Successful Tracheal Transplantation with Fresh Allografts in a Rabbit Model

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Abstract

Purpose. Immunogenicity and the restoration of blood supply to the donor graft remains a clinical challenge in tracheal allotransplantation. We conducted a study on 20 rabbits of a genetically similar strain to eliminate the risk of rejection caused by immunogenicity.

Methods. We examined the histomorphological changes related to revascularization and the immunogenetic reaction of the fresh allografts after tracheal transplantation. Histomorphological assessment was conducted by investigating the anastomotic sites, graft necrosis, and epithelization. Cellular changes, including the infiltration of granulocytes, histiocytes, and fibroblast proliferation related to a granulation tissue-like reaction, were also assessed, with lymphocyte infiltration which is an indicator of graft rejection. All of these characteristics, apart from epithelization, were graded semiquantitatively as none (0), mild (1), moderate (2), and severe (3). Epithelization was graded as 0, indicating no epithelization; 1, ≤20%; 2, ≤40%; 3, ≤60%; 4, ≤80; 5, complete epithelization of the entire graft.

Results. Morphologic integrity of the trachea was completely retained in 16 (80%) animals. The overall rating score of epithelization was 3.6 ± 1.0, while those of the granulation tissue-like reaction and lymphocyte infiltration were 4.8 ± 0.6 and 1.5 ± 0.7, respectively.

Conclusion. These findings demonstrate that tracheal allotransplantation is possible with fresh allografts in genetically similar strains of rabbits.

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Introduction

Although resection followed by end-to-end anastomotic reconstruction of the trachea is feasible, the use of a tracheal graft becomes indispensable to restore the respiratory conduit following resection of more than 50% of the trachea.1 Investigations on tracheal substitution achieved by a prosthesis, autogenous tissue, or an allotransplant have been carried out, but as yet it remains a clinically unsolved surgical problem. Several studies on prosthetic grafts have been reported with unsatisfactory results.2–4 Similarly, repair of the tracheal defect with autogenous tissue usually results in failure because of the difficulty in maintaining a patent airway.5–7 Furthermore, autogenous tissue transplantation is a complicated and multistaged procedure, which makes its use of limited value in clinical application.2,8 For these reasons, tracheal allotransplantation appears to be the most promising method for tracheal replacement.

The major obstacles for a successful tracheal allotransplantation involve the immunogenicity and restoration of adequate blood supply to the donor graft.9,10 Thus, vascularization procedures and immunosuppression may help the allograft to retain its viability. On the other hand, as the trachea is a comparatively simple organ and with low immunogenicity, restoration of the blood supply has become a more challenging problem. Hence, attempts have been focused on achieving adequate vascularization of the donor graft for successful tracheal allotransplantation.

In the present study, we investigated the histomorphological changes related to the revascularization and immunogenic effects of fresh allografts after tracheal allotransplantation in 20 rabbits of a genetically similar strain.
Materials and Methods

Experimental Animals

A total of 20 adult chinchilla rabbits of both sexes, which were genetically similar, having been reproduced in a closed colony system for 12 generations, weighing 3–5 kg (average 4.0 ± 0.8 kg) were used in this study. All animals were treated with humane care according to guidelines that complied with the Principles of Laboratory Animal Care of the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals formulated by the National Academy of Sciences. The Ankara University Animal Committee on Animal Research approved this study.

Allotransplantation of the Trachea

Preoperatively, the animals were given 100 mg of intramuscular cephtriaxone. They were premedicated with 10 mg/kg of intramuscular ketamine hydrochloride and anesthesia was induced with 3 mg/kg of intramuscular xylazine. The animals were operated on in pairs simultaneously, under aseptic conditions, using spontaneous ventilation without an endotracheal tube. The trachea was exposed through a midline neck incision, and an eight-ring tracheal segment, approximately 1.5 cm in length, was resected from each of the two animals and it was replaced with the other one’s. We performed end-to-end anastomoses by using continuous 6-0 polypropylene sutures to reestablish the respiratory conduit. The intramuscular administration of 100 mg cephtriaxone was continued daily for 5 days after transplantation. No immunosuppressants or steroids were given to any of the animals during the course of the experiment.

Histomorphological Examination

The animals were killed with an overdose (100 mg/kg) of intravenous thiopental sodium on postoperative day (POD) 21. The grafts, including the proximal and distal anastomotic sites, were retrieved and opened longitudinally at the cartilaginous trachea for gross inspection of the luminal surface (Fig. 1). Allograft viability was assessed macroscopically and microscopically. Macroscopically, graft status was evaluated in relation to the anastomotic sites and graft necrosis, with anastomotic sites being graded as good, moderate, or poor. The grafts were then fixed with 10% natural buffered formalin and embedded in paraffin. Thereafter, sagittal cross sections were cut at a thickness of 4 μm and stained with hematoxylin–eosin (H&E). Microscopically, epithelization, a granulation tissue-like reaction with secondary findings such as the infiltration of granulocytes, histiocytes, and fibroblast proliferation, were assessed under a light microscope. Lymphocyte infiltration, which is closely associated with the immunogenic response of the graft, was also assessed. All of these characteristics, apart from epithelization, were graded as none (0), mild (1), moderate (2), and severe (3) according to the semiquantitative rating scale described previously. Epithelization of the grafts was graded as 0, indicating no epithelization; 1, ≤20%; 2, ≤40%; 3, ≤60%; 4, ≤80%; 5, complete epithelization of the entire graft. The granulation tissue-like reaction was calculated as the total mean of the semiquantitative results of the granulocyte infiltration, histiocyte infiltration, and fibroblast proliferation scores for each rabbit. Assessments were done in a double-blind fashion by two pathologists. Data are expressed as mean ± standard deviation (SD).

Results

All but two of the animals survived until the day they were scheduled to be killed. One animal died from tracheal stenosis and the other of unknown cause on PODs 7 and 13, respectively. At autopsy, gross necrotic changes and stenosis were seen in the graft of the former animal, whereas the graft of the latter animal had retained its viability and showed good healing.

In 16 (80%) of the remaining 18 animals, morphologic integrity of the trachea was completely normal, while 1 showed malacia and 1, dissolution. The inner surface was shiny and the grafts had been incorporated by the recipient trachea in these 16 animals. The anastomotic sites were assessed as good in 7 grafts, moderate in 9, and poor in 4.

The two animals that died before the day they were scheduled to be killed were excluded from the micro-