EXPERIMENTAL TRACHEAL AND TRACHEOESOPHAGEAL ALLOTRANSPPLANTATION

We investigated the effects of allograft perfusion with a preservative technique and of combined thyrotracheoesophageal implantation on airway epithelium of long segments of thyrotracheal grafts allotransplanted on their own vascular pedicles into immunosuppressed pigs. Four groups of five animals each underwent heterotopic (into the neck) thyrotracheal (group 1) and thyrotracheoesophageal (group 2) and orthotopic thyrotracheal (group 3) and thyrotracheoesophageal (group 4) allotransplantation. Allograft revascularization included (1) interposition of donor right subclavian artery—incorporating the inferior thyroid artery—to recipient right carotid artery (end-to-end fashion) and (2) end-to-side anastomosis of donor anterior vena cava to recipient right external jugular vein. All thyrotracheoesophageal blocks were harvested after inferior thyroid artery perfusion with 4°C Euro-Collins solution. The overall lengths of tracheal and esophageal grafts were 10.7 ± 2.7 cm and 13.4 ± 3.6 cm, respectively. In the heterotopic groups, all allografts were viable and histologically normal at postmortem examination and the incidence and severity of airway ischemia and rejections (at equal residual levels of cyclosporine) were not different between groups 1 and 2. In the orthotopic groups, the first two pigs died of airway collapse with histologically normal grafts. In the remaining pigs, temporary airway stenting was inserted and allografts remained viable and histologically intact for their entire length 30 days after transplantation. Transplanted tracheal smooth muscles had concentration-dependent contractions and relaxations similar to those of nontransplanted (native) tracheas. This study documents the feasibility of allotransplanting long tracheal and esophageal segments on their own vascular pedicles and demonstrates that allograft preservation and thyrotracheoesophageal transplantation are equally effective in minimizing airway ischemia. Thyrotracheoesophageal transplantation does not enhance recipient alloimmune response compared with thyrotracheal transplantation alone. (J THORAC CARDIOVASC SURG 1995;110:1037-46)

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Successful clinical tracheal allotransplantation is still in its experimental infancy because of the lack of a surgical technique enabling harvest and reimplantation of the fragile tracheal vascular network.1 We recently developed a heterotopic model for direct revascularization and venous drainage of thyrotracheal allografts in immunosuppressed pigs. All grafts appeared histologically normal at postmortem examination, but early and transient airway ischemia occurred mostly into the posterior tracheal wall.2 The mechanism underlying these ischemic phenomena was attributed to the absence of donor treatment with preservative and section of esophageal arteries nourishing the posterior tracheal wall...
Fig. 1. Drawing of tracheal revascularization in pigs, right system. ITA, Inferior thyroid artery; SA, subclavian artery; VC, anterior vena cava; Ao, ascending aorta; E, esophagus.

Accordingly, we hypothesized that donor treatment with a perfusate would minimize the sensitivity of airway epithelium to reimplantation response after transplantation, and that the vascular supply of the posterior tracheal wall could be increased without unbalancing recipient’s alloimmune response by engrafting the esophagus. Single and combined tracheal and thyrotracheoesophageal allotransplantation were therefore performed first in a heterotopic and then in an orthotopic pig model. Surgical, histologic, and immunologic findings are presented.

Materials and methods

Animals. Twenty young Large White pigs of both sexes, weighing 15 to 30 kg, were used in this study. They were divided into four groups of five animals each: heterotopic tracheal (group 1) and tracheoesophageal (group 2) and orthotopic tracheal (group 3) and tracheoesophageal (group 4) allotransplantation. All received care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 85-23, revised 1985). Donors and recipients were matched only for weight. Animals were medicated with intramuscular ketamine hydrochloride (25 mg/kg) and anesthetized with intravenous sodium pentobarbital (25 mg/kg). After orotracheal intubation, anesthesia was maintained with inhaled halothane and the animals’ lungs were ventilated (Laboz Ventilator; Laboz Inc., Pau, France) with an equal gas mixture of oxygen and protoxide at a tidal volume of 250 ml and a rate of 20 breaths/min. Jugular and femoral venous catheters were placed for infusion of crystalloid solutions, and adequacy of ventilation and oxygenation was assessed by means of arterial blood gas analysis and pulse oximetry.

Harvesting and preservation technique. The fundamental principle for harvesting long segments of tracheal grafts is to preserve as much as possible their vascular supply provided by the right inferior thyroid artery (Fig. 1), a branch of the right subclavian artery. 2 Briefly, after completion of a median cervicosternotomy, dissection included two basic components. (1) Venous; all subclavian, jugular, vertebral, first intercostal and mammary veins were isolated and ligated distally on both sides, the right and left azygos veins were isolated and ligated at their confluence into the anterior vena cava (AVC), and the AVC and posterior vena cava were isolated and encircled. (2) Arterial; the first intercostal, axillary, vertebral, and carotid arteries were isolated on both sides and encircled. The internal mammary arteries were dissected and ligated on each side.

The ascending aorta and pulmonary artery were separated from one other, the pig was heparinized (3 mg/kg), a 4-0 polypropylene (Prolene; Ethicon, Inc., Somerville, N.J.) purse-string stitch was placed on the anterior surface of the ascending aorta, and an aortic root cannula (DLP, Inc., Grand Rapids, Mich.) was inserted for infusion of tracheoplegia Euro-Collins solution (EC). After cannulation and just before inflow occlusion, the thoracic aorta was isolated 10 cm distally to the origin of the left subclavian artery and all previously isolated cervical vessels were divided between ligatures. The subsequent steps were as follows: (1) inflow occlusion (clamping of the AVC and ligation of the posterior vena cava), (2) cross-clamping of the aorta before the origin of innominate artery and administration of a high-potassium chloride solution into the aortic root, (3) venting the right side of the heart (transecting the AVC at its confluence into the right atrium), (4) venting the left side of the heart (amputating the tip of the left appendage), (5) ligation of the distal thoracic aorta, and (6) tracheal perfusion (through the closed aortic segment, the EC [total volume 65 ml/kg at 4 °C at a perfusion pressure of 80 mm Hg]
entered the innominate and left subclavian arteries and all
their previously ligated primary and secondary branches,
including the inferior thyroid arteries, and thus flushed
the thyroid gland, cervicothoracic trachea, and esopha-
gus). The trachea was then harvested en bloc as previously
detailed and was placed in cold (4°C) storage with EC.

**Implantation technique.** To avoid an upper sternot-
omy, extensive dissection of the anterior mediastinum,
and manipulation of the phrenic and recurrent laryngeal
nerves, we simplified our original technique of implanta-
tion. Operation was accomplished through a median
cervicotomy alone, and revascularization of the grafts was
made after (1) excision of the cervical thymus gland and
the underlying sternothyroid and sternomuscular muscles, (2)
resection and encirclement with tourniquets of the right
external jugular vein, and (3) isolation of the proximal and
distal right carotid artery. On completion of these steps,
the pigs were heparinized (5 mg/kg) and the recipient’s
right carotid stumps by an end-to-end 6-0 polypropy-
ylene (Prolene) continuous anastomosis. The clamps
then interposed between the proximal and distal recipi-
ent’s right carotid stumps by an end-to-end 6-0 polypro-
ylene (Prolene) continuous anastomosis. The clamps
were then released, allowing the graft to reperfuse, after
injection of 240 mg intravenous methylprednisolone ace-
tate. Thereafter the recipient’s right external jugular vein
was clamped and anastomosed to the donor’s AVG with
an end-to-side 7-0 Prolene suture. During implantation,
allograft hypothermia was further maintained by topical
application of cold saline solution.

Implantation of the trachea and esophagus differed in
heterotopic and orthotopic allotransplantation. In the
heterotopic groups, the esophagus was peeled off over its
entire length as close as possible to its muscular layer in
pigs belonging to group 1 and was left in place in pigs of
Group 2. Thereafter, the proximal and distal grafts or-
ifices were, depending on the site and geometry of the
vascular suture, Anastomosed either in a transverse or
perpendicular position to the adjacent skin vessels, and thus
flushed from the prevertebral plane, and (3) transected trans-
versely (approximately 1 to 2 cm away from the tracheal
transections, avoiding the overlapping of the tracheal and
esophageal anastomoses). The esophageal lumens were
scrubbed with povidone-iodine solution and the donor’s
esophagus was then anastomosed proximally and distally
with a one-layer interrupted suture of 2-0 Vycril. After
completion, the donor’s trachea was interposed to the
recipient’s trachea in the manner described previously.
Once the tracheal and esophageal anastomoses were
completed, grafts were revascularized as previously de-
scribed. The right sternothyroid muscle, preserved before
implantation, was pedicled and interposed between the
lower tracheal anastomosis and the right carotid artery to
avoid arterial erosion. In all recipients, a central venous
catheter was placed through a jugular vein and the cervical
incision was closed with a two-layer 2-0 Vycril suture after
a single drainage of the cervical region.

**Posttransplantation management.** Animals received in-
travenous cephalothin (500 mg/gal), oral acetylsalicylic
acid (100 mg/daily), prednisolone (40 mg, days 1 and 3
after operation) and low–molecular weight heparin (0.2
ml subcutaneously) for the first 10 postoperative days.
They were immunosuppressed with intramuscular cyclo-
sporine (5 to 10 mg/kg per day) to maintain plasma
concentrations of 200 to 250 ng/ml and with oral azathio-
prine (2.5 mg/kg/daily). Rejections were intentionally not
untreated. The first dose of cyclosporine was given three
hours before induction of anaesthesia. Animals were
placed in cages and fed standard laboratory pig food and
water ad libitum.

**Posttransplant monitoring.** Fiberoptic examinations
and tracheal and esophageal biopsies were performed
routinely at posttransplant days 2, 5, and 7. Exocrine
tracheal allograft function was assessed by determining
the presence of mucous secretions within the tracheal
conduit. Animals receiving heterotopic allotransplanta-
tion were killed with intravenous 26% pentobarbital so-
dium (0.5 ml/kg) at a set time of 14 days after transplan-
tation. This interval was selected because our previous
experience suggested that new ischemic lesions were
unlikely to occur after this period of investigation. Animals
receiving orthotopic allotransplantation were killed
30 days after transplantation; grafts were then removed
for evaluation of their potency and final histology. Patency
of tracheal allografts was expressed as a proportional
cross-sectional area (CSA) of the most stenotic site in the
proximal anastomosis first with a continuous 4-0 polydiox-
anone (PDS; Ethicon) suture on the posterior tracheal
wall and interrupted 3-0 Vycril sutures on the cartilagi-
nous wall, the knots of which were tied outside. After
completion, the original orotracheal tube was guided back
through the glottis and inserted into the distal trachea,
and the distal anastomosis was made in the manner
described previously.

In recipients undergoing orthotopic tracheoesophageal
transplantation, the anterior aspect of the esophagus was
exposed after transection of the trachea with the preced-
ing steps. Thereafter, the esophagus was (1) dissected free
over a length of approximately 15 cm, with great care
taken to maintain its lateral vascular supply, (2) freed
from the prevertebral plane, and (3) transected trans-
versely (approximately 1 to 2 cm away from the tracheal
transections, avoiding the overlapping of the tracheal and
esophageal anastomoses). The esophageal lumens were
scrubbed with povidone-iodine solution and the donor’s
esophagus was then anastomosed proximally and distally
with a one-layer interrupted suture of 2-0 Vycril. After
completion, the donor’s trachea was interposed to the
recipient’s trachea in the manner described previously.
Once the tracheal and esophageal anastomoses were
completed, grafts were revascularized as previously de-
scribed. The right sternothyroid muscle, preserved before
implantation, was pedicled and interposed between the
lower tracheal anastomosis and the right carotid artery to
avoid arterial erosion. In all recipients, a central venous
catheter was placed through a jugular vein and the cervical
incision was closed with a two-layer 2-0 Vycril suture after
a single drainage of the cervical region.

**Posttransplantation management.** Animals received in-
travenous cephalothin (500 mg/daily), oral acetylsalicylic
acid (100 mg/daily), prednisolone (40 mg, days 1 and 3
after operation) and low–molecular weight heparin (0.2
ml subcutaneously) for the first 10 postoperative days.
They were immunosuppressed with intramuscular cyclo-
sporine (5 to 10 mg/kg per day) to maintain plasma
concentrations of 200 to 250 ng/ml and with oral azathio-
prine (2.5 mg/kg/daily). Rejections were intentionally not
untreated. The first dose of cyclosporine was given three
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experience suggested that new ischemic lesions were
unlikely to occur after this period of investigation. Animals
receiving orthotopic allotransplantation were killed
30 days after transplantation; grafts were then removed
for evaluation of their potency and final histology. Patency
of tracheal allografts was expressed as a proportional
cross-sectional area (CSA) of the most stenotic site in the
Histopathologic studies. Biopsy and postmortem specimens were immediately fixed in Bouin's solution and in 10% buffered formalin, respectively. After embedding in paraffin, 5 μm thick sections were stained with hematoxylin and eosin and assessed histologically in a blinded fashion. The status of the thyroid gland was evaluated by investigating the presence or absence of follicular atrophy. Vascular patency was evaluated by means of preliminary angiography.

Histologic status of thyrotracheal allograft biopsy samples after heterotopic transplantation (group 1)

Table III. Histologic status of thyrotracheal allograft biopsy samples after heterotopic transplantation (group 1)

<table>
<thead>
<tr>
<th>Pig no.</th>
<th>Day 2</th>
<th>Day 5</th>
<th>Day 7</th>
<th>Trachea</th>
<th>Thyroid</th>
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</thead>
<tbody>
<tr>
<td>40139</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>Normal</td>
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<tr>
<td>40208</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>40391</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>Normal</td>
</tr>
<tr>
<td>40464</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>40422E</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation. 0, Normal tracheal wall; 1, isolated lesions of the epithelium; 2, ischemic necrosis with or without hemorrhage of the lamina propria; 3, ischemic necrosis of the submucosa; 4, ischemic necrosis of the cartilage. *Indicates allograft reversible infection with Streptococcus pyogenes. †Reversible infection with Enterobacter cloaceae.

Histologic status of thyrotracheal allograft biopsy samples after heterotopic transplantation (group 1)

Table III. Histologic status of thyrotracheal allograft biopsy samples after heterotopic transplantation (group 1)

<table>
<thead>
<tr>
<th>Pig no.</th>
<th>Day 2</th>
<th>Day 5</th>
<th>Day 7</th>
<th>Trachea</th>
<th>Thyroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>40139</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>Normal</td>
</tr>
<tr>
<td>40208</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>40391</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>Normal</td>
</tr>
<tr>
<td>40464</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>40422E</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation. 0, Normal tracheal wall; 1, isolated lesions of the epithelium; 2, ischemic necrosis with or without hemorrhage of the lamina propria; 3, ischemic necrosis of the submucosa; 4, ischemic necrosis of the cartilage. *Indicates allograft reversible infection with Streptococcus pyogenes. †Reversible infection with Enterobacter cloaceae.
Table IV. Histologic status of tracheal, esophageal and thyroid allograft biopsy samples after heterotopic transplantation (group 2)

<table>
<thead>
<tr>
<th>Pig no.</th>
<th>Trachea</th>
<th>Esophagus</th>
<th>Trachea</th>
<th>Esophagus</th>
<th>Trachea</th>
<th>Esophagus</th>
<th>Trachea</th>
<th>Esophagus</th>
<th>Thyroid</th>
</tr>
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<tbody>
<tr>
<td>32167</td>
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<td>2</td>
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<td>0</td>
<td>0</td>
<td>Normal</td>
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<tr>
<td>32334</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>32485B</td>
<td>0</td>
<td>0</td>
<td>1*</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>32425B</td>
<td>0</td>
<td>0</td>
<td>0†</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>40138</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>TOTAL</td>
<td>0</td>
<td>0</td>
<td>0.6 ± 0.8</td>
<td>0.4 ± 0.8</td>
<td>0</td>
<td>0</td>
<td>0.2 ± 0.4</td>
<td>0</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation. 0, Normal wall; 1, isolated lesions of the epithelium; 2, ischemic necrosis with or without hemorrhage of the lamina propria; 3, ischemic necrosis of the submucosa; 4, ischemic necrosis of the cartilage (trachea) or muscularis (esophagus).

*Indicates reversible allograft infection with S. pyogenes.
†Reversible infection with Escherichia coli.

isometric recordings and expressed in grams. The maximal responses to carbachol or isoproterenol and the concentration of agonist yielding 50% of maximal response were interpolated from the individual concentration-effect curves. Relaxation was expressed as the percentage of decrease in tension of carbachol-elicited constriction.

Statistical analysis. Data are expressed as mean ± standard deviation or standard error of n observations. Statistical analysis was performed with the Mann-Whitney test or one-way analysis of variance (repeated measures) with Fisher’s protected least significance difference, Sheffe’s F-test, and Dunnett’s t test for multiple comparison. All analyses were performed with a software package (STATISTICA, STATSOFT, Paris, France). The a priori level of statistical significance was accepted at p < 0.05.

Results

All animals survived operation. The original vascular supply always originated from the right inferior thyroid artery. The overall mean ischemic time, tracheal length, esophageal length, and recipient carotid artery clamping time were 183 ± 45.1 minutes, 10.7 ± 2.7 cm, and 13.4 ± 3.6 cm, and 22.1 ± 4.3 minutes, respectively. None of the pigs had injury of either recurrent laryngeal nerve or postoperative neurologic deficits related to carotid artery manipulation. All animals receiving heterotopic transplants were in good condition when they were killed. The first two animals receiving orthotopic transplants died 5 and 2 days after grafting of airway collapse with histologically normal grafts; accordingly, in all subsequent animals a modified silicone T-tube (Axion, Aubagne, France) was inserted into the interposed tracheal lumen at the end of operation and its vertical limb protruded through the cervical wound. The tube was surgically removed a mean of 4.5 ± 1.3 days after transplantation.

Heterotopic transplantation. The overall lengths of the engrafted trachea and esophagus was 12.5 ± 2.7 and 15.8 ± 2.8 cm, respectively. The operative differences between the two groups are listed in Table II. As shown, tracheal grafts belonging to group 2 were significantly longer than those of group 1. All vascular anastomoses were patent at pre-mortem angiography. Graft surveillance showed the following: (1) Exocrine graft function started 2.2 ± 0.4 (group 1) and 1.2 ± 0.4 (group 2) days after transplantation (p = 0.03). (2) In both groups, early ischemic lesions of tracheal grafts were located exclusively at the levels of the tracheocutaneous and tracheoesophagocutaneous anastomoses (Tables III and IV). (3) The overall incidence (n = 7, 35% vs n = 3, 15%; p = 0.15) and severity (0.4 ± 0.5 vs 0.2 ± 0.5; p = 0.26) of ischemic lesions were lower in tracheoesophageal than tracheal allografts. (3) No graft had permanent bacterial colonization. (4) At postmortem evaluation, all thyroid and esophageal grafts appeared histologically normal; all tracheal grafts appeared histologically normal except at the level of tracheocutaneous anastomoses (n = 2) and tracheoesophagocutaneous anastomoses (n = 1) where ischemic lesions of the epithelium were recorded.

Table V. Rejection episodes documented after thyrotacheoesophageal heterotopic allotransplantation (group 2)

<table>
<thead>
<tr>
<th>Tracheal rejection</th>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>9</td>
<td>3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mild</td>
<td>—</td>
<td>8*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Moderate</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Severe</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>TOTAL</td>
<td>9 (46%)</td>
<td>11 (54%)</td>
<td>—</td>
<td>—</td>
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</table>

*Once, a mild rejection of the inferior thyroid artery was associated.
Table VI. Operative profile of pigs undergoing thyrotracheal (group 3) and thyrotracheoesophageal (group 4) orthotopic allotransplantation

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group 3 (n = 5)</th>
<th>Group 4 (n = 5)</th>
<th>p value</th>
<th>Overall (n = 10)</th>
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</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>34.2 ± 3.4</td>
<td>37.5 ± 12.1</td>
<td>NS</td>
<td>35 ± 8.5</td>
</tr>
<tr>
<td>Harvesting time (min)</td>
<td>109 ± 11.9</td>
<td>92.8 ± 2.2</td>
<td>0.07</td>
<td>94.9 ± 25.3</td>
</tr>
<tr>
<td>Ischemic time (min)</td>
<td>176 ± 29.02</td>
<td>251 ± 12.4</td>
<td>0.008</td>
<td>213.5 ± 44.7</td>
</tr>
<tr>
<td>Tracheal length (cm)</td>
<td>9 ± 0.7</td>
<td>9.3 ± 2.1</td>
<td>NS</td>
<td>9.15 ± 1.4</td>
</tr>
<tr>
<td>Esophageal length (cm)</td>
<td>—</td>
<td>10.5 ± 2.16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation. NS, Not significant.

Table VII. Outcome of pigs undergoing thyrotracheal (group 3) and thyrotracheoesophageal (group 4) orthotopic allotransplantation

<table>
<thead>
<tr>
<th>Group</th>
<th>Pig no.</th>
<th>Trachea</th>
<th>Esophagus</th>
<th>Survival (days)</th>
<th>Cause of death</th>
<th>Tracheal status</th>
<th>Stenotic site (%)</th>
<th>Esophageal status</th>
<th>Thyroid status</th>
<th>Rejection</th>
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<tbody>
<tr>
<td>3</td>
<td>40410</td>
<td>9</td>
<td>10</td>
<td>5</td>
<td>AC</td>
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<td>100</td>
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<td>Normal</td>
<td>None</td>
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<tr>
<td>3</td>
<td>40392</td>
<td>8</td>
<td>9</td>
<td>30</td>
<td>K</td>
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<td>None</td>
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<td>9</td>
<td>30</td>
<td>K</td>
<td>Normal</td>
<td>100</td>
<td>Normal</td>
<td>Trachea (mild) None</td>
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<tr>
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<td>9</td>
<td>10</td>
<td>30</td>
<td>K</td>
<td>Normal</td>
<td>Distal anastomosis 91</td>
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<td>Trachea (mild) None</td>
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AC, Airway collapse; K, killed; TE, tracheoesophageal.

*Percentage of patency of the tracheal graft was expressed as proportional cross-sectional area of the most stenotic site in the graft to the third ring below the distal anastomosis of the recipient's trachea.

Immunehistologically confirmed mild rejections were diagnosed in 20 (33%) of 60 tracheal (n = 40) and esophageal (n = 20) biopsy specimens obtained. Nine rejections (45%) occurred in the 20 tracheal biopsy specimens from pigs belonging to group 1; one of these also had a mild vascular rejection. Eleven rejections occurred in tracheoesophageal allografts (Table V), eight (8/20, 40%) occurred synchronously in the tracheal and esophageal grafts, and three were dyssynchronous esophageal rejection only. The residual levels of cyclosporine were not significantly different (p = 0.2) between groups 1 (243.04 ± 119.75 μg/ml) and 2 (204.4 ± 112.2 μg/ml). The blood CD4+/CD8+ lymphocyte ratio was significantly higher (p = 0.01) in pigs with 0.876 ± 0.1 than without 0.57 ± 0.16 rejection.

Orthotopic transplantation. The overall lengths of the engrafted trachea and esophagus were 9.1 ± 1.4 and 10.5 ± 2.1 cm, respectively (Table VI). The outcomes of these two types of allotransplantation are depicted in Table VII. Except for the two pigs that died of airway collapse, these animals underwent open tracheal graft biopsy at the time of removal of the T-tube, and all showed histologically normal tracheal walls. All vascular anastomoses were patent when the animals were killed. All tracheal and esophageal grafts were viable and histologically intact for their entire length 30 days after transplantation (Fig. 2). Only one tracheal graft showed a clinically asymptomatic stenosis of the distal site of the tracheal anastomosis. Maximal smooth muscle contraction and relaxation responses did not differ among transplanted, native, and control tracheas (Fig. 3).

Mild rejections were diagnosed in two tracheal and three tracheoesophageal grafts; there was one mild vascular rejection in each group. The residual levels of cyclosporine did not differ (p = 0.5) between groups 3 (215.2 ± 69.2 μg/ml) and 4 (270 ± 159.4 μg/ml).

Discussion

Presently available techniques of circumferential resection and primary reconstruction have greatly expanded the indications for operation and dramatically improved the quality of survival for both
children and adults with benign and malignant diseases involving up to 50% of tracheal length.\textsuperscript{6,7} Unfortunately, many patients have more extensive diseases, for which complex operations are currently under investigation\textsuperscript{8} or surgery is contraindicated because the length of the residual trachea would not permit primary reconstruction.\textsuperscript{9} To maintain tracheal patency, these long-segment diseases necessitate repeated dilation, tracheal T-tubes, or a definitive tracheostomy, all procedures associated with some degree of compromise of normal respiration, deglutition, or phonation and social acceptability.

Because of this problem, and encouraged by the success of transplantation efforts with other organs, many investigators have evaluated replacement of the tracheal conduit. Tracheal substitution grafts have taken three forms: (1) not revascularized,\textsuperscript{11-18} (2) indirectly revascularized,\textsuperscript{19-22} and (3) directly revascularized.\textsuperscript{23} Except for the study of Khalil-Marzouk,\textsuperscript{23} these attempts had disappointing results; all long-segment tracheal grafts transplanted without their vascular pedicles became ischemic, necrotic, and infected. We recently detailed a surgical technique for direct revascularization of unpreserved long segments of tracheal allograft in immunosuppressed pigs.\textsuperscript{2} Revascularization included interposition of the donor's subclavian artery (incorporating the origin of the inferior thyroid artery) to the recipient's subclavian artery and a single cava-caval anastomosis; grafts were then implanted heterotopically into the neck. Unlike the study of Khalil-Marzouk,\textsuperscript{23} our results demonstrated that the thyrotracheal allograft's viability depended on both arterial and venous revascularization and that early ischemic lesions, located mainly in the posterior tracheal wall, disappeared after epithelial regeneration. In this study, we have investigated how to (1) reduce the early allograft ischemic phenomena by developing a technique of allograft preservation before extraction, (2) increase the vascular supply to the posterior tracheal wall, and (3) simplify our original demanding technique of allograft revascularization.

This technique of thyrotracheoesophageal perfusion was extrapolated from that described by LoCicero and colleagues\textsuperscript{24} for perfusion of the bronchial circulation in donor lung procurement. We postulated that the thyrotracheoesophageal circulation could be accessed by clamping the ascending and distal thoracic aorta beyond the origin of the bronchial artery and by infusing, after distal ligation of all cervical vessels, the perfusate into a closed aortic segment incorporating the origins of the innominate and left subclavian arteries. With this technique, the perfusate circulates selectively through the right and left carotid, subclavian, and inferior thyroid arteries; the thyroid gland; the anterolateral surface of the cervicothoracic trachea up to the carina; the esophageal arteries vascularizing the posterior tracheal wall and esophagus; and the contralateral anterolateral surface of the tracheal allograft, finally flushing through the descending cervical veins into the AVC. As a result of perfusion with EC through this tracheal circulation, all group 1 grafts appeared histologically normal for their entire length during the period of investigation, except for scattered areas of ischemia. These ischemic areas were located exclusively at the level of
Fig. 3. A, Contractile responses to increasing doses of carbachol in tracheal smooth muscle rings from control tracheas (9.9 ± 0.7 gm), native tracheas (11.4 ± 1.5 gm) and transplanted grafts (11.3 ± 2.6 gm; p not significant). Fifty percent effective values were also similar: control (0.87 × 10⁻⁶ ± 0.76); native (0.67 × 10⁻⁶ ± 0.3), and transplanted (1.4 × 10⁻⁶ ± 0.9). (B) Relaxation of tracheal smooth muscle rings precontracted with carbachol and stimulated with increasing doses of isoprenaline. The relaxation responses are presented for control (91.7% ± 7.8%), native (91.6% ± 13.2%), and transplanted (64% ± 12%; p not significant). Each transplanted, native, and control group includes eight samples. Control tracheas were freshly obtained from other experimental procedures performed at our laboratory.

Fig. 4. Drawing of the vascularization of the posterior tracheal wall by small esophageal arteries.

the tracheocutaneous anastomosis and probably were related to formation of fibrotic tissue. This demonstrates that allograft perfusion with a preservative solution before extraction minimizes the ischemia-reperfusion injury at the level of the airway epithelium observable in unperfused tracheal allografts. Another piece of indirect evidence supporting this conjecture is that the incidence of histologically documented rejection in this group was rather high (45%); this could be related to excellent preservation of the graft mucosae and submucosae, where experimental and clinical studies have demonstrated that major histocompatibility complex class II antigens, the trigger cells of alloimmune response, are regularly distributed. This hypothesis is also offered as a clarification for the extremely low incidence (0.3%) of graft rejection observed in our previous work, in which more frequent and severe ischemic lesions of the mucosae and submucosae may have suppressed the expression of class II major histocompatibility complex molecules.

Simultaneous thyrotracheoesophageal transplantation resulted in an amelioration of the tracheal vascularization compared with thyrotracheal transplantation alone, but the small sample size may have precluded statistical significance. Nevertheless, the fact that tracheal grafts belonging to group 2 had, despite their significantly longer length, almost twofold lower incidence and severity of ischemic lesions and significantly earlier exocrine function than did group 1 grafts suggests that the esophagus increases the vascular network of the trachea by supplying important nutrients to the posterior tracheal wall (Fig. 4), intratracheal airway epithelium, and walls...
of the "tracheal" arteries and veins. It is noteworthy that this increased tracheal vascularization was obtained without major immunologic repercussions related to the combined thyrotracheal and esophageal transplantation. It is well known that two organs may be more immunogenic than a single one because of the increased lymphatic mass transplanted, and previous clinical and experimental studies have provided evidence for this assumption. Our results showed no significant differences, however, at similar cyclosporine levels, in the incidence and severity of tracheal graft rejection between either form of transplantation. This suggests that the engrafted esophagus does not increase the immunologic response of the recipient. Interestingly, there were three dysynchronous rejections; we speculate that factors other than major histocompatibility complex alloantigen expression predispose the alloimmune response to a greater degree in one organ than the other of a pair.

In our previous model, allograft revascularization was made by interposing the donor's subclavian artery to the recipient's subclavian artery. This was a technically demanding procedure that included an upper sternotomy, dissection of the anterosuperior mediastinum and thoracic inlet, manipulation of the phrenic and vagus nerves and parietal pleurae, and ligation of almost all branches of the recipient's subclavian artery. Clinical experience with tracheal surgery has clearly demonstrated that the cervical trachea and upper two thirds of the mediastinal trachea can be managed through a simple collar incision into the neck. Consequently, we have developed this implantation technique in which the donor's subclavian artery, incorporating the inferior thyroid artery, is interposed between the right common artery after a simple cervical incision and dissection. This not only reduces the ischemic time but greatly facilitates the performance of the vascular anastomoses and avoids sacrifice of important subclavian arterial branches, including the vertebral artery. Moreover, the venous return is made by a simple anastomosis between the donor's AVC and the external jugular vein, which in pigs has a greater size than the internal jugular vein; this avoids cross-clamping of the brachiocephalic confluence and thus eliminates potential risks inherent in the reduction of venous return.

On the basis of our experience with the heterotopic groups, we performed orthotopic transplantation of long-segment tracheal allografts, either alone or in combination with the esophagus. The first two pigs in both groups had early expiratory dyspnea and so were killed immediately. Their tracheal grafts appeared normal at pathologic examination. The only plausible explanation for this observation was that the long-segment tracheal grafts were less rigid than usual and tended to collapse, producing airway obstruction during expiration. Consequently, a temporary stenting of the airway was inserted, and none of the pigs had airway obstruction thereafter. All grafts were histologically viable when the animals were killed, none had airway bacterial colonization, and tracheal smooth muscle function was well preserved. This and the fact that only one had stenosis or granulation formation at the anastomotic sites further support the belief that grafts were adequately perfused during the period of investigation. One could argue that the operative hazards of adding two esophageal anastomoses to the tracheal transplantation could be obviated by simply transplanting along with the trachea the entire or to a lesser degree only the anterior muscular wall of the esophagus, denuded of its mucosa. Although we have no evidence to confirm this hypothesis, its clinical relevance is evident, and we believe that this method would probably be equally effective in preserving the blood supply to the posterior wall of the trachea.

In conclusion, our presented results clearly demonstrate that one of the major concerns of tracheal transplantation, the surgical mechanisms leading to revascularization, has been resolved. By applying our harvesting and implantation techniques, long segments of tracheal allografts can be successfully engrafted with their own vascular pedicles. Although the underlying pathophysiologic mechanisms are different, this experience suggests that donor pretreatment with EC and combined thyrotracheoesophageal (without increasing recipient alloimmune response) transplantation are equally effective in reducing airway ischemia after transplantation. Orthotopic transplantation of long tracheal segments necessitates temporary stabilization of the tracheal wall to prevent airway collapse. However, two other major areas of concern must still be addressed before human tracheal transplantation can be considered feasible in clinical practice. These are (1) prevention of host rejection and (2) justification.

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REFERENCES