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Successful Tracheal Transplantation with Fresh Allografts in a Rabbit Model

ERKAN DİKMEN[1], MURAT KARA[1], HÜSEYİN ÇAKMAK[1], DİCLEHAN ORHAN[2], AYLİN OKÇU HEPER[2], and ADEM GÜNGÖR[1]

Departments of [1]Thoracic Surgery and [2]Pathology, Ankara University School of Medicine, İbn-i Sina Hospital, TR-06100, Sıhhiye, Ankara, Turkey

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 immunogenic 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.

Key words Trachea · Allograft · Transplantation

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 immunsuppression 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.

Reprint requests to: M. Kara, Güvenlik Caddesi, Esenlik sokak 7/10, TR-06540, Aşağıayrancı, Ankara, Turkey
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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.[11] 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).

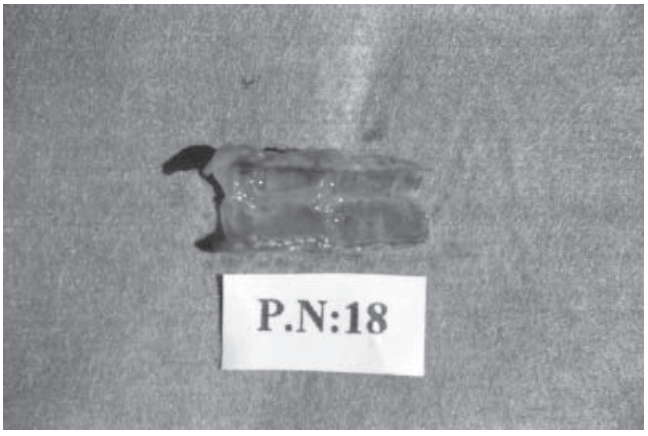

Fig. 1. Macroscopic view of the luminal surface of an allograft showing intact mucosa and good healing

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-

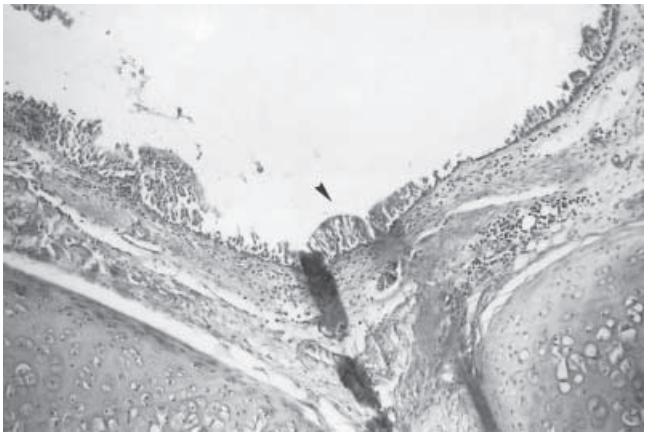

Fig. 2. Complete epithelization covering the anastomotic site (*arrowhead*) graded as 5 (H&E, ×50)

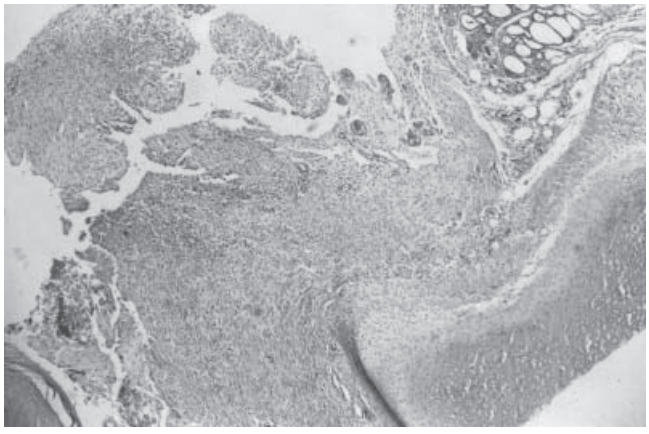

Fig. 3. Severe granulation tissue-like reaction in the anastomotic site graded as 3 (H&E, ×20)

Table 1. Histomorphological features of the tracheal allografts

Rabbit no.	Cause of death	Graft status	Anastomotic sites	Epithelization	Granulation tissue-like reaction	Lymphocyte infiltration
1	Killed	Healed	Good	4	5	1
2	Killed	Healed	Moderate	4	5	1
3	Killed	Healed	Good	5	6	2
4	Killed	Healed	Good	4	5	1
5	Killed	Healed	Good	5	5	1
6	Killed	Healed	Moderate	3	4	1
7	Killed	Healed	Moderate	3	4	3
8	Killed	Healed	Moderate	2	5	3
9	Tracheal stenosis	Malacia	Poor	—	—	—
10	Unknown	Healed	Moderate	—	—	—
11	Killed	Healed	Poor	2	5	2
12	Killed	Healed	Moderate	4	5	1
13	Killed	Healed	Moderate	3	4	2
14	Killed	Healed	Good	5	6	1
15	Killed	Healed	Moderate	3	4	2
16	Killed	Malacia	Poor	3	5	1
17	Killed	Healed	Moderate	4	5	1
18	Killed	Healed	Good	5	5	1
19	Killed	Dissolution	Poor	2	6	2
20	Killed	Healed	Good	4	4	2

scopic assessment. In the remaining 18 grafts, epithelization was scored as 5 in 4, 4 in 6, 3 in 5, and 2 in 3, the overall epithelization score being 3.6 ± 1.0 (Fig. 2). The granulation tissue-like reaction score, including granulocyte and histiocyte infiltration with fibroblast proliferation, was 4 in 5, 5 in 10, and 6 in 3 grafts (Fig. 3). Lymphocyte infiltration score was 1 in 10, 2 in 6, and 3 in 2 grafts. The granulation tissue-like reaction and lymphocyte infiltration scores were 4.8 ± 0.6 and 1.5 ± 0.7, respectively (Table 1).

Discussion

Numerous studies have been conducted on tracheal allotransplantation,[10–13] with most investigators concluding that the prevention of graft rejection caused by immunogenic effects and restoration of the blood supply to the donor trachea is the key to successful tracheal allotransplantation.

Although immunosuppression using cryopreservation, irradiation, or immunsuppressive therapy is advocated as a preventive measure against graft rejection caused by immunogenicity,[12–14] the trachea is generally believed to have weak antigenicity because

structurally, it is a comparatively simple organ consisting of epithelium, cartilage, and connective tissue. The tracheal epithelium produces HLA-DR antigens, which may play an important role in the course of graft rejection. On the other hand, the tracheal cartilage does not seem to produce these HLA-DR antigens consistently.[15] The tracheal cartilage has low immunogenicity as does other cartilaginous tissue, and its major role is thought to be support of the tracheal structure. A weak rejection response of the tracheal allograft is likely, even in the presence of major histoincompatibility.[14,16] Most of the diseases preceding an indicator for tracheal transplantation are malignant. Thus, a short course of immunosuppression that will allow long-term viability of tracheal allografts is desirable.[14] Similarly, our results showed a weak antigenic response and we were able to eliminate the risk of immunologic response of the recipients by using a genetically similar strain of rabbits. Lymphocyte infiltration, as a feature, showing the graft rejection, was grade 1 in 10 of the 18 animals and the overall score was reasonable at 1.5 ± 0.7.

Graft ischemia caused by inadequate blood supply to the donor trachea appears to be the major cause of failure in tracheal allotransplantation. The degree of healing of a graft is related to the blood supply to the organ rather than the immunogenic effect of the donor trachea.[13] Tracheal substitution grafts have been transplanted in three forms: as nonvascularized, indirectly vascularized, and directly vascularized. Although experiments using directly vascularized allografts showed satisfactory results, these procedures include both tracheal and microvascular anastomosis, which make their use of limited value in clinical application.[17,18] Indirectly vascularized grafts have two major sources of blood supply, namely, those arising from the anastomotic sites, and those arising from flaps such as omentum, muscle, and fascia. Nonvascularized tracheal grafts receive their blood supply both from the mediastinal collateral circulation and the anastomotic sites. Furthermore, mucosal blood flow and the mitotic index are more important in the anastomotic sites than in the midportion of the tracheal grafts which are supported by the omentopexy.[13,19] Similarly, grafts longer than 4 cm showed malacia in the midportion, implying the significance of the blood supply arising from the anastomotic sites.[20]

Reperfusion of tracheal allografts begins in the submucosa on the fourth posttransplant day and is completed around the 10th day. The most effective procedure to promote vascularization is wrapping of the allograft with omentum, but even then, revascularization is still delayed for up to 4–5 days.[21] Thus, tracheal allografts are subjected to relative ischemia for a 4-day period and the optimal revascularization period is likely to occur between 14 and 20 days posttransplant.[10] Hence, studies on growth factors such as PGI_2 (prostacycline) and bFGF (basic fibroblast growth factor), which promote revascularization, have been conducted to overcome this early ischemia problem following tracheal transplantation. Results have shown that these factors were capable of enhancing the revascularization and epithelial regeneration of tracheal autografts even without any vascularization procedures.[21,22] The effect of these growth factors on tracheal allografts needs further elucidation.

In conclusion, the immunogenic effect of the donor graft constitutes a small component in the process of graft rejection following tracheal allotransplantation. The main challenge should be directed toward restoration of the blood supply of the donor trachea to achieve success. This study showed that tracheal allotransplantation is possible with fresh allografts in a genetically similar strain of rabbits.

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