BRIEF REPORT

Tracheal Allotransplantation after Withdrawal of Immunosuppressive Therapy

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SUMMARY

Reconstruction of long-segment tracheal defects requires a vascularized allograft. We report successful tracheal allotransplantation after indirect revascularization of the graft in a heterotopic position. Immunosuppressive therapy was administered before the operation, and the tracheal allograft was wrapped in the recipient's forearm fascia. Once revascularization was achieved, the mucosal lining was replaced progressively with buccal mucosa from the recipient. At 4 months, the tracheal chimera was fully lined with mucosa, which consisted of respiratory epithelium from the donor and buccal mucosa from the recipient. After withdrawal of immunosuppressive therapy, the tracheal allograft was moved to its correct anatomical position with an intact blood supply. No treatment-limiting adverse effects occurred.

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Airway defects that are 1 to 5 cm long can be bridged by end-to-end anastomoses, whereas longer defects pose a major reconstructive challenge.[1-3] Currently, there are few therapeutic options for repairing defects longer than 5 cm. Several studies, both experimental[4,5] and clinical,[6] support tracheal allotransplantation as a viable option for treating long-segment tracheal defects. However, since the trachea lacks an identifiable vascular pedicle that would allow direct vascular anastomosis to the blood vessels in the recipient's neck, the major challenge to successful tracheal transplantation is the safe restoration of the graft's blood supply.[7]

Recently, an avascular, tissue-engineered tracheal transplant was used to relieve stenosis in a main-stem bronchus,[8] but such tissue may heal unpredictably when it is used for tracheal reconstruction. Other reconstructions that use composite tissue transplants[9] require a method for restoring the blood supply to the tracheal transplant.

Indirect revascularization of the trachea is possible, as evidenced by successful revascularization of a tracheal allograft after heterotopic wrapping in omentum[6] and successful transplantation of tracheal allografts and autografts involving vascularized fascial flaps in laboratory animals[4,5] and humans.[10,11] This type of revascularization can be achieved by wrapping the tracheal allograft in heterotopic tissue from the recipient that is well vascularized and perfused by an identifiable vascular pedicle.

Experiments in immunosuppressed rabbits showed complete revascularization and restoration of mucosal lining in tracheal allografts after 2 to 4 weeks of heterotopic revascularization in the lateral thoracic area.[4] On the basis of our experience with tracheal allotransplantation in animals and tracheal autotransplantation in patients, we decided to reconstruct a long-segment tracheal defect in a patient by using an allograft that was initially revascularized by heterotopic wrapping in vascularized fascia.

CASE REPORT

The patient was a 55-year-old woman who had been involved in a car accident 25 years earlier and had undergone tracheotomy. She had a long history of tracheal stenosis and placement of stents to preserve the lumen. When we first saw the patient, in October 2006, she had two stents, each 4 cm long, supporting her airway (Fig. 1A). She sought treatment for numerous stent-related problems, including episodes of bronchitis and pneumonia. Nearly continuous antibiotic therapy was necessary to control the chronic infection around the stented airway. The patient coughed continually to try to clear the mucus that accumulated at the end of the lower stent, and bad breath attributed to stent colonization was a serious problem. At regular intervals, laser resection was necessary to remove the granulation tissue that formed at the ends of the stent.

To avoid the risk of total airway collapse on removal of the stents, we opted to treat this patient with tracheal allotransplantation. The plan was to place an 8-cm-long tracheal allograft in the recipient's forearm (Fig. 2A) to allow it to revascularize for later use in correcting the tracheal defect. The airway stents could be removed once the allograft had achieved full mucosal revascularization. The procedure was approved by the institutional review board of the University Hospital Leuven, and the patient provided written informed consent.

METHODS AND RESULTS

HETEROTOPIC IMPLANTATION OF THE GRAFT FOR REVASCULARIZATION

After a 9-month search, we located a suitable trachea from a deceased male donor with the same blood group (November 2007). Thoracic surgeons harvested the double-lung block as well as an 8-cm-long tracheal segment. At the same time, a full-thickness skin graft was harvested from the supraclavicular area, and the donor tissue was stored in University of Wisconsin solution and ice during transport (time from harvest to the site of transplantation, less than 2 hours). The 8-cm-long donor trachea was placed over the dissected subcutaneous tissue in the recipient's left forearm in the proper orientation for revascularization (Fig. 2A). The allograft was circumferentially wrapped with fascia and subcutaneous tissue. Simultaneously, the full-thickness skin graft from the donor was sutured into a small skin defect that had been created behind the patient's left pinna. Immunosuppressive therapy with tacrolimus (to obtain a trough level of 12 to 15 μg per liter), azathioprine (100 mg per day), and corticosteroids (0.4 mg per kilogram of body weight per day) was begun intravenously and then maintained orally thereafter (tacrolimus, 6 mg per day; azathioprine, 100 mg per day; and methylprednisolone, 4 mg per day).

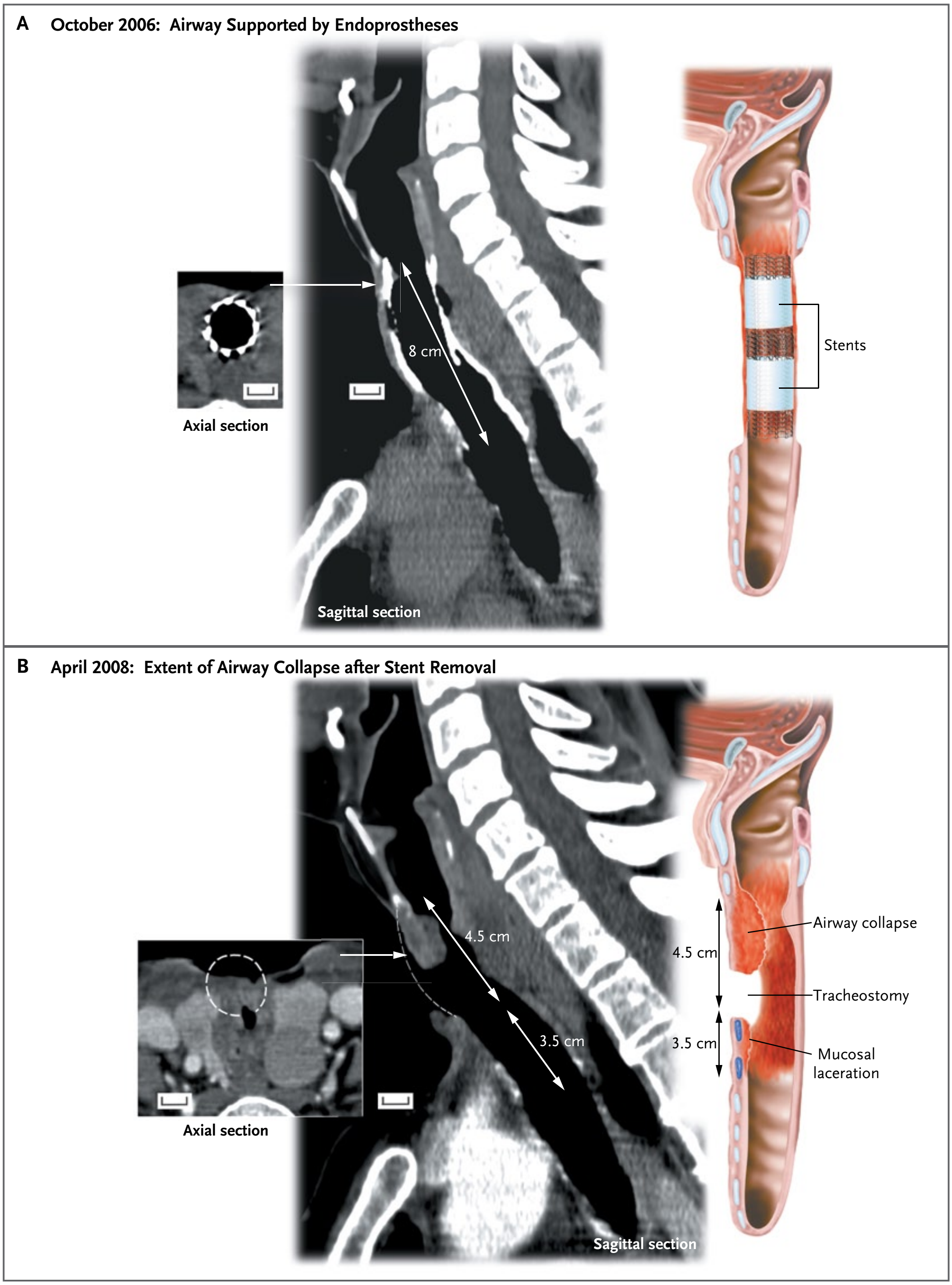
Every 2 weeks, the forearm dressing was removed to determine mucosal viability and to remove intraluminal secretions (see video, available with the full text of this article at NEJM.org). In the first weeks, the posterior membranous sheath underwent avascular necrosis. Thirty-four days after transplantation, during one of the scheduled fortnightly examinations, we removed the necrotic posterior tracheal wall and placed buccal mucosa from the recipient in this part of the trachea (Fig. 2B).

A video showing a functional transplant is available at NEJM.org

After 4 months, the tracheal allograft had a full mucosal lining (Fig. 2C). At this time, we removed the airway stents, and a second tracheotomy was created in the caudal neck area to permit respiration. A computed tomographic (CT) scan obtained after the stents were removed showed that the upper segment of the airway had collapsed, whereas the lumen of the lower segment was preserved (Fig. 1B). Therefore, the allograft was shortened to 4.5 cm to cover the tracheal defect (Fig. 2D), which allowed histologic evaluation of the excised 3.5-cm segment. Since the procedure involved transplantation from a man to a woman, we were able to detect donor respiratory epithelial cells by the presence of the Y chromosome on fluorescence in situ hybridization (FISH) analysis. Immunostaining and FISH were performed as described previously.[12]

EVALUATION OF THE ALLOGRAFT AND WITHDRAWAL OF IMMUNOSUPPRESSIVE THERAPY

Gross examination of the excess tracheal tissue showed that the elasticity of the cartilage rings



was preserved (see video). Microscopical examination showed that the tracheal rings were composed of viable cartilage tissue. The epithelial lining consisted of squamous epithelium and respiratory epithelium that originated from the recipient's buccal mucosa and the donor's tracheal epithelium, respectively. FISH analysis of the respiratory epithelium showed male donor cells,

Figure 1 (facing page). Computed Tomographic Scans and Diagrams of the Tracheal Defect in the Patient.

Panel A shows the airway when the patient first presented in October 2006. Axial and sagittal computed tomographic (CT) scans and the diagram of the airway in sagittal section show the two metallic endoprostheses (each 4 cm in length and 18 mm in diameter). Panel B shows these same views after stent removal and subsequent airway collapse in April 2008. The upper 4.5 cm of the trachea needed to be reconstructed because thickening of the tissue lining the airway was limiting airflow and closing off the tracheotomy site. The lower 3.5-cm segment showed mucosal laceration. The white dashed lines in Panel B indicate the extent of the planned airway augmentation to be achieved after orthotopic tracheal transplantation. Bars in both panels represent 1 cm.

but the Y chromosome was not detected in the squamous epithelial lining (Fig. 2C). The donor skin graft behind the recipient's outer ear showed no signs of rejection (Fig. 2D). On the basis of the presence of recipient cells in the allograft, immunosuppressive therapy was tapered and stopped, with the last dose administered 229 days after transplantation (Fig. 3A). The skin graft was rejected after immunosuppressive therapy was discontinued; it became necrotic after 4 weeks and sloughed off 2 weeks later. The resulting skin defect healed by second intention.

ORTHOTOPIC TRACHEAL ALLOTRANSPLANTATION

At the time of orthotopic transplantation, the tracheal graft had viable cartilage fully lined with squamous epithelium. No signs of rejection were detected within the transplant (Fig. 3A). FISH analysis did not reveal Y chromosomes within the mucosal lining. The donor tracheal cartilage was surrounded by recipient blood vessels and had a mucosal epithelial lining that originated from the recipient. The revascularized allograft was dissected with the radial artery and two radial veins. One ring of the transplant was removed for histologic and FISH analyses. After incision and expansion of the collapsed airway segment, the tracheal defect was 4.5 cm long, a length that was adequate to cover the original defects as well as the tracheostomy site we had created. Orotracheal intubation was maintained while the transplant was sutured into the airway defect and the radial vascular pedicle was sutured to the neck vessels (the radial artery end-to-end to the superior thyroid artery and the radial veins end-to-side to the internal jugular vein). The distal mucosal graft was placed within the skin incision in the neck to serve as an external indicator of the viability of the buried fascial flap (Fig. 3B).

The patient remained intubated transorally but was weaned from mechanical ventilation within 6 days, at which time the orotracheal tube was removed. After the procedure, axial and sagittal CT images showed that the airway had been restored by the tracheal transplant (Fig. 3B). The patient was discharged after 1 week.

Since the removal of the airway stents, the patient has had no further episodes of bronchitis or pneumonia. One year after tracheal reconstruction, the patient was satisfied with the outcome. Pulmonary-function tests at that time showed a forced vital capacity of 3.85 liters (121% of the predicted value), a forced expiratory volume in 1 second of 2.21 liters (81% of the predicted value), and a peak expiratory flow rate of 5.23 liters per second (80% of the predicted value), findings that were consistent with the absence of significant functional upper-airway obstruction. CT scans obtained 1 year after complete cessation of immunosuppressive therapy were similar to those obtained 1 week after transplantation.

DISCUSSION

Since the blood supply to the trachea makes it unsuitable for direct revascularization, most previous attempts at tracheal transplantation have been performed after indirect revascularization. In 1979, Rose et al. reported the first allogeneic tracheal transplantation in a human.[13] The donor trachea was implanted heterotopically in the sternocleidomastoid muscle of the recipient and was transferred to the orthotopic position 3 weeks later. However, the recipient was not given immunosuppressive therapy. Moreover, this report did not document the viability of the allograft, and no information has been made available about the long-term outcome. In 2004, Klepetko et al.[6] reported preserved viability of a heterotopically revascularized allograft. The graft was revascularized in the omentum of a patient who received a lung transplant from the same donor. Ultimately, the tracheal transplant was not used, but its viability was documented after 60 days.

Recently, successful reconstruction of a mainstem bronchus by means of a tissue-engineered transplant was reported by Macchiarini et al.[8] In this study, reconstruction was performed with a

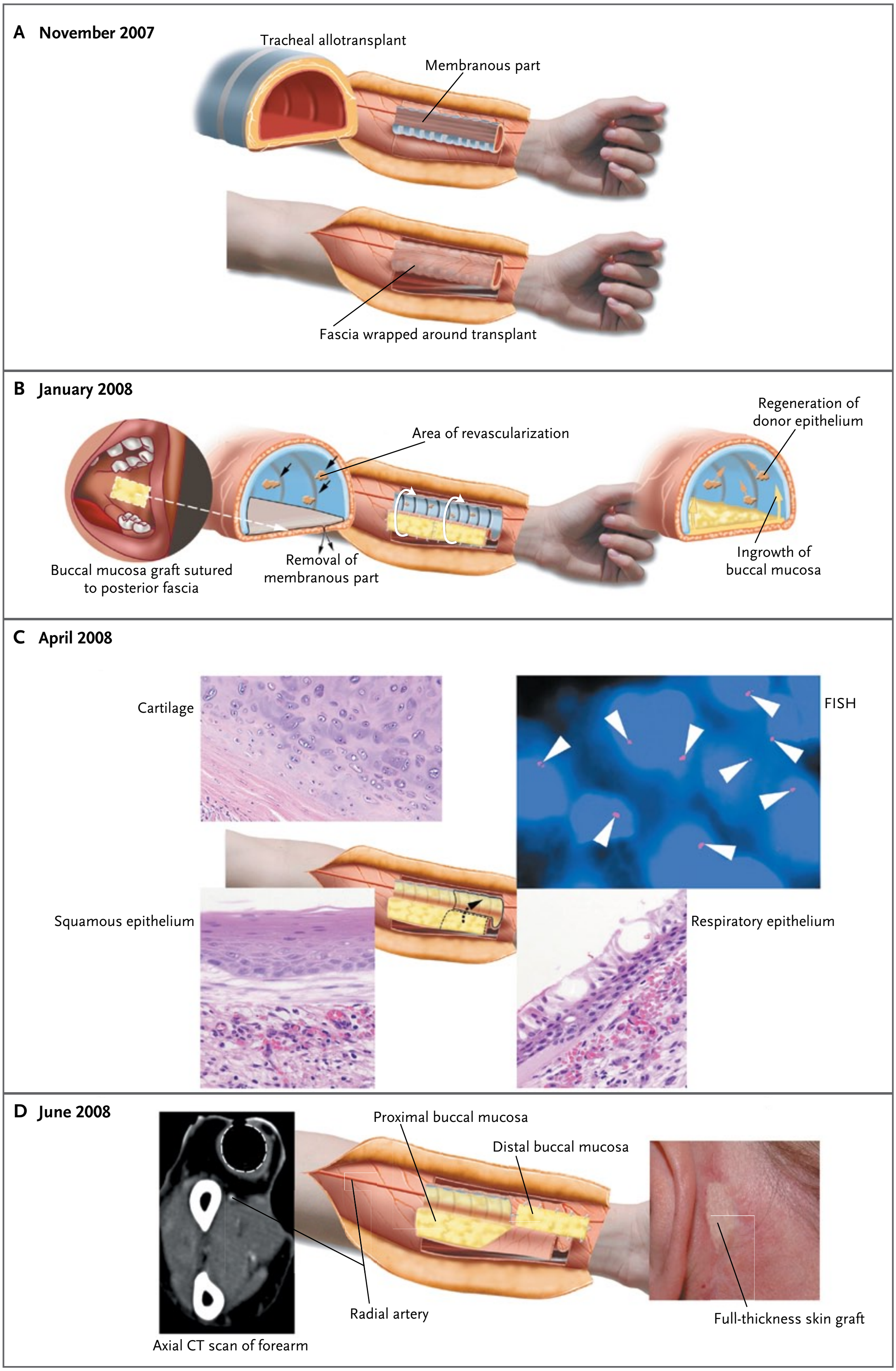

A November 2007
Tracheal allotransplant
Membranous part
Fascia wrapped around transplant
B January 2008
Regeneration of donor epithelium
Area of revascularization
Buccal mucosa graft sutured to posterior fascia
Removal of membranous part
Ingrowth of buccal mucosa
C April 2008
Cartilage
FISH
Squamous epithelium
Respiratory epithelium
D June 2008
Proximal buccal mucosa
Distal buccal mucosa
Radial artery
Full-thickness skin graft
Axial CT scan of forearm

Figure 2 (facing page). Procedure for Heterotopic Tracheal Allotransplantation.

Panel A shows the 8-cm-long tracheal allograft in place, with its cartilaginous part positioned over the subcutaneous tissue in the recipient's forearm. A cross section of the avascular allograft is also shown. Subcutaneous tissue and fascia dissected from the underlying muscles were wrapped around the tracheal allograft to cover the membranous part. Panel B shows that the steps taken after 4 weeks of fascial wrapping had resulted in tracheal revascularization and reestablishment of a mucosal lining. The cross-sectional view at the level of a cartilage ring shows the denuded cartilage framework with small areas of revascularization (black arrows). The membranous posterior wall of the allograft underwent avascular necrosis and was removed from the underlying fascia (double arrows). Two full-thickness buccal mucosa grafts measuring 1 cm×4 cm were sutured to the fascia that was in contact with the posterior tracheal wall (dashed white arrow). The mucosa-covered fascia was then sutured to the cartilaginous trachea within the forearm (curved arrows). Regeneration of donor epithelium and progressive ingrowth of buccal mucosa over the cartilaginous framework took place over the next few weeks (cross-sectional view at far right). Panel C shows the chimerism achieved within the allograft after 4 months of heterotopic revascularization. The buccal mucosa that had been sutured onto the fascia at the site of the excised segment was implanted distally to the preserved tracheal allograft (dashed arrow). Photomicrographs of an excised segment of excess trachea show viable cartilage tissue as well as a new mucosal lining consisting of both squamous and respiratory epithelium (hematoxylin and eosin). Fluorescence in situ hybridization (FISH) with a Y-specific centrometric DNA probe shows that the nuclei of the respiratory cells are of donor origin (arrowheads indicating Y-chromosome staining with CEP Y SpectrumOrange). (For more details, see Fig. 1 in the Supplementary Appendix, available with the full text of this article at NEJM.org.) Panel D shows the allograft before immunosuppressive therapy was withdrawn. During subsequent orthotopic transplantation, the proximal buccal mucosa was removed (see Fig. 3A), and the distal buccal mucosa graft was folded outward to allow for direct visual monitoring of flap viability (see Fig. 3B). The axial CT scan shows the tracheal allograft in the forearm (radial [upper] and ulnar [lower] bones shown in cross section), with the area available to serve as a tracheal lumen indicated by the white dashed line. The site of the full-thickness skin graft (used to monitor the tracheal graft for rejection) is visible behind the pinna of the left ear.

tracheal transplant colonized by the recipient's epithelial and chondrogenic mesenchymal cells without the use of immunosuppressive therapy. The main drawback of this procedure was the lack of an intrinsic blood supply. An avascular, tissue-engineered trachea would be unsuitable for use inside a major tracheal defect, where the graft would be exposed to the airway lumen and to continuous movements during respiration, swallowing, and coughing.[14] As with other composite-tissue allografts,[9] restoration of arterial inflow and venous outflow is essential for the survival of the tracheal allograft.

In the case we describe, arterial inflow and venous outflow were established by means of heterotopic revascularization in the recipient's forearm. Immunosuppressive therapy during revascularization of the tracheal transplant was necessary for establishing connections between the donor's capillary network around the trachea and the recipient's fascial blood vessels. This occurred quickly enough to maintain viability of the cartilaginous trachea while the posterior membranous trachea underwent avascular necrosis. The ingrowth of the cartilaginous trachea could have resulted from the low metabolic requirements of cartilage tissue.[15] We speculate that in future tracheal transplantations, it might be safer to remove the posterior membranous trachea during heterotopic implantation in order to avoid tissue necrosis within the transplant.

Chimerism occurred after the recipient buccal mucosa was introduced, which grew progressively over the lumen of the cartilaginous tracheal transplant. The endothelial and respiratory cells that originated from the donor disappeared shortly after the withdrawal of all immunosuppressive therapy. We think that this immunologic rejection occurred silently because of repopulation by the recipient's surrounding vascular network and buccal mucosal cells. The result was a viable cartilaginous allograft that received nutrients from the recipient's forearm tissue, making possible safe tracheal transplantation in the orthotopic position.

We speculate that the cartilaginous framework was not recognized by the immune system because adult cartilage lacks blood vessels. The cells in the tracheal cartilage are highly differentiated chondrocytes encased in a dense collagen–proteoglycan extracellular matrix. In our patient, the viability of the tracheal cartilage was maintained after all immunosuppressive drugs had been discontinued and after rejection of the skin graft, supporting the hypothesis that intact cartilage allografts are resistant to rejection and that tracheal cartilage remains viable when surrounded by well-vascularized recipient tissue. Experiments

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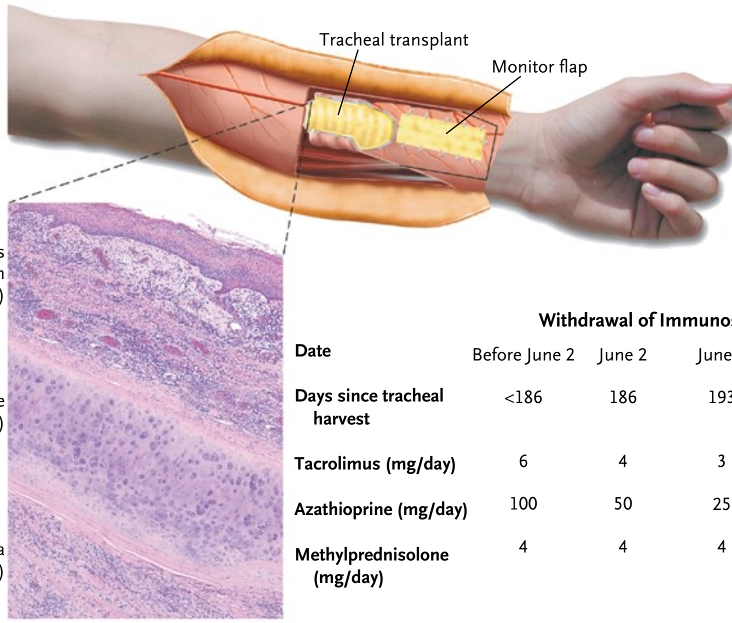

Withdrawal of Immunosuppressive Therapy

Date	Before June 2	June 2	June 9	June 30	July 15
Days since tracheal harvest	<186	186	193	214	229
Tacrolimus (mg/day)	6	4	3	2	0
Azathioprine (mg/day)	100	50	25	0	0
Methylprednisolone (mg/day)	4	4	4	0	0

B

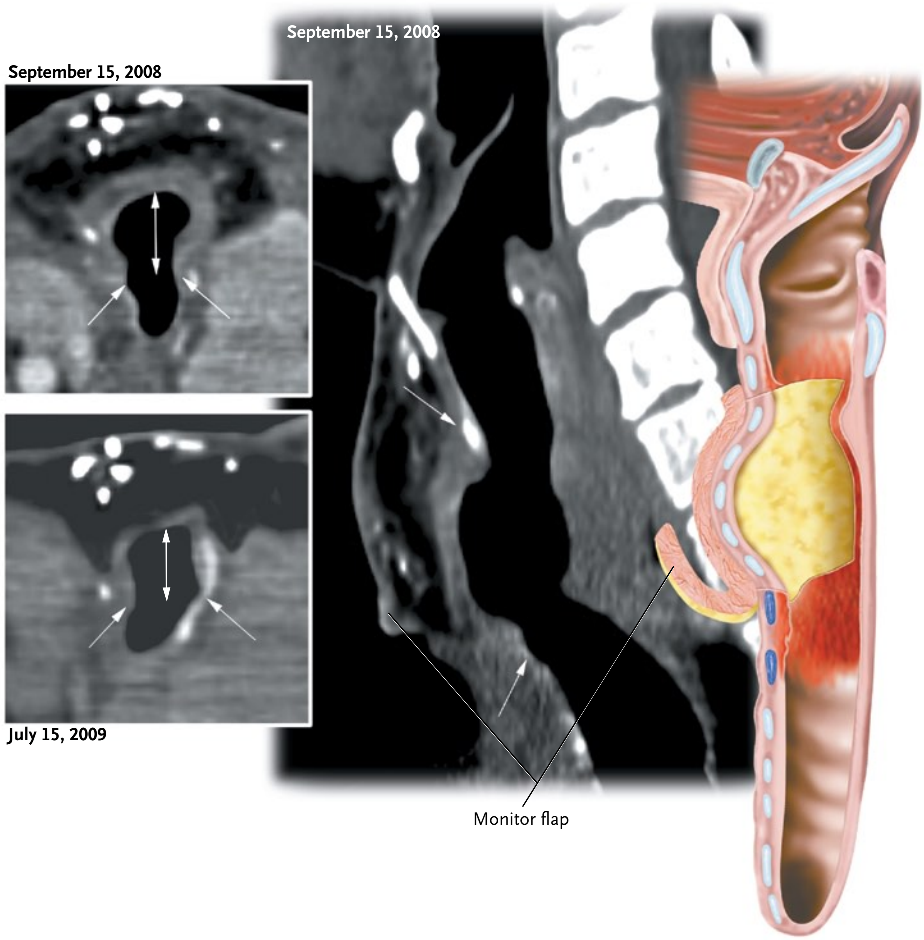

Figure 3 (facing page). Orthotopic Tracheal Allotransplantation after Withdrawal of Immunosuppressive Therapy.

The allograft was dissected with its new vascular pedicle, consisting of the radial artery and its venae committantes, and the uppermost cartilage ring was removed for histologic evaluation. Panel A shows the schedule of withdrawal of immunosuppressive therapy, and a photomicrograph of the excised cartilage ring (hematoxylin and eosin). The graft consisted of viable cartilage fully lined with squamous epithelium that originated from the recipient's buccal mucosa. (See Fig. 2 in the Supplementary Appendix for more details.) Panel B shows a sagittal CT scan of the graft and an accompanying diagram of the reconstruction 1 week after orthotopic transplantation. The arrows delineate the tracheal graft. The mucosal monitor flap serves as an external indicator of graft viability. The two axial CT scans at the left show the graft 1 week after transplantation (top) and 1 year after the complete cessation of immunosuppressive therapy (bottom); in both scans, the single-headed arrows point to the suture line between graft and native tissue, and the two-headed arrows indicate the airway lumen gained with the tracheal graft.

in animal models have shown that articular cartilage allografts are resistant to rejection because humoral antibodies cannot penetrate the intact matrix to reach the chondrocytes, whereas isolated chondrocytes possess transplantation antigens that provoke rejection.[16]

The unique characteristic of a vascularized cartilaginous tracheal transplant is that it restores the lumen of a severely damaged airway. Our findings suggest that allotransplantation of the cartilaginous tracheal framework may bring relief for selected patients who have long-segment tracheal stenoses or restenosis after segmental resection, without the immunosuppression-related risks to which recipients of other composite-tissue allografts are exposed.

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Appendix

The members of the Leuven Tracheal Transplant Group included E. Annys, B. Vanacker, W. Coosemans, C. Dooms, N. Ectors, P. Ferdinande, E. Hauben, R. Hermans, M. Jorissen, P. Nafteux, A. Lerut, T. Roskams, K. Segers, C. Vanclooster, S. Vander Borght, V. Vander Poorten, F. Van Gelder, E. Verbeken, D. Van Hees, and M. Waer.

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