and neck transplantation (Chaired by Professor Marshall Strome, USA). Its aims are to describe the progress made in this field as well as the distance yet to be covered in the attempt to make laryngeal transplantation a viable clinical alternative for those with irreversible laryngeal disease, including, ultimately, cancer.

The Cleveland Patient

In 1998, the Cleveland group performed the first true human laryngeal transplantation in a 40-year-old man who had suffered severe trauma to the throat 20 years earlier (1). A fully matched laryngo-pharyngeal complex, including thyroid, parathyroid and five rings of trachea, was transplanted and both superior and one recurrent laryngeal nerves were anastomosed. Prior to the operation, cyclosprin, azathioprine and methylprednisolone were administered and thereafter, immunosuppression was maintained with cyclosporine, mycophenalate mofetil, methylprednisolone and anti-CD3 antibody (week 1 only). After a brief episode of presumed rejection at 15 months, cyclosporine was replaced by tacrolimus. Three episodes of tracheo-bronchitis, one involving pneumocysits carinii, were documented in the first 15 months. A brief

episode of laryngeal edema occurred thereafter, coinciding with a reduction in tacrolimus trough levels. No further rejection or side effects have been encountered and doses of medication have been progressively reduced. At more than 7 years, the patient swallows normally and has a high quality of life. Objective voice measures 16 months post-transplant were, and remain, within normal limits for intensity, maximum phonation time and airflow, and he has a vocal range of one octave, giving a highly serviceable voice (9).

However, this isolated success has not yet been repeated. Reasons for this include the need for a large, trained multidisciplinary team and an institution prepared to support the considerable costs, concerns over optimizing nerve function and immunosuppression and the need to identify 'ideal' candidates for the first trials. These issues are discussed below.

Indications

This operation was performed for purely quality of life purposes. This was the ideal initial recipient, being a relatively fit, young trauma victim. There are only a few hundred such candidates in most countries, but these would, ideally, form the first targets of clinical trials. Other suitable, though rare, early targets would be those with large benign or low grade malignant tumors of the larynx, or those developing laryngeal malignancy who are already on a post-transplant immunosuppression regimen. Persons who have already undergone laryngectomy for cancer (the International Association of Laryngectomees mails 250 000 persons per annum) might be candidates if their superior laryngeal nerves could be located, adequate organ suspension can be achieved, and there is no sign of recurrent cancer for 5 years or more. However, ultimately, the biggest pool of patients who stand to benefit are those presenting with locally advanced laryngeal cancer (7000 patients *per annum* in the United States). When a non-revascularized partial laryngeal transplant was performed in 1969, and again when a tongue transplant (analogous in this setting) was performed in 2003, both in patients with advanced squamous cancer, both patients rapidly succumbed to recurrent disease. Hence, it is essential for this latter group that more specific immunomodulation be developed before transplantation can be considered ethical.

Andrews and colleagues have recently introduced the concept of the partial laryngeal transplant. Although this would be faced by many of the same problems as a total transplant, the preservation of one functioning arytenoid-muscular unit would extend the potential recipient pool to a larger number of cancer patients, as well as theoretically provide a functionally improved result based on an immediately mobile hemilarynx (34).

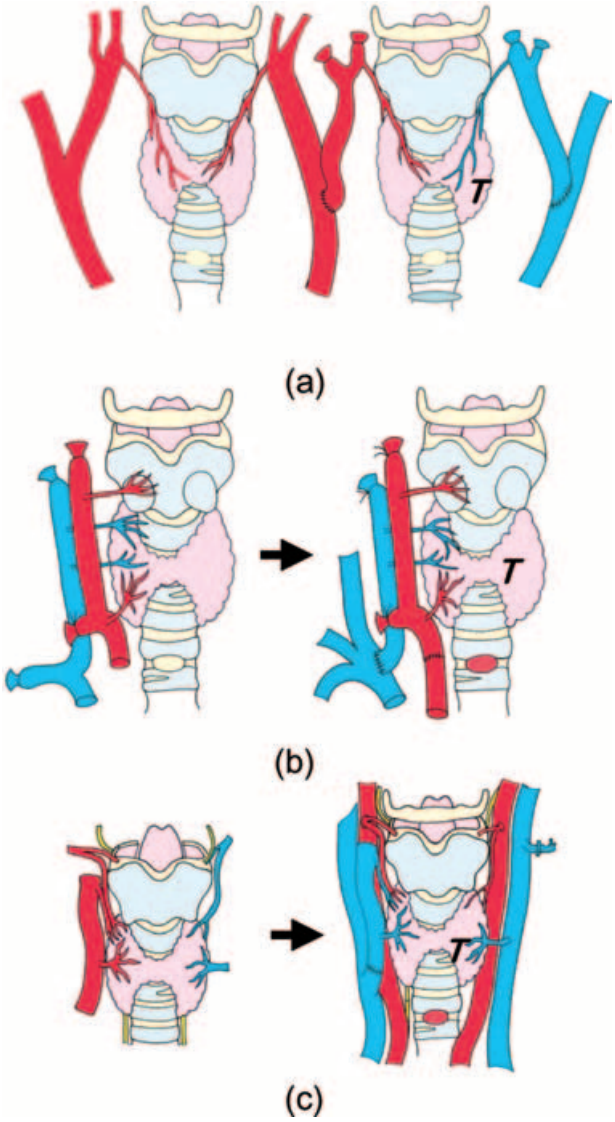

Figure 1: Comparison of replantation techniques in animal models and man. (A) Small animal model (rat: after Lorenz, 2002). (B) Large animal model (pig: after Birchall, 2002). (C) Human (after Strome, 2001).

Replantation

Figure 1 compares methods of replantation of the larynx in small (heterotopic, rat) and large (orthotopic, pig) animal models, together with the method used by the Cleveland team in their human recipient.

Early experiments into laryngeal transplantation used the dog. Berke's group applied modern microvascular techniques to extend this orthotopic model in the mid-1990s (2). Haughey's subsequent successful development of a canine tongue transplant model, the only substantive study of this closely related technique, supports the continuing utility of dog models in head and neck transplantation

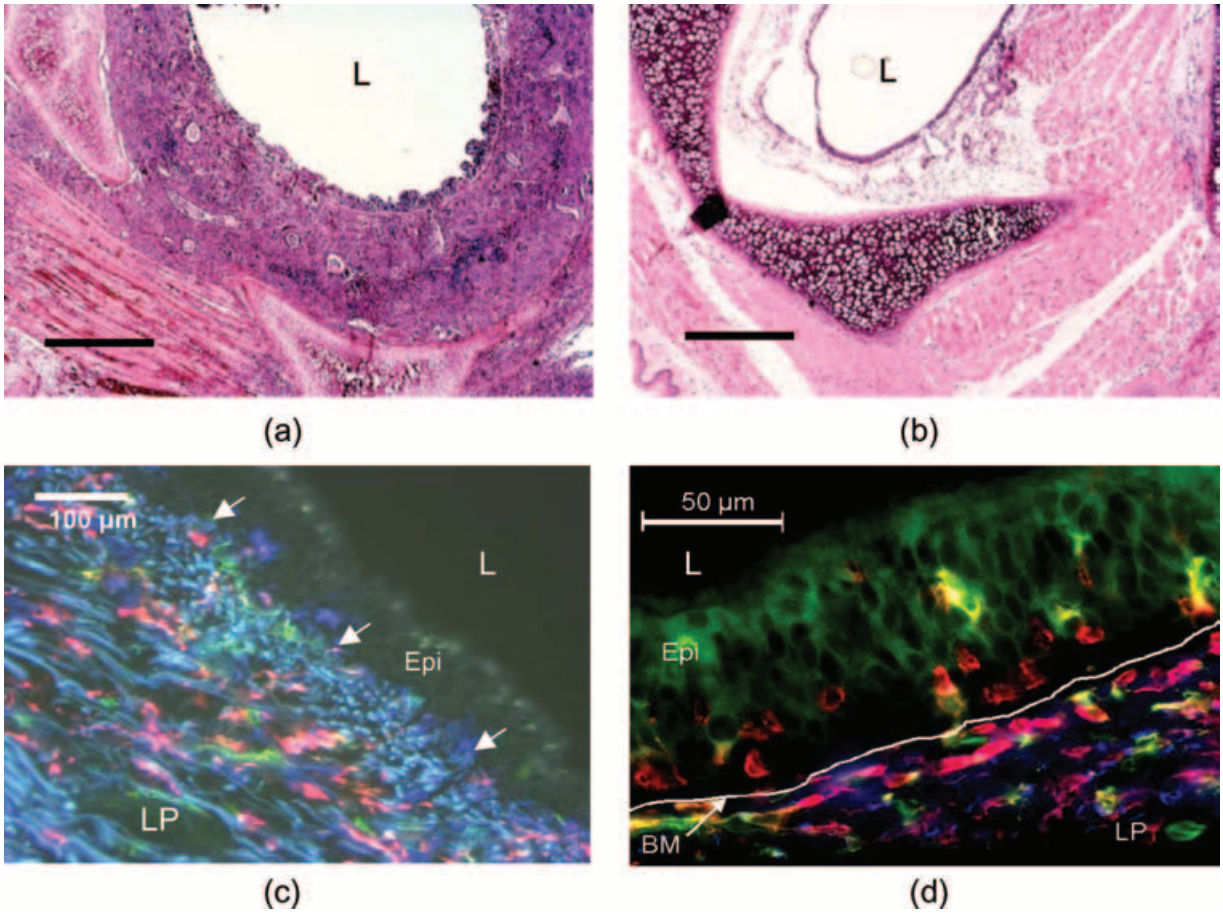

Figure 2: Responses of laryngeal allografts to transplantation. Haematoxylin and eosin image of cross-section through rat laryngeal allograft at 7 days (a) with and (b) without immunosuppression. (A) Profuse infiltration by mononuclear cells and vascular occlusion. (B) Normal architecture and patent microvessels. (C) Three-colour immunofluorescence histology of pig laryngeal mucosa showing dendritic cells with varying levels of activation in a laryngeal allograft 8 h after reperfusion (blue = MHC class II; green = CD172; red = CD163) and (D) MHC-II expression in man (green = HLA-DR; red = CD45). Arrows indicate basement membrane. L (a,b,d) = laryngeal lumen.

research (3). From 1987 onward, Strome's group developed and used the heterotopic rat laryngeal transplant model. This has the advantage of being inexpensive, straightforward and avoids the need for tracheostomies and gastrostomies, and the model has yielded a large amount of experimental data on laryngeal transplantation (4) (Figures 1A and 2A). However, the donor larynx forms a closed mucosal sac and reinnervation studies are impractical. Genden's murine model of tracheal transplantation has recently provided exciting data which are extrapolatable to laryngeal work (5). Birchall's group has developed an orthotopic pig model which is robust and allows detailed assessment of immunology in an open airway, coupled with functional reinnervation (6,7) (Figures 1B and 2C). However, as with the dog, this model is expensive in time and labor and requires tracheostomy and gastrostomy, at least in the short term. These models all offer complimentary information forming the basis on which to build further human studies.

Reinnervation

The main barrier to successful laryngeal transplantation is restoration of normal laryngeal speech and breathing functions. The larynx has the most complex motor function of any muscular-based system in the human body as may be appreciated by listening to high-performance singing. This complexity is reflected by the comparatively high density of motor fibres in the recurrent nerve, as compared to the small size of the muscles it innervates. While sensation is certainly important in eliciting cough, restoration of near-normal mobility of the arytenoids, and hence of the vocal cords, is essential.

Sensory Supply

The superior laryngeal nerve principally supplies sensation to the superior part, helping to prevent aspiration of saliva and food. One old study suggested that repair of the superior nerves alone would be sufficient to prevent aspiration in dogs. Happily, direct repair of these nerves seems to effectively restore this aspect of function, as observed in multiple animal models (8) and in the Cleveland patient (1,9). Experience with humans with vagal nerve damage, however, suggests that superior nerve function alone, while very helpful, is insufficient for normal function probably because the evolution of speech has left us with a low lying larynx. Hence the need for replacement of appropriate motor function.

Motor Supply

The superior laryngeal nerve only supplies one muscle, the cricothyroid. Successful direct repair of these nerves in the Cleveland patient has provided him with the ability to shorten and lengthen his vocal cords, with the resultant ability to vary the pitch of his voice (9).

However, it is the recurrent laryngeal nerve which provides most of the motor drive to the larynx and it is restoration of the functions it supplies that causes the greatest barrier to laryngeal transplantation. Bilateral paralysis (as following transplantation) generally results in cords lying together (opposed) with useful, though phoniatrically limited, speech, and poor breathing. Unfortunately, direct repair of the recurrent nerve results in synkinesis (mass-movement) (10) of muscles as repair in adult animals fails to recapitulate the successful axonal pathfinding of embryogenesis. Thus, although recent electromyography of the Cleveland patient has confirmed some recurrent nerve reinnervation on the right side (9), the significant abduction that would be required to allow closure of his tracheostomy is still lacking.

Pacemakers

The use of laryngeal pacemakers has been explored by Zelear's group (11). Implantation of a device based on a cardiac pacemaker allowed the removal of the tracheostomy in three of six patients with bilateral recurrent nerve damage implanted, five of whom were reported to have good cord movement. Although the reported system was bulky, groups in the USA and Europe are presently obtaining encouraging results with refined models. These devices may ultimately either provide short-term function and retention of muscle bulk ('baby-sitting') while nerve regeneration proceeds or be used as a permanent solution.

Anatomical Reinnervation

The second means of replacing recurrent nerve function is anatomical reinnervation. There is only one muscle that causes laryngeal abduction and posterior motion of the arytenoids, the posterior cricoarytenoid (PCA). The unique position of this muscle to laryngeal function, particularly breathing, has given its reinnervation greater prominence than that of the other intrinsic muscles. Fex was the first to show that the phrenic nerve could be re-routed in animals to drive the PCA (12). This has been confirmed several times in both animals and man (7,13), and has been given impetus by the Berke-Sercarz group's detailed anatomical description, which gives a firm rationale for this approach (15), although reports also suggest that the technique (and related re-routing techniques using other motor nerves) may still result in aberrant reinnervation of adjacent muscles as well as the target abductors with functionally suboptimal results (16).

Innervation of the other, adductor, branch of the recurrent nerve, using either branches of the ansa cervicalis or the residual recurrent nerve trunk, will add tone and bulk to the vocal cords, with resultant phoniatric gain. This strategy may also, theoretically, reduce the opportunities for delayed aberrant reinnervation of fibres intended for the PCA, although this needs confirming in appropriate animal models. Clinical trials of nerve transfer using phrenic-abductor branch and ansa-adductor branch repairs are presently underway in France and Korea, where at least 10 patients with bilateral recurrent nerve paralysis have been reinnervated to date (Crumley, personal communication). However, the final reports, with follow-up of more than 2 years, from these studies are required before purely anatomical reinnervation can be assumed to be the answer to laryngeal transplant's greatest challenge.

Neurobiology

Another, complimentary, possibility is to work in partnership with peripheral nerve biologists to achieve faster, more accurate reinnervation of a laryngeal graft. Nerve growth factors, such as BDNF, NT-3 and NT-4, have all shown promise in speeding up peripheral nerve repair (17) and are presently being applied in models of laryngeal reinnervation. The application of insulin-like growth factor-1 (a potent myotrophic stimulus) to laryngeal muscles, using a viral vector, is also being tried as a means of retaining end-organ function when reinnervation eventually occurs (18). Important areas to be investigated are which growth factors to use when, and how to retain them at the repair or end-organ site. Importantly, a detailed appraisal of type and sequence of gene expression by nucleus ambiguus motor nuclei after recurrent nerve section and repair is required, as well as studies of pathfinding in embryogenesis (19).

Gold and colleagues have reviewed studies of the effects of neuroimmunophilin compounds (including tacrolimus) on peripheral nerve repair. Although these studies use sub-immunosuppressive doses, more than one study has shown beneficial neurotrophic effects following neurotmesis (20). If this effect could be confirmed using therapeutic immunosuppressive doses, this would add weight to the use of tacrolimus and related agents as a first-line immunosuppressive drug for reinnervated organ grafts, such as larynx and hand.

Preservation Fluids and Ischaemia-Reperfusion Injury

Ischaemia-reperfusion injury (IRI) is an important cause of early graft loss in other transplant settings and may increase the incidence and severity of acute rejection. Therefore, one early research goal has been to minimize this effect. In a controlled rat study, perfusion with the University of Wisconsin solution prevented the histological evidence of tissue damage seen after 6 h of preservation with saline alone (21). In both this study and a later dog study (2), perfusion with the University of Wisconsin solution appeared to permit ischaemic intervals as long as 24 h, as assessed macroscopically and with light microscopy. In a minipig model, Birchall's group showed no significant increase in immunologically active cell numbers following a 5-h cold-ischaemia time (22,23) (Figure 2C). Although these studies show no immediate evidence of IRI, longer term follow-up studies are required.

Immunosuppression and Immunomodulation

Rat studies by the Strome group laid the foundation for the choice of immunosuppressive regimen used in the 1998 transplant. Follow-up studies have demonstrated that the addition of steroids reduces the required dose of cyclosporine to 2 mg/kg (24) and that, as single agent, cyclosporin trough levels should not fall below 250 ng/ml (25). They have also compared the use of tacrolimus (0.1–0.6 mg/kg) with and without mycophenolate mofetil (MM: 15–40 mg/kg) in the same rat model. They showed a significant dose-response relationship between tacrolimus alone and decreasing histological rejection scores, and that the addition of MM allowed the use of low dose (0.2 mg/kg) tacrolimus without increased rejection (26). In a parallel study, a short (7-day) course of tacrolimus combined with anti-alpha-beta T-cell receptor antibody was able to prevent rejection of 10 unmatched rat laryngeal grafts for up to 100 days (27) (Figure 2A, B). Skin grafting, mixed lymphocyte reaction and flow cytometry in this study suggested that tolerance was neither donor-specific nor related to systemic immunocompromise. Finally, this group has most recently performed studies suggesting that rapamycin, in addition to its action in prolonging graft survival (28), actually suppresses the growth of squamous cancer cells in a mouse model. This is particularly interesting since the main hope is that laryngeal transplantation will eventually be used as primary reconstruction following cancer resection, and the use of such an agent might reduce the risk of cancer recurrence.

Genden showed that a single injection of UV-B irradiated donor splenocytes was sufficient to prevent rejection in a rat tracheal graft model (29). Lorenz described how 800 cGy of irradiation prior to implantation significantly reduced histological rejection scores at 15 days in the Strome rat model. However, this effect was not additive to that of cyclosporine (2). The long-term effects of either of these approaches has, however, not yet been studied.

Using multiple colour immunofluorescence, Birchall's group has described a dense, organised network of immunologically active cells, including many dendritic cells in pig (22,23) (Figure 2C) and man (Figure 2D) (31) laryngeal mucosae. Human epithelial cells also express MHC molecules. Thus, all the indications are that at least the mucosal element of the larynx will represent a strong graft. In support, Genden's studies of murine tracheal grafts have shown that replacement of the epithelium by host epithelial cells completely prevents rejection after withdrawal of immunosuppression (4). As re-epithelialization therefore appeared as an attractive strategy for airway grafts, this group went on to show that subsequently the process could be significantly speeded up with the application of vascular endothelial growth factor (VEGF) carried by a fibrin matrix to the mucosa (31). Further circumstantial evidence of the importance of the epithelial immune system to graft rejection comes from a hand transplant recipient who failed to take immunosuppressant medication. When the hand was eventually amputated, only the epithelial component showed significant signs of rejection (33). This view is tempered, however, by the observation that rat laryngeal grafts appear to exhibit early rejection throughout all tissues (24).

These observations suggest that tissue (mucosa)—specific interventions may be highly effective in preventing laryngeal (or other mucosal organ) allograft rejection in due course, and thus removing the need for conventional immunosuppression. Such strategies would also open the door to transplantation for the large pool of potential recipients with laryngeal cancer.

Signs of rejection of the human transplant recipient were assessed qualitatively by endoscopy and by serial biopsies of graft tracheal mucosa during the first month. Based on an observation of acute laryngeal oedema responsive to steroids, the human transplant recipient was reasonably assumed to have had a single episode of rejection. To improve the precision of identification of rejection in the

animal model, it has been proposed to monitor the function of transplanted parathyroids (26). An increased knowledge of normal and pathological laryngeal mucosal immunology (31) as well as systemic immune responses should, in future, allow highly precise monitoring of graft status from mucosal biopsies and blood samples.

The Future for Laryngeal Transplantation

There is a steadily increasing volume of laboratory research providing pre-clinical information on laryngeal transplantation. The qualified success of the sole human laryngeal transplant to date has provided us with some of the answers needed before full clinical trials, as well as highlighted the problems still to be overcome. These are functional laryngeal reinnervation (especially but not exclusively abductor), and the need for organ-specific immunosuppression strategies. Other questions involve longer term work on ischaemic injury and improved methods of diagnosing rejection, both acute and chronic. Conventional reconstruction and prosthetics have regularly failed to replace the complex functions of the human larynx, thus keeping laryngeal transplantation as a major research goal for restoring quality of life for patients with irreversible laryngeal disease. With continuing advances in mucosal and transplant immunology and in peripheral nerve neurobiology, the future for this technique, either total or partial (34), is cautiously optimistic.

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References

1. Strome M, Stein J, Esclamado R, Hicks D, Lorenz RR, Braun W et al. Laryngeal transplantation and 40-month follow-up. N Engl J Med 2001; 344: 1676–1679.
2. Kevorkian KF, Sercarz JA, Ye M, Kim YM, Hong KH, Berke GS et al. Extended canine laryngeal preservation for transplantation. Laryngoscope 1997; 107: 1623–1626.
3. Haughey BH, Beggs JC, Bong J, Genden EM, Buckner A. Microneurovascular allotransplantation of the canine tongue. Laryngoscope 1999; 109: 1461–1470.
4. Lorenz RR, Fritz MA, Strome M. Special feature: ccurrent state of laryngeal transplantation. Curr Opin Otolaryngol Head Neck Surg 2001; 9: 381–386.
5. Genden EM, Iskander A, Bromberg JS, Mayer L. The kinetics and pattern of tracheal allograft re-epithelialization. Am J Respir Cell Mol Biol 2003; 28: 673–681.
6. Birchall MA, Bailey M, Barker EV, Rothkotter HJ, Otto K, Macchiarini P. Model for experimental revascularized laryngeal allotransplantation. Br J Surg 2002; 89: 1470–1475.
7. Birchall M, Idowu B, Murison P et al. Laryngeal abductor muscle reinnervation in a pig model Acta Otolaryngol 2004; 124: 839–846.
8. Blumin JH, Ye M, Berke GS, Blackwell KE. Recovery of laryngeal sensation after superior laryngeal nerve anastomosis. Laryngoscope 1999; 109: 1637–1641.
9. Lorenz RR, Hicks DM, Shields RW Jr., Fritz MA, Strome M. Laryngeal nerve function after total laryngeal transplantation. Otolaryngol Head Neck Surg 2004; 131: 1016–1018.
10. Crumley RL. Laryngeal synkinesis revisited. Ann Otol Rhinol Laryngol 2000; 109: 365–371.
11. Zealear DL, Billante CR, Courey MS , Netterville JL, Paniello RC, Sanders I et al. Reanimation of the paralyzed human larynx with an implantable electrical stimulation device. Laryngoscope 2003; 113: 1149–1156.
12. Fex S. Functioning remobilization of vocal cords in cats with permanent recurrent laryngeal nerve paresis. Acta Otolaryngol 1970; 69: 294–301.
13. Crumley RL. Phrenic nerve graft for bilateral vocal cord paralysis. Laryngoscope 1983; 93: 425–428.
14. Marie JP, Dehesdin D, Ducastelle T, Senant J. Selective reinnervation of the abductor and adductor muscles of the canine larynx after recurrent nerve paralysis. Ann Otol Rhinol Laryngol 1989; 98: 530–536.
15. Damrose EJ, Huang RY, Ye M, Berke GS, Sercarz JA. Surgical anatomy of the recurrent laryngeal nerve: implications for laryngeal reinnervation. Ann Otol Rhinol Laryngol 2003; 112: 434–438.
16. Lewis WS, Crumley RL, Blanks RH, Pitcock JK. Does intralaryngeal motor nerve sprouting occur following unilateral recurrent laryngeal nerve paralysis? Laryngoscope 1991; 101: 1259–1263.
17. Terenghi G. Peripheral nerve regeneration and neurotrophic factors. J Anat 1999; 194: 1–14.
18. Flint PW, Nakagawa H, Shiotani A, Coleman ME, O'Malley BW Jr.. Effects of insulin-like growth factor-1 gene transfer on myosin heavy chains in denervated rat laryngeal muscle. Laryngoscope 2004; 114: 368–371.
19. Huber AB, Kolodkin AL, Ginty DD, Cloutier JF. Signaling at the growth cone: ligand-receptor complexes and the control of axon growth and guidance. Annu Rev Neurosci 2003; 26: 509–563.
20. Gold BG, Udina E, Bourdette D, Navarro X. Neuroregenerative and neuroprotective actions of neuroimmunophilin compounds in traumatic and inflammatory neuropathies. Neurol Res 2004; 26: 371–380.
21. Strome M, Wu J, Strome S, Brodsky G. A comparison of preservation techniques in a vascularized rat laryngeal transplant model. Laryngoscope 1994; 104: 666–668.
22. Gorti GK, Birchall MA, Haverson K, Macchiarini P, Bailey M. A preclinical model for laryngeal transplantation: anatomy and mucosal immunology of the porcine larynx. Transplantation 1999; 68: 1638–1642.
23. Barker EV. Ischaemia-reperfusion injury in a pig laryngeal transplant model. PhD thesis, University of Bristol, UK 2003.
24. Lorenz RR, Dan O, Haug M 3rd, Strome M. Effects of adding steroids, in vitro irradiation, or both to cyclosporine immunosuppression in the murine laryngeal transplantation model. Ann Otol Rhinol Laryngol 2002; 111: 455–9.
25. Haug M 3rd, Dan O, Wimberley S, Fritz M, Lorenz RR, Strome M. Cyclosporine dose, serum trough levels, and allograft preservation in a rat model of laryngeal transplantation. Ann Otol Rhinol Laryngol 2003; 112: 506–510.

26. Nelson M, Fritz M, Dan O, Worley S, Strome M. Tacrolimus and mycophenolate mofetil provide effective immunosuppression in rat laryngeal transplantation. Laryngoscope 2003; 113: 1308–1313.
27. Akst LM, Siemionow M, Dan O, Izycki D, Strome M. Induction of tolerance in a rat model of laryngeal transplantation. Transplantation 2003; 76: 1763–1770.
28. Khariwala SS, Kjaergaard J, Lorenz RR, Van Lente F, Shu S, Strome S. Everolimus (RAD) inhibits *in vivo* growth of murine Squamous cell carcinoma [abstract]. Proceedings of the American Academy of Otorhinolaryngology-Head and Neck Surgery; 2005.
29. Genden EM, Mackinnon SE, Yu S, Hunter DA, Flye MW. Portal venous ultraviolet B-irradiated donor alloantigen prevents rejection in circumferential rat tracheal allografts. Otolaryngol Head Neck Surg 2001; 124: 481–488.
30. Lorenz RR, Dan O, Fritz MA, Nelson M, Strome M. Immunosuppressive effect of irradiation in the murine laryngeal transplantation model: a controlled trial. Ann Otol Rhinol Laryngol 2003; 112: 712–715.
31. Rees LE, Ayoub O, Haverson K, Birchall MA, Bailey M. Differential major histocompatibility complex class II locus expression on human laryngeal epithelium. Clin Exp Immunol 2003; 134: 497–502.
32. Govindaraj S, Gordon R, Genden EM. Effect of fibrin matrix and vascular endothelial growth factor on re-epithelialization of orthotopic murine tracheal transplants. Ann Otol Rhinol Laryngol 2004; 113: 797–804.
33. Kanitakis J, Jullien D, Petruzzo P et al. Clinicopathologic features of graft rejection of the first human hand allograft Transplantation 2003; 76: 688–93.
34. Andrews RJ, Berke GS, Blackwell KE, Jakobsen M, Wang MB, Sercarz JA. Hemilaryngeal transplantation in the canine model: technique and implications. Am J Otolaryngol 2000; 21: 85–91.