EXPERIMENTAL TRACHEAL ALLOGRAFT REVASCULARIZATION AND TRANSPLANTATION

The feasibility of tracheal allotransplantation with a fascial vascular carrier was examined in three groups with varied dose sequences of immunosuppression. A control group (group 1) received no medication. The three experimental groups were given daily cyclosporine intramuscular doses of 5 mg/kg (group 2), 5 mg/kg plus 3 mg/kg methylprednisolone (Solu-Medrol) (group 3), and 10 mg/kg (group 4) for 6 weeks or until death. Grafts were assessed by silicone dye infusion of the artery of the fascial flap to examine their microcirculation and by quantitative histologic study. Group 1 evidenced complete rejection after a heterotopic revascularization period of 14 days. The allografts of the experimental groups remained viable after 14 days of revascularization and could be transplanted orthotopically after this period. After transplantation, the viability of group 2 tracheas was unpredictable with changes ranging from mild to complete rejection. Group 3 evidenced well-preserved transplant viability with infection-induced necrosis at the anastomoses caused by the corticosteroid component. All group 4 animals survived the follow-up period with normal allograft viability. Cyclosporine in a dosage of 10 mg/kg per day can effectively suppress the immune response after transplantation of vascularized tracheal allografts. This experimental model will allow future studies to examine airway wall immunogenicity. (*J THORAC CARDIOVASC SURG* 1995;110:728-37)

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Although more than 50% of the trachea may be resected with direct end-to-end reconstruction, the occasional occurrence of extensive disease has led to continued interest in tracheal replacement, by means of either a prosthesis or an allotransplant. The major obstacles to successful clinical transplantation of the trachea have been the immunogenicity of the tracheal wall and the restoration of its blood supply.

Experimental transplantation models may be use-

ful to study both revascularization and rejection. However, the distinction between avascular and rejection necrosis in these models is possible only if the blood supply to the transplant is fully restored. Because direct revascularization of tracheal vessels can be expected to be associated with significant technical problems, indirect revascularization by a pedicled flap of omentum or muscle has been used.

The omentum has been used intensively in experimental tracheal revascularization with different results. Both orthotopic and heterotopic revascularization studies with omentum are available. These two different revascularization models may in part be responsible for the confusion around the possibility of tracheal revascularization.

The majority of the studies on tracheal revascularization were done orthotopically with tracheal autografts that were isolated and immediately reimplanted wrapped by the transposed omentum.¹⁻³ Consequently, the blood supply of the grafts was derived from two sources, the neovascularity from the recipient trachea at both ends of the graft and the supply from the omentum. From these studies, it seemed that only the synergistic effects of the two

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supplies succeeded in preserving the viability of the graft at both ends near the anastomosis. The mid-portion of the autograft failed to receive the synergistic effect and became necrotic.

Another possibility is to study heterotopic tracheal revascularization.^{4,5} These grafts are completely immobile during revascularization and are not exposed to the airway during mucosal regeneration and give better angiogenetic results compared with orthotopic revascularization. A disadvantage is that only short follow-up times may be studied. Mucosal overgrowth at both ends of the graft and mucus accumulation will result in intraluminal infection early after heterotopic transplantation.

We developed an experimental model that combined the advantages of both orthotopic and heterotopic revascularization without the disadvantages of the two locations. A previous animal study on tracheal autograft revascularization showed that a large arteriovenous bundle and its surrounding vascularized island of fascia can be induced to perfuse a heterotopic transplanted trachea and that this allows its orthotopic transfer as a prefabricated flap.⁶ Tracheal revascularization occurred to a greater extent by angiogenesis from the fascia through the membranous part in a circumferential, posterior-anterior direction and to a lesser extent through the intercartilaginous ligaments. The versatility of the model allowed for the step-by-step evaluation of the revascularization process of heterotopic transplanted tracheal autografts. It was established that revascularization and epithelial regeneration are concomitant processes. The timetable of the tracheal revascularization process was investigated and an optimal revascularization period was determined to be between 14 and 20 days. Mucosal disintegration was seen if the grafts were isolated for a longer period than the optimal revascularization period because of stagnation of respiratory secretions and intraluminal infection. At the optimal time the revascularized segments were orthotopically transplanted on the fascial vascular pedicle.⁷

Encouraged by the data on autotransplantation we wanted to investigate the feasibility of tracheal orthotopic allotransplantation with the lateral thoracic fascial flap used as a vascular carrier. The first objective of this study was to evaluate the timetable of acute tracheal rejection of unprotected rabbit allografts. The second objective was to develop an apparently reliable form of experimental tracheal transplantation by the combined use of heterotopic

and orthotopic graft implantation made possible by vascular ingrowth from a fascial flap. The minimal immunosuppressive dose that allows for long-term experimental tracheal allotransplantation in the orthotopic position was determined. This model will allow future studies to examine reliably factors that affect graft rejection.

Material and methods

The experimental animals used were New Zealand White rabbits and Dutch belted rabbits (weight 3000 gm). This combination provides a strong histocompatibility barrier. Dutch belted rabbits served as donors and New Zealand White rabbits were the recipients. Thirty-seven rabbits were used. Sixteen tracheal allografts were studied in the control group. Twenty-one animals randomly divided into three groups ($N = 7$) and having different immunosuppressive protocols underwent tracheal allotransplantation in the experimental group.

First stage: heterotopic fascia revascularization. Anesthesia was induced in the donor and recipient rabbits with intravenous pentobarbital and maintained with inhaled halothane and oxygen through mask ventilation. At the recipient site, the left thoracic fascia was isolated on the lateral thoracic vessels. The vascular anatomy and dissection technique of this subcutaneously, axially perfused sheet of fascia have been described previously.⁶ At the donor site a cervical tracheal allograft was excised through a midline incision in the neck. The trachea was divided five rings caudally to the larynx and a segment of 2.5 cm was resected. On the outside, the graft was cleared from most of the connective tissue and the tracheal lumen was flushed with 10 ml of sterile saline solution. The tracheal allograft was transferred to the dissected lateral thoracic fascial flap of the recipient animal. The donor animal was killed with an overdose of pentobarbital sodium (Nembutal) (100 mg/kg intravenously). The tracheal graft was brought over the dissected fascial flap and was stretched to its original length (2.5 cm) by attachment to the underlying thoracic muscles with Vicryl polyglactin 5/0 (Ethicon, Inc., Somerville, N.J.) sutures to avoid shrinkage of the allograft (Fig. 1). The proximal end of the transplant was located at the medial side of the flap. The tracheal segment was wrapped in the vascularized fascia and then secured with polypropylene stitches. The lateral thoracic skin was closed with interrupted sutures.

Histomorphometry of the allografts in the control group ($N = 16$). To study the timetable of tracheal rejection, every second day after heterotopic transplantation from day 2 to day 16 two rabbits were killed. After the follow-up period, the rabbits were premedicated and anesthetized. The left axillary region was opened and the left thoracic artery of the fascial flap was flushed through the cannula with 10 ml of heparinized, normal saline solution (10 IU of heparin per milliliter) followed by 5 ml of blue silicone dye (Microfil, Canton Bio-Medical Products Inc., Boulder, Colo.). This material formed a cast of the vascular architecture of the fascial flap and the established vascular connections with the tracheal allograft. After these injections were completed, the tracheal segments were re-

Fig. 1. Schematic presentation of orthotopic tracheal transplantation. 1, Tracheal allograft, stretched to its original length (2.5 cm), is wrapped by fascial flap for heterotopic revascularization; 2, resection of 2 cm segment of native trachea; 3, transplantation of 2 cm revascularized allograft segment by transposition of pedicled fascial flap after 14-day revascularization period.

moved and fixed in 10% neutral buffered formalin solution for light microscopy. Two representative cross sections were taken from the middle of each allograft. The tissues were embedded in paraffin and 4 μ m thick cross sections were cut and stained with hematoxylin-eosin (H&E) stain.

Reperfusion of the allograft after 2, 4, 6, 10, 12, 14, and 16 days was assessed histomorphometrically by the distribution of blood vessels in the lamina propria of the tracheal segments that were perfused with blue silicone dye. Revascularization occurred in a continuous, circumferential posterior-anterior direction and was expressed as a percentage of the total tracheal circumference showing reperfusion. The values of the two cross sections in the two animals for each even day after unprotected revascularization were added and averaged.

The amounts of squamoid and ciliated epithelium were scanned on the histologic slides and presented as a mean percentage and standard error of the total tracheal circumference.

Intermediate angiographic studies in the experimental group. The tracheal viability after a revascularization period of 14 days was investigated in two animals of groups 2, 3, and 4. The rabbits were anesthetized, the left axillary region was opened, and the left lateral thoracic artery was injected with 5 ml of blue silicone dye. The tracheal segments were removed and fixed in 10% neutral buffered formalin solution for light microscopy.

Second stage: orthotopic transplantation. Five animals of each experimental group underwent orthotopic transplantation after the 14-day revascularization period. The tracheal segment and its surrounding fascia were resected and the composite island flap pedicled on the lateral thoracic vessels was subcutaneously rotated to the neck region. The mucosal overgrowth at both ends of the allograft was removed and the remaining tracheal allograft of 2 cm length was orthotopically transplanted after resection of a 2 cm long segment of native trachea (Fig. 1). The transplant was oriented in its original proximal-distal direction to allow for mucociliary clearance over the transplanted region. The upper transplant anastomosis was done after some sutures were placed on the posterior wall of the lower anastomosis. The transplantation was completed with closure of the anterior lower anastomosis with interrupted polypropylene (Prolene, Ethicon) sutures and closure of the skin incision. The animals breathed spontaneously during the entire operation. Antibiotics were administered for 4 postoperative days in both operation stages. Rabbits were housed in separate quarters in an environment-controlled facility and fed a standard rabbit diet.

Cyclosporine (Sandimmune, Sandoz) for intravenous infusion was diluted to 10 mg/ml. The three experimental groups were given cyclosporin A intramuscularly in a daily dose of 5 mg/kg (group 2), 5 mg/kg plus methylprednisolone (Solu-Medrol) 3 mg/kg (group 3), and 10 mg/kg (group 4), beginning the day of initial graft transfer. After the orthotopic transplantation the animals were followed up for 4 weeks or until death. After the follow-up period, the rabbits were premedicated and anesthetized with pentobarbital sodium (40 mg/kg intraperitoneally). The lateral thoracic artery of the fascial flap was exposed and cannulated with a 14-gauge catheter and perfused with blue silicone rubber at a pressure of 150 mm Hg. Viability was assessed with both clinical and histologic parameters on the injected tracheal specimen. The same injection technique was used immediately after death in the animals that died before the end of the follow-up period. In the animals that survived for 4 weeks, a high-resolution gadolinium-pentetic acid enhanced T1-weighted spin echo sequence was used before the animals were killed to obtain a magnetic resonance imaging (MRI) study in the coronal, sagittal, and axial planes from the transplanted region.

All animals received humane 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"

Fig. 2. Allograft revascularization, squamoid epithelium, and ciliated epithelium scores during heterotopic revascularization in control group. Allograft revascularization score acutely decreased after 10 days and after initial progressive reperfusion. Epithelial regeneration from squamoid to ciliated cells was seen with progressive vascularization. Ischemic mucosal degeneration evolved from ciliated cells over squamoid cells and basal cells to completely denuded avascular submucosa. Revascularization with mucosal regeneration and rejection-mediated cessation of microcirculation with mucosal dedifferentiation were significantly linked. Rejection process terminated when submucosal capillaries of graft involuted and could no longer deliver fresh immune cells.

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Results

Control group ($N = 14$). Results are shown in Fig. 2 and Fig. 3, *A*, *B*, *C*, and *D*.

Mucosal revascularization. Submucosal thrombosis was noticed in the grafts at 2 days. Revascularization started after 4 days in the membranous region and evolved in a circumferential posterior-anterior direction from day 4 to day 10. A progressive mononuclear cell migration into the lamina propria was seen from day 6 on. After 8 days, areas of revascularized blood vessels displayed a mononuclear vasculitis with reactive intimal changes. After 10 days, the majority of the vessels were thrombosed. After 12 days, no flow and no silicone dye were observed in the submucosa of any of the grafts and mononuclear cell infiltration decreased rapidly within the avascular submucosa.

Mucosal regeneration. From day 4 on, a squamoid epithelial recovery was seen in the posterior region preceding the revascularization process by 2 days. The squamoid epithelium recovered from the basal cells of the respiratory epithelium that were most resistant against ischemia. A differentiation of squa-

moid to ciliated epithelium was seen with progressive revascularization. The mucosal regeneration and differentiation evolved, like the revascularization process, in a circumferential posterior-anterior direction. A breakpoint was reached after 10 days. A mucosal degeneration was seen in the allografts with progressive damage of the microcirculation. The ciliated epithelium curve followed the downward slope of the vascularization curve with ischemic metaplasia of ciliated cells to squamoid cells. The avascular submucosa after 12 days was lined with squamoid and basal cells that completely disappeared after 14 days. Mucosal regeneration and degeneration were dependent on the condition of the submucosal microcirculation and were not directly damaged by the cell-mediated rejection process.

Cartilage component. Histologic changes similar to those in the submucosal microcirculation were observed at the external part of the cartilage rings in the small contact area with the fascial flap. The peritracheal connective tissue between cartilage and the fascia was repopulated by blood vessels from day 2 to day 10. Mononuclear cell invasion around the regenerated blood vessels, followed by vasculitis and thrombosis, occurred from day 10 to day 14. After 14 days, a fibrous, avascular connective tissue band

Fig. 3. Photomicrographs of allograft revascularization and rejection in group 1: rejection controls. **A**, Revascularization of tracheal submucosal thrombosis (H&E stain, original magnification $\times 25$). Progressive thrombus revascularization occurred from left thrombus (2: located posteriorly) to right thrombus (1: located anteriorly). Arrow indicates posterior (P) to anterior (A) direction of graft. Right thrombus is populated by silicone spots (small arrows) without apparent endothelial lining. Endothelial cells or subendothelial cells (visible as black spots) are located in thrombus. Silicone dye-injected vessels are coalesced and obtained endothelial lining from donor origin in left thrombus (asterisks). Thrombus undergoes further resorption with progressive reperfusion and vessel with magnitude similar to thrombosed vessel will ultimately be formed. Mucosal lining is ciliar (ce) over left thrombus and squamoid (se) over right thrombus, indicating concomitant mucosal-vascular regeneration evolving in posterior-anterior direction. TL, Tracheal lumen. **B**, Photomicrograph of allograft revascularization and rejection after 8 days (H&E stain, original magnification $\times 25$) shows well-defined perivascular mononuclear cell infiltration on both sides of cartilage graft (white arrows). F, Fascia; TL, tracheal lumen. **C**, Photomicrograph of allograft after 12 days of revascularization (H&E stain, original magnification $\times 25$). Cessation of submucosal microcirculation with fibrinoid material is seen within vessels (arrows). Epithelial lining shows ischemic degeneration to monocellular layer. Cell-mediated rejection is not completely extinguished at external side of tracheal cartilage (asterisk) after 12 days. F, Fascia; TL, tracheal lumen. **D**, Photomicrograph of allograft after 16 days of revascularization (H&E stain, original magnification $\times 25$). End stage of tracheal rejection with cartilage allograft surrounded by two nearly avascular bands (both double arrows). Lamina propria contains vasculonecrotic lesions with significant fibrinoid material. Epithelial lining has completely disappeared. Cartilage allograft and fascia (F) are separated by fibrous connective tissue (lower double arrows). Cartilage component shows early signs of ischemic necrosis with eosinophilic ground substance and resorption. TL, Tracheal lumen.

isolated the cartilage graft from the fascia. Early signs of avascular necrosis of the cartilage component with eosinophilic changes of the ground substance were observed after 16 days. The end stage was a cartilage transplant surrounded by avascular fibrous tissue at both the submucosal and fascial side.

Experimental group (N = 21)

Intermediate angiographic studies. The viability of the tracheal transplant after a revascularization period of 14 days was preserved in each of the three groups without major differences among the groups. The submucosal layer was completely revascularized and the segments were lined with respiratory epithelium. Some graft-invading cells consisting primarily of lymphocytes and macrophages were seen at both sides of the cartilage rings. The cartilage component had a normal histologic appearance.

Graft status after transplantation

GROUP 2. Three animals did not survive the follow-up period and died 12, 15, and 17 days, respectively, after transplantation of respiratory distress. Postmortem macroscopic evaluation displayed complete necrosis of the transplant mucosa. There was an abrupt transition area between the necrotic transplant and the native trachea without apparent problems at the anastomosis (Fig. 4, A). Histologic evaluation showed a complete rejection of the donor tissues with full-thickness avascular necrosis of the tracheal wall.

Two animals survived the follow-up period. Apart from a more pronounced mononuclear cell infiltration in the lamina propria, these two animals displayed the macroscopic and histologic pattern of the group 4 animals.

GROUP 3. The mean survival period of the five group 3 animals after orthotopic transplantation was 6 days. They died of considerable neck infection in combination with infection-induced necrosis at the upper and lower transplant anastomoses. On macroscopic evaluation, the transplants had a normal viability. The mucosal layer was completely colored by the blue silicone dye but necrosis at both anastomoses led to tracheal obstruction (Fig. 4, B).

On histologic examination, it was shown that the fascial wrap protected the transplant from the infectious process. Polynuclear cells were visible at the outside of the fascia. No fascial protection was available at both anastomoses that were damaged by the infection in the neck. The middle part of the transplant was completely revascularized and lined with ciliated epithelium.

GROUP 4. All the animals survived the follow-up period without respiratory problems. After 4 weeks

the animals were anesthetized and an MRI study was obtained with a gadolinium-pentetic acid enhanced T1-weighted spin echo sequence.

The transplanted and the native tracheas had comparable airway lumens (Fig. 5, A). On the axial image, the mucosal lining and the revascularized lamina propria of the transplant were visible as an enhancing inner circle that bordered the tracheal lumen. The fascial wrap was clearly visible as an enhanced outer circle around the tracheal transplant (Fig. 5, B).

Macroscopic evaluation of longitudinal sections displayed an intact mucosal lining in the area of the transplant-native trachea anastomosis. A sharp demarcation was found between the native tracheal epithelium and the transplant mucosa injected with the blue silicone dye. A lower density of silicone-injected blood vessels was present in the native tracheal submucosa near the transplant anastomosis, indicating the neovascular capacity of the tracheal transplant (Fig. 4, C).

The histologic features of the transplant were similar to those of the findings on intermediate angiographic studies. The difference between transplant and native tracheal morphologic features was seen at the anastomotic region. The transplanted trachea could be distinguished by the silicone-injected submucosal vessels and the slight lymphocytic infiltration of the lamina propria (Fig. 5, C).

Discussion

The results in the control group showed that the trachea is an organ subject to the same immunologic laws as all other allogeneic tissues. The most important component in tracheal rejection appears to be cell-mediated rejection and the presumed prime target cell population is allograft endothelium. Microvascular thrombosis is the end point in acute tracheal rejection 10 days after initial progressive revascularization and mucosal regeneration.

The mucosal rejection process is related to the submucosal microcirculation. Mononuclear cells, provided by the revascularized submucosal blood vessels, attack the lamina propria and destroy the vessel wall with thrombosis and blockage of the microcirculation and subsequent mucosal degeneration.

The cartilaginous component of the allograft is rejected, like the epithelial cells, as a result of vascular changes. The cell-mediated vascular rejection occurs at both sides of the cartilage component. The revascularized connective tissue between cartilage and fascial flap displayed microvascular rejection

Fig. 4. Macroscopic view of tracheal transplant anastomosis. **A**, Group 2 animal. Abrupt transition area is seen between necrotic allograft mucosa and normal native tracheal mucosa. No granulation tissue has formed at anastomosis. Fascial wrap around transplant (*arrow*) is injected with silicone dye. This macroscopic picture corresponds with complete necrosis induced by chronic rejection of initially revascularized graft. **B**, Group 3 animal. Internal lining of transplant is colored by silicone dye, indicating normal mucosal viability. Necrosis is seen at anastomosis, an area without fascial protection. Growth of blood vessels from transplanted to native trachea is prevented by infectious disruption at anastomosis. **C**, Group 4 animal. Viable transplant mucosa (colored with blue silicone dye) with completely healed anastomosis (*arrows*). Silicone-injected blood vessels, induced by fascial flap, are visible on native tracheal mucosa.

Fig. 5. Radiologic and histologic evaluation of group 4 allografts. **A**, Coronal T1-weighted spin echo MRI section through tracheal transplant (gadolinium-enhanced) shows upper (*U*) and lower (*L*) transplant anastomoses. Transplanted region (2 cm) represents majority of cervical trachea. **B**, Axial enhanced T1-weighted spin echo MRI demonstrates revascularized tracheal segment with fascial wrap (*F*). Tracheal lumen is lined by hyperintense ring representing revascularized mucosa. **C**, Photomicrograph of *outlined segment* on **A** (H&E stain, original magnification $\times 10$). Transplant anastomosis reveals difference between native and transplanted trachea. Dense, silicone-injected submucosa and lymphocytic infiltration of lamina propria allow for identification of transplanted allograft. Mononuclear cells are present at both sides of cartilage in transplanted segment. *A*, Allograft; *N*, native trachea; *F*, fascia. *Arrow* indicates polypropylene suture.

signs similar to those of the tracheal submucosa with mononuclear vasculitis and thrombosis. The immunologically induced avascularity around the cartilage rings isolates the cartilaginous component in a fibrous cocoon with problematic metabolic diffusion. Cartilaginous ischemic necrosis results from pericartilaginous avascularity.

This observation suggests that the endothelium of the revascularized submucosal and pericartilaginous blood vessels is the site of the prime manifestation of the rejection process. The endothelial cells of the capillary network, at both sides of the tracheal cartilage, are derived from donor vascular endothelial cells. The donor endothelial cells grow into the thrombosed vessels after graft isolation and relined the revascularized thrombi, a phenomenon induced by the fascial wrap. This explains the allogeneic perivasculitis of the regenerated microcirculation.

Heterotopic revascularized tracheal allografts may successfully be transplanted only when protected by immunosuppressive drugs. The immunosuppressive dosages in the three groups were chosen to conform with immunosuppressive protocols used in other experimental transplantation studies.⁸ Cyclosporine at 10 mg/kg was clearly capable of preserving the viability of transplanted tracheas with normal healing of anastomoses. Cyclosporine administered at 5 mg/kg resulted in differing histologic patterns of rejection after transplantation ranging from moderate to complete rejection. The association with methylprednisolone in group 3 animals seemed to successfully suppress the immune response but was responsible for the infection seen at the operation wounds, which led to airway problems at the anastomoses.

Vascularized fascia is as effective as the omentum in the revascularization of airway segments. Possibly, the nature of the tissue used as a vascular carrier is less important. Of major importance is that the vascular carrier have axial perfusion and a reliable and easily transferable vascular pedicle. For tracheal transplantation, one needs a vascular carrier with as little bulk as possible to adapt the flap within the small tracheoesophageal septum. For this purpose, the use of a vascularized sheet of fascia seems useful.

A reliable vascular carrier and a heterotopic revascularization stage form the keystone for successful orthotopic transplantation. Tracheal grafts are revascularized through the membranous and intercartilaginous ligaments. They are revascularized through the same mechanisms as skin grafts

with an additional obstacle formed by the cartilage rings.⁶ For optimal revascularization, it is of utmost importance to have a close and immobile contact between the graft and vascular bed, and this cannot be fulfilled in an orthotopic position in which the graft moves with each respiration and swallowing act. The heterotopically located transplants are revascularized equally over the total fascia-enwrapped area without restriction on the length of the transplant.

This model may become important in the study of the morphologic and pathophysiologic features of airway wall rejection. Because understanding the pathophysiologic process of airway wall rejection is largely dependent on animal models, it is important to consider the potential shortcomings of the various laryngotracheal rejection models. Larynx and trachea contain mucosa and cartilage and may both be used to study the immunogenetic requirements for airway wall rejection. Heterotopic laryngeal⁸ and tracheal transplant⁹ models were introduced and have been used successfully to study the morphologic basis of acute rejection. With a heterotopic laryngeal transplantation model it was concluded that cyclosporine at 7.5 mg/kg and 10 mg/kg was capable of preserving the viability of transplanted larynges. In a study on rat tracheal allografts that were heterotopically revascularized by omental wrapping, it was shown that tracheal transplant viability was improved with an optimum combination of cyclosporine and methylprednisolone.⁹ The accumulation of mucus secretions in the aerodynamic afunctional larynx or trachea is, however, an important factor that enhances the development of graft infection and graft necrosis.

Orthotopic laryngeal transplantation and orthotopic tracheal revascularization are problematic for different reasons. A denervated larynx will result in intractable aspiration and orthotopic tracheal revascularization will result in unpredictable and insufficient viability.¹⁻³ Orthotopic tracheal revascularization has some disadvantages that can influence the revascularization process. The shearing movements of respiration and swallowing prevent close adhesion between graft and vascular bed and the regenerating, fragile mucosal component is exposed to respiration during early revascularization.

There are reports of successful transplantation of five-ring segments in dogs with administration of the immunosuppressant FK 506¹⁰ or after 10,000 cGy irradiation of the allograft.¹¹ Orthotopic revascular-

ization, however, seems length dependent, which has led to the statement that tracheal grafts are revascularized from their ends rather than from their midportion.³

With a combined model of tracheal heterotopic revascularization followed by orthotopic transplantation it is possible to investigate the union between donor and recipient tracheas and the histologic pathologic conditions can be related to graft function within the respiratory process. Another advantage of this transplantation model is that it allows for the study of allograft rejection focused on the microcirculation of the transplant. The vascular pedicle of the transplant is provided by the receptor without the possibility of complications related to vascularity that might interfere with the rejection process, such as thrombosis of the vascular pedicle anastomosis.

Tracheal orthotopic transplantation after fascial revascularization will further be used to test hypotheses regarding host immune tolerance, tissue preservation, and pharmacologic agents.

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