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ORIGINAL ARTICLE

The orthotopic left lung transplantation in rats: a valuable experimental model without using cuff technique

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Summary

Advances in the field of clinical lung transplantation must rely on observations made in animal models. In this study, we introduced a new procedure in the rat, orthotopic left lung transplantation without using the cuff technique, in which the donor pulmonary artery, pulmonary vein, and membranous parts of the bronchus were anastomosed continuously in the lumen using a mattress suture under a surgical microscope; meanwhile, a second, low-pressure perfusion through the pulmonary artery and turnover of the vascular stump were made, which also made the vessel anastomosis easy. Transplantations were completed in 68 rats (89.5%), the mean time used for suturing the left lung hilar structure was 23.5 ± 4.6 min. All lung grafts had good life-sustaining function because of there being no cuff-induced granulation tissue in bronchial anastomotic stoma, and three out of 12 allografts were observed with active bronchiolitis obliterans lesions at 8 weeks after transplantation. This model is a simple, valuable experimental model for studying lung transplantation and new therapies for preventing acute or chronic rejection.

Introduction

Lung transplantation is an important therapeutic option in end-stage lung disease; the overall survival rate after lung transplantation has improved because of improvements in organ preservation and immunosuppressive treatment [1], however, bronchiolitis obliterans syndrome (BOS) remains the major complication affecting more than half of the recipients [2,3]. The mechanisms that contribute to BOS are still poorly understood.

Advances in clinical lung transplantation must rely on observations made in animal models. Many different experimental models have been used to study different aspects of lung transplantation; but the lung transplantation model in rats is more universal. It may be classified into heterotopic tracheal transplantation and orthotopic vascularized aerated lung transplantation. In the heterotopic tracheal transplant model, the mouse trachea has been transplanted into subcutaneous locations [4], the peritoneal cavity [5], or inserted directly into the pulmonary parenchyma [6]. Despite its advantages, such as technical simplicity and reproducibility, the tracheal allograft is not aerated and vascularized; therefore, it may be difficult to interpret changes that are observed in the small airways of whole lung allografts. This system is also limited by the availability of reagents and transgenic technology. The orthotopic tracheal allograft model has the advantage of exposure to air; however, in some reports it failed to develop fibrotic obliteration because of the re-epithelialization of the allograft with recipient epithelium [7]. It seemed that a rat model of orthotopic aerated vascularized lung transplantation, which was first reported

in 1971 [8] and then in 1982 [9], would be the optimal choice; but it was difficult to directly suture the hilum of the left lung under a surgical microscope. In 1989, the refinement of the cuff technique made the model more reproducible and more attractive [10,11]. Because of its simplicity, many investigators have frequently used this model to study ischemia-reperfusion injury and active rejection of lung transplants. However, in some reports, the bronchial cuff failed to develop BOS, causing concern that the bronchial cuff had been related to cuff-induced granulation tissue, which could occlude the bronchial lumen resulting in short- or long-term graft failure [12,13].

In this article, we describe a new procedure in the rat model for orthotopic vascularized aerated lung transplantation without using cuff techniques. The pulmonary artery, pulmonary vein, and the membranous parts of the bronchus were anastomosed in the lumen using a continuous mattress suture under a surgical microscope. Some allografts exhibited bronchiolitis obliterans (BO) at 8 weeks after surgery.

Materials and methods

Animals

Male Sprague–Dawley and Lewis rats weighing 250–300 g (Vital River, Inc, Peking, China) were used. Donor animals were Sprague–Dawley for allografts ($n = 40$) or Lewis rats for isografts ($n = 36$); Lewis rats were used as recipients.

All animals received care in compliance with the 'Principles of Laboratory Animals Care' formulated by the Science and Technology Committee of the People's Republic of China and the 'Guide for the Care and Use of Laboratory Animals' (Publication No 028, Revised 1997, China Government printing office, Hunan Province). The Animal Care Committee of the Second Xiangya Hospital Research Institute approved the experimental protocol.

Extraction of the donor lungs

We extracted the donor lungs as previously described [14]. Five hundred units of heparin were administered via the inferior vena cava. The lungs were flushed with 10 ml of cold (4 °C) low-potassium dextran glucose through the main pulmonary artery. Subsequently, the left lung was harvested at end-tidal volume. The inflated left lung was placed into cold (4 °C) low-potassium dextran glucose and a 20-GA intra-arterial catheter (Vasocan Braunüle, B. Braun Inc, Bethlehem, PA, USA) was cannulated in the pulmonary artery and snared with #1 silk suture (Ethicon Inc, Somerville, NJ, USA). The lung was stored in low-potassium dextran glucose at 4 °C until transplantation.

Recipient procedure

Recipient animals were anesthetized, intubated, and sterilely prepped in a fashion similar to that used for the donor animals as described above. The animals were ventilated with a 1.5% halothane and 100% oxygen mixture for adequate sedation at a rate of 70 breaths/min, with a tidal volume (V_T) of 6 ml/kg and a positive end-expiratory pressure of 2 cmH_2O. A left thoracotomy was performed in the fourth intercostal space to approach the hilar structure. The hilum of the left lung was dissected, the left pulmonary artery and main bronchus were clamped together with a microvascular clamp, and the pulmonary vein was occluded with a slip knot (#1 silk suture; Ethicon Inc.). The native lung was removed to allow the stump of the hilar structure to be at a corresponding length for suturing.

The donor lung was implanted into the thorax of the recipient and covered with wet gauze. The bronchial stump was dissected to appropriate lengths and sutured continuously with 9-0 Prolene (Ethicon Inc) under a surgical microscope (SMI CO, LTD, Shanghai, China). The first needle was sutured in the end of the 'C-shaped' cartilage of the bronchus and knotted out of the wall (Fig. 1a). Subsequently, the membranous portion of the bronchus was anastomosed continuously into the lumen using mattress sutures, which keep the bronchial endomembrane very smooth (Fig. 1b). The distance between two sutures was 0.2–0.3 mm, and 12–14 sutures were needed. When the anastomosis of the membranous portion of the bronchus was completed, the needle was inserted through the two other ends of the 'C-shaped' cartilage and the suture line was drawn slightly to recover the native shape of the bronchial annular cartilage; then, a loop knot was tied to prevent the line from loosening (Fig. 1c). Subsequently, the cartilage of the bronchial stump was anastomosed continuously with the telescoping technique described by Marck *et al.* (Fig. 1d) [9].

After the bronchial anastomosis was completed, the pulmonary artery was flushed continuously with warm (24 °C), low-potassium dextran glucose at a pressure of 3–5 cmH_2O through the catheter cannulated in the pulmonary artery to make the donor pulmonary vein turgid and easy to suture. Moreover, the vessel stumps were opened while they were kept immersed in the solution. The excess solution was sucked out by an assistant to prevent overflow of the liquid wetting the animals and operation table. The pulmonary vein was anastomosed with 10-0 Prolene (Ethicon Inc) as described previously for suturing the membranous portion of the bronchus. The distance from the needle to the vessel stump in the proximal end was more than double that in the distal end (Fig. 2a). The engorged

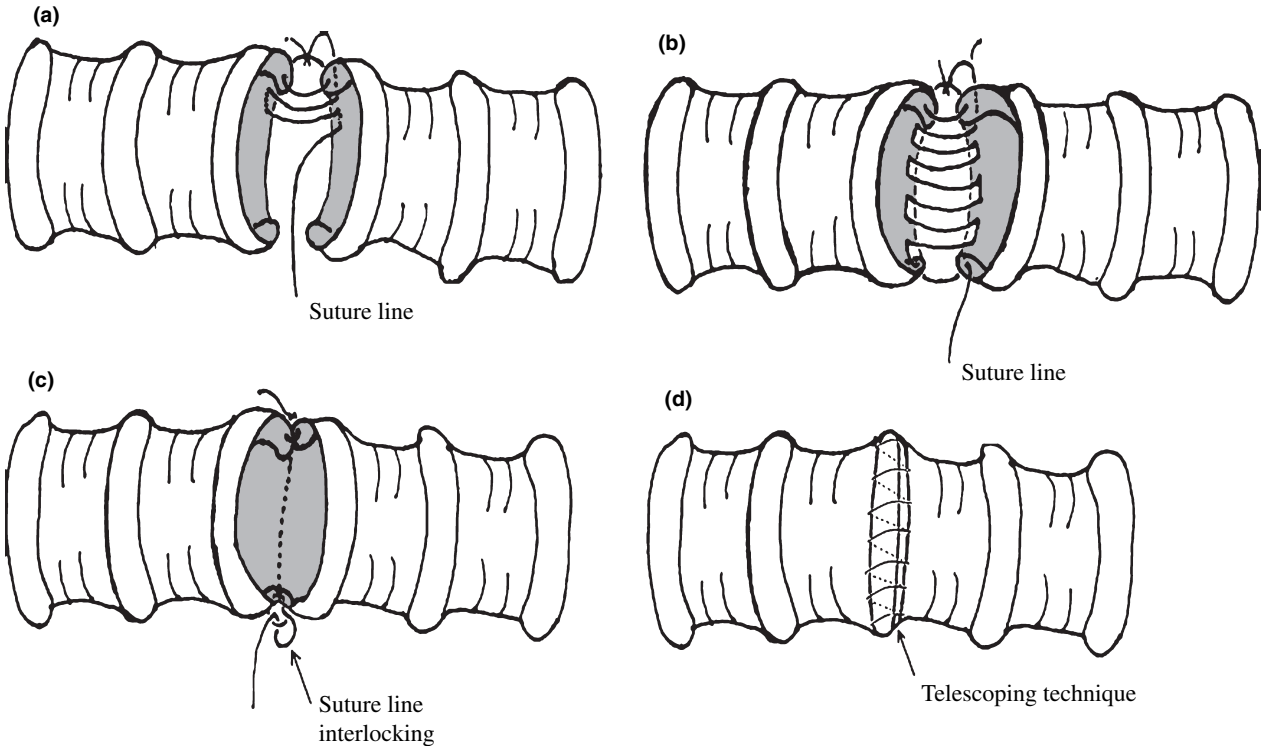

Figure 1 The procedure of bronchial anastomoses (a–d). The suture at the starting point (a); then bronchial membranous portion were anastomosed continuously into the lumen by using mattress suture (b), drew the suture line slightly and recovered the bronchial annular cartilage to the native shape, then tied a knot to avoid the line loosening (c). The running suture was used to suture the cartilages of the stumps by telescoping technique (d).

pulmonary vein could obviate stenosis in the anastomotic stoma. When the anastomosis was completed, the proximal end was everted over the distal end (Fig. 2b and c) to prevent blood leakage in the anastomotic stoma. The catheter was removed and the pulmonary artery was trimmed to appropriate lengths for anastomosis as described above. The pulmonary artery wall was stretched horizontally to over 1.5 times its diameter to prevent excessive narrowing in the anastomotic stoma ahead of the knot.

The transplanted lung was re-inflated and re-perfused by releasing the slip knot on the pulmonary vein and unclamping the microvascular clamp on the recipient bronchus and pulmonary artery. Temporary hyperinflation and a positive end-expiratory pressure are necessary to remove partial atelectasis and check for air leakage in the anastomotic stoma, which have been reported elsewhere [15].

Once the lung was implanted, the left thoracotomy incision was closed over a pleural drainage tube with #4 silk sutures (Ethicon Inc). The recipients were ventilated with 100% oxygen until they were awake. When spontaneous respiration resumed, the endotracheal tube was removed from the airway, and the pleural drainage tube was removed under pressure. After surgery, the animals were kept in an oxygenated cage for the first 24 h [16]. Allograft recipients received 2.0 mg/kg/day cyclosporine A (Sandimmun; Novartis, Basel, Switzerland) by subcutaneous injection and 30 mg/kg/day mycophenolate mofetil (CellCept; Novartis) by mouth. All animals that died after surgery were examined postmortem.

Functional assessment of the graft

All recipients were maintained until 8 weeks after transplantation, at which time the surviving animals were killed for functional graft assessment and histologic observations. The animals were anesthetized with 50 mg ketamine (i.p.) and ventilated on a FiO_2 of 0.5. A median sternotomy was performed. The right pulmonary hilum was occluded for 10 min, and subsequent blood samples from left ventricle were analyzed by a blood gas analyzer (NOVA Biomedical, Waltham, MA, USA).

Histopathology

At the time of killing, the transplanted lungs were harvested, fixed in 4% paraformaldehyde, embedded in

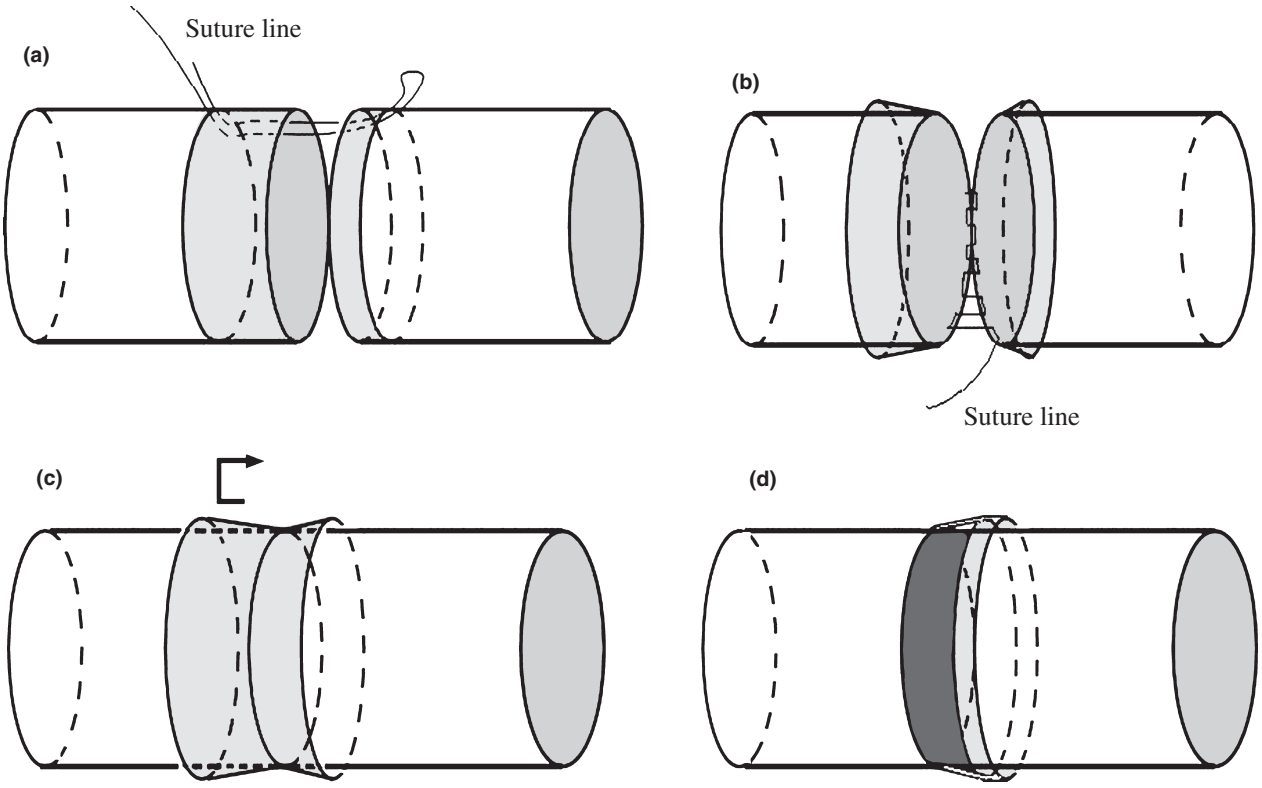

Figure 2 The procedure of vessel anastomoses (a–d). The mattress suture was used at the starting point, left the vessel stump in the proximal end which was more than double that in the distal, and ligated at the proximal end (a), then anastomosed the vessel contiguously by using mattress suture in lumen (b).While completing the anastomoses, turned over the proximal stump to overlay the distal stump of vessel (c and d).

paraffin, sectioned, and stained with van Gieson's and hematoxylin-eosin. A semiquantitative grading system formulated by the Lung Rejection Study Group [17] and Lee *et al.* [18] was used to assess rejection and the severity of small airway injury in a blinded fashion.

Grade I: Bronchiolar epithelial denuding, mild to moderate inflammation within the small airway wall and surrounding interstitium.

Grade II: Bronchiolar epithelial denuding, destruction of elastica and muscular wall hyperplasm with extensive inflammation extending into the peribronchiolar tissue.

Grade III: Intraluminal cellular proliferation, nascent granulation tissue and fibrosis with partial occlusion of the airway (less than 50% of the lumen diameter).

Grade IV: Intraluminal projection or plugging with nascent granulation tissue and fibrosis with obliteration (at lest 50% of the lumen diameter).

The active BO includes Grades III and IV.

Statistical analysis

Data were expressed as mean ± SEM. Differences between groups were determined by analysis of variance (ANOVA). Results were considered statistically significant at $P < 0.05$.

Results

We performed 100 rat lung transplants with a technical failure rate of 50% in the first 24 transplantations. During this period of model development, eight recipients died from lacerations on the wall of the pulmonary vein, and four recipients died in the immediate postoperative period. In the later transplantations, when the pulmonary artery was perfused continuously with low-potassium dextran glucose at low pressure, incidents of laceration of the pulmonary vein were rare. During the subsequent 76 transplants, three deaths occurred because of intraoperative bleeding, two deaths because of air leakage in the lung parenchyma because of uncontrolled temporary hyperinflation, and three recipients died in the immediate postoperative period, resulting in a technical success rate of 89.5% (68/76). However, seven recipients died within 6–48 h postoperatively from complications associated with the surgery. Only one recipient with pulmonary artery thrombosis was found at postmortem examination.

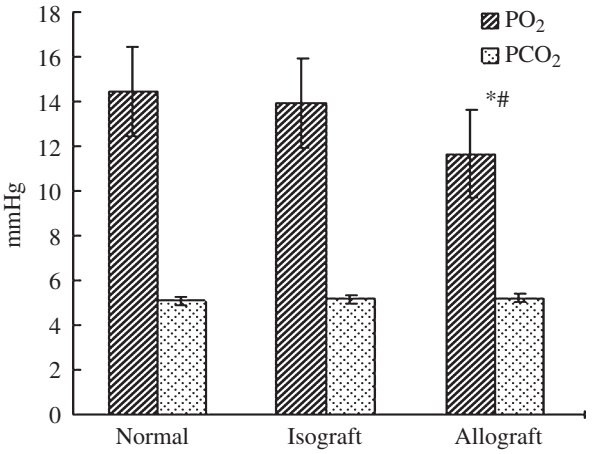

Figure 3 The arterial PO_2 level of lung isografts ($n = 6$) did not differ from the normal rats ($n = 6$), but higher slightly than that of lung allografts ($n = 6$) ($P < 0.05$). Blood was collected from left ventricle after a 10-min occlusion of the right pulmonary hilum. *Compared with normal rat, $P < 0.05$; #Compared with isografted recipients, $P < 0.05$.

The time required for suturing the left lung hilar structure was 23.5 ± 4.6 min, and the total surgical time was 78.4 ± 5.2 min. All animals were sacrificed at 8 weeks after surgery, and their lung grafts were examined functionally or histologically.

To assess the functional status of the lung grafts, the PO_2 and PCO_2 levels were measured 8 weeks after transplantation. Blood was collected from the left ventricle after a 10-min occlusion of the right pulmonary hilum. The arterial PO_2 levels of the lung isografts did not differ from those of normal rats but were slightly higher than those of the lung allografts ($P < 0.05$). The PCO_2 level in none of the samples was statistically significant (Fig. 3).

At 8 weeks after transplantation, the histological examinations of the lung allografts showed moderate to severe inflammatory infiltration including lymphocytes and polymorphonuclear cells, which invaded the bronchial wall and surrounding interstitium (Grades I–II). Denuding of the bronchial epithelium was found, but active BO lesions (Grades III–IV) was observed in only three of the 12 lung allografts (Fig. 4).

Discussion

The orthotopic vascularized aerated lung transplantation model in rats is analogous to clinical lung transplantation. First attempted in 1971 [8], then in 1982, Marck *et al.* used microsurgery with some modifications to directly suture the hilar structures, but the technical complexity of vascular and bronchial sutures remained an obstacle [9]. Mizuta *et al.* [10] developed their experimental model in rats using cuffs, and investigators have frequently used this model, with some modifications [11,19,20], to study ischemia-reperfusion injury in lung transplantation. However, in some reports the bronchial cuff failed to develop BOS, causing concern that the bronchial cuff was related to cuff-induced granulation tissue, which could occlude the bronchial lumen resulting in short- or long-term graft failure [12,13]. In this article, we have established a new procedure in the rat model of orthotopic vascularized aerated lung transplantation without using cuff techniques, and observed histologically that the allografts manifested some features of BO at 8 weeks postoperatively (Fig. 4), which will enable the study of the exact pathogenesis and of new therapies for BO.

In our experimental model, 89.5% of the recipients survived the surgery. The time needed for suturing the left lung hilum and the overall duration of surgery were not longer than those required by the cuff technique for experimental lung transplantation in rats as described by other authors [12]. Bronchial anastomosis is critical for maintaining adequate aeration of the grafts; we used a continuous mattress suture in the lumen to anastomose the membranous portion of the bronchus (Fig. 1), and none of the recipients was found with bronchial leakage during the transient hyperinflation or after surgery. The bronchial epithelium and mucous membrane were smooth at the stoma, which caused fewer deaths in the animals from obstruction of the trachea by mucus or granulation tissue. The bronchus was applanned and narrowed when the membranous portion was anastomosed by interrupted sutures resulting from the elasticity of bronchial annular cartilage. However, the surgical procedure described in our experimental model could restore the native shape of the bronchial annular cartilage. Moreover, the elasticity of the bronchial annular cartilage kept the suture line taut thus avoiding bronchial air leakage.

The wall of the pulmonary vein in the rat is very thin and easily torn, especially if it adheres to itself when blood infusion is unavailable. We continuously infused at constant temperature, low-potassium dextran glucose solution at low pressure through the donor pulmonary artery during anastomosis of the pulmonary vein, which not only made the vessel stumps open and easy to suture, but the second rinse also helped to reduce organic cell injury [21–23]. When anastomosis of the vessel was completed, we everted it over the longer proximal end to overlay the anastomotic stoma and distal vessel (Fig. 2), which prevented blood leakage even in cases where the suturing technique was not perfect. During the anastomosis of the blood vessel, injury to the vascular endothelium at the stump was inevitable because of the iterative positioning of the ring clamp. Many studies have confirmed that injury to the endothelium could accelerate thrombosis and intimal proliferation [24–26]. Therefore, we left

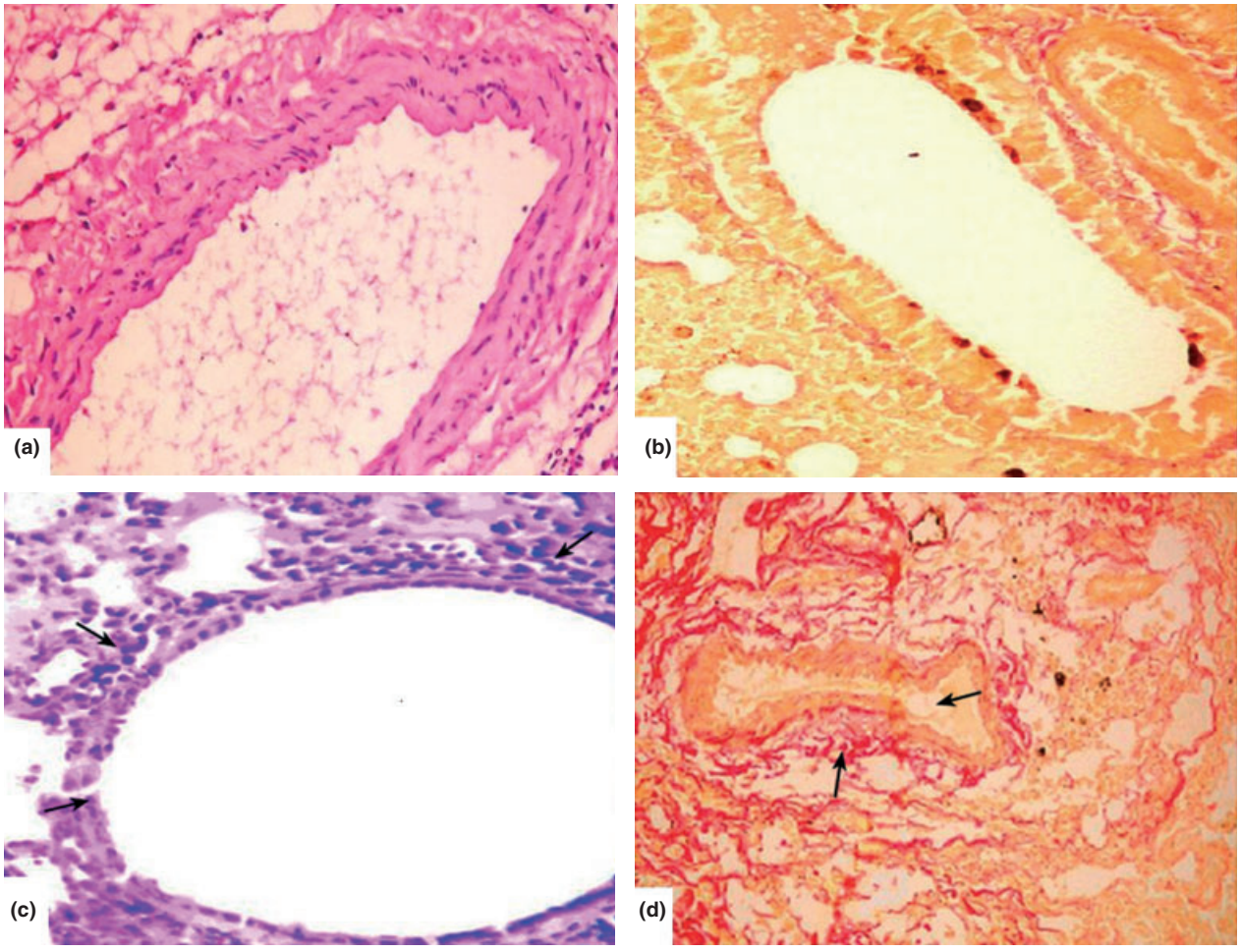

Figure 4 Histologic observations in animal groups. (a) and (b) Almost normal histology in the isograft; (c) the extensive inflammatory cellular infiltrates into interstitium and peribronchiolar tissue, bronchiolar epithelial denuding (indicated by arrows); (d) active OB-like lesion in respiratory bronchioles; (e) a semiquantitative grading to assess rejection and the severity of small airway injury in allografts at 4 week after lung transplantation (a and c, hematoxylin and eosin × 200; b and d, van Gieson ×200).

the injured intima out of the vessels, kept the blood running though the healthy and smooth endothelium, and few animals suffered thrombosis at the postoperative examination. These techniques used in this experimental model resulted in the same functional status for lung isografts as for normal animals at 8 weeks after transplantation (Fig. 3).

To elucidate the pathogenesis of BO, a reliable animal model is required. According to the literature, the orthotopic lung transplantation from Fischer 344 to Wistar

Kyoto rats is the only model that reliably results in BO without a further stimulus [27–29]. However, Hirschburger *et al.* presumed that this model was not suitable to study BO because BO did not develop in grafts with life-sustaining function [13]. In our study, the lung allografts of the recipients still had good function at 8 weeks after transplantation (Fig. 3). During pathologic evaluation of allograft rejection, all allografts exhibited extensive inflammatory infiltration and evidence of epithelial loss, which plays a pivotal role in the process of BO [30], however active BO was observed in only three of 12 allografts, which was in conformity with the literature reported by Lee [18].

In summary, we established a new procedure for orthotopic lung transplantation in the rat that differs from the cuff technique. The membranous portion of the bronchus and the vessels were anastomosed with a continuous mattress suture in the lumen; meanwhile, a second pulmonary artery perfusion and vascular stump turnover technique were used during vessel anastomosis. The above techniques made the experimental model very simple and reliable, and more analogous to clinical lung transplantation. Histopathologic observation demonstrated some features of BO at 8 weeks after transplantation. Moreover, if the strains from Fischer 344 to Wistar Kyoto rats could be introduced in this model, this will be a more valuable animal model for studying the mechanisms of and new therapies for acute and chronic rejection.

Authorship

X-JX: designed research/study. Q-CZ, D-JW: performed research/study. NY, B-LY: contributed important reagents. Y-HW: collected data. R-XF: analyzed data. Q-CZ wrote the paper and it was revised by X-JX.

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References

1. Hosenpud JD, Bennett LE, Keck BM, Boucek MM, Novick RJ. The registry of the international society for heart and lung transplantation: Eighteenth Office Report-2001. *J Heart Lung Transplant* 2001; **20**: 805.
2. Burke CM, Theodore J, Dawkins KD, *et al.* Post-transplantation obliterative bronchiolitis and other late lung sequelae in human heart-lung transplantation. *Chest* 1984; **86**: 824.
3. Husain AN, Siddiqui MT, Holmes EW, *et al.* Analysis of risk factors for the development of bronchiolitis obliterans syndrome. *Am J Respir Crit Care Med* 1999; **159**: 829.
4. Hertz MI, Jessurun J, King MB, Savik SK, Murray JJ. Reproduction of the obliterative bronchiolitis lesion after heterotopic transplantation of mouse airways. *Am J Pathol* 1993; **142**: 1945.
5. Boehler A, Chamberlain D, Kesten S, Slutsky AS, Liu M, Keshavjee S. Lymphocytic airway infilitration as a precursor to fobrous obliteration in a rat model of bronchiolitis obliterans. *Transplantation* 1997; **64**: 311.
6. Dutly AE, Andrade CF, Verkaik R, *et al.* A novel model for post-transplant obliterative airway disease reveals angiogenesis from the pulmonary circulation. *Am J Transplant* 2005; **5**: 248.
7. Fernández FG, Jaramillo A, Chen C, *et al.* Airway epithelium is the primary target of allograft rejection in murine obliterative airway disease. *Am J Transplant* 2004; **4**: 319.
8. Asimacopoulos PJ, Molokhia FA, Pegg CA, Norman JC. Lung transplantation in rat. *Transplant Proc* 1971; **3**: 583.
9. Marck KW, Wildevuur CR. Lung transplantation in the rat: I. Technique and survival. *Ann Thorac Sur* 1982; **34**: 74.
10. Mizuta T, Kawaguchi A, Nakahara K, Kawashima Y. Simplified rat lung transplantation using a cuff technique. *J Thorac Cardiovasc Surg* 1989; **97**: 578.
11. Reis A, Giaid A, Serrick C, Shennib H. Improved outcome of rat lung transplantation with modification of the non suture external cuff technique. *J Heart Lung Transplant* 1995; **14**: 274.
12. Mizobuchi T, Sekine Y, Yasufuku K, Fujisawa T, Wilkes DS. Comparison of surgical procedures for vascular and airway anastomoses that utilize a ''modified'' non-suture external cuff technique for experimental lung transplantation in rats. *J heart and Lung Tansplant* 2004; **23**: 889.
13. Hirschburger M, Greschus S, Kuchenbuch T, *et al.* Lung transplantation in the Fischer 344–>Wistar Kyoto rat strain combination is not suitable to study bronchiolitis obliterans. *J Heart Lung Transplant* 2007; **26**: 390.
14. Maclean AA, Liu M, Fischer S, Suga M, Keshavjee S. Targeting the angiotensin system in posttransplant airway obliteration: the antifibrotic effect of angiotensin converting enzyme inhibition. *Am J Respir Crit Care Med* 2000; **162**: 310.
15. Sekine Y, Yasufuku K, Heidler KM, *et al.* Monocyte chemoattractant protein-1 and RANTES are chemotactic for graft infiltrating lymphocytes during acute lung allograft rejection. *Am J Respir Cell Mol Biol* 2000; **23**: 719.
16. Belperio JA, Keane MP, Burdick MD, *et al.* Role of CXCL9/CXCR3 chemokine biology during pathogenesis of acute lung allograft rejection. *J Immunol* 2003; **171**: 4844.

17. Yousem SA, Berry GJ, Cagle PT, *et al.* Revision of the 1990 working formulation for the classification of pulmonary allograft rejection: Lung Rejection Study Group. *J Heart Lung Transplant* 1996; **15**: 1.
18. Lee AG, Wagner FM, Chen MF, Serrick C, Giaid A, Shennib H. A novel charcoal-induced model of obliterative bronchiolitis-like lesions: implications of chronic nonspecific airway inflammation in the development of posttransplantation obliterative bronchiolitis. *J Thorac Cardiovasc Surg* 1998; **115**: 822.
19. Okazaki M, Krupnick AS, Kornfeld CG, *et al.* A mouse model of orthotopic vascularized aerated lung transplantation. *Am J Transplant* 2007; **7**: 1672.
20. Oto T, Rabinov M, Negri J, *et al.* Techniques of reconstruction for inadequate donor left atrial cuff in lung transplantation. *Ann Thorac Surg* 2006; **81**: 1199.
21. Serrick CJ, Jamjoum A, Reis A, Giaid A, Shennib H. Amelioration of pulmonary allograft injury by administering a second rinse solution. *J Thorac Cardiovasc Surg* 1996; **112**: 1010.
22. Halldorsson AO, Kronon M, Allen BS, *et al.* Controlled reperfusion prevents pulmonary injury after 24 hours of lung preservation. *Ann Thorac Surg* 1998; **66**: 877; discussion 884-885.
23. Koike N, Takeyoshi I, Ohki S, Tsutsumi H, Matsumoto K, Morishita Y. The effect of short-term coronary perfusion using a perfusion apparatus on canine heart transplantation from non-heart-beating donors. *J Heart Lung Transplant* 2003; **22**: 810.
24. Gawaz M. Role of platelets in coronary thrombosis and reperfusion of ischemic myocardium. *Cardiovasc Res* 2004; **61**: 498.
25. Schwartz RS, Henry TD. Pathophysiology of coronary artery restenosis. *Rev Cardiovasc Med* 2002; **3**(Suppl. 5): S4.
26. Eguchi H, Ikeda H, Murohara T, *et al.* Endothelial injuries of coronary arteries distal to thrombotic sites: role of adhesive interaction between endothelial P-selectin and leukocyte sialyl LewisX. *Circ Res* 1999; **84**: 525.
27. Matsumura Y, Marchevsky A, Zuo XJ, Kass RM, Matloff JM, Jordan SC. Assessment of pathological changes associated with chronic allograft rejection and tolerance in two experimental models of rat lung transplantation. *Transplantation* 1995; **59**: 1509.
28. Yasufuku K, Heidler KM, Woods KA, *et al.* Prevention of bronchiolitis obliterans in rat lung allografts by type V collagen induced oral tolerance. *Transplantation* 2002; **73**: 500.
29. Suga M, Maclean AA, Keshavjee S, Fischer S, Moreira JM, Liu M. RANTES plays an important role in the evolution of allograft transplant-induced fibrous airway obliteration. *Am J Respir Crit Care Med* 2000; **162**: 1940.
30. Boehler A, Estenne M. Post-transplant bronchiolitis obliterans. *Eur Respir J* 2003; **22**: 1007.

