



Review

Technical Aspects and Benefits of Experimental Mouse Lung Transplantation[☆]



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ARTICLE INFO

Article history:

Received 4 February 2016

Accepted 15 April 2016

Available online 1 November 2016

Keywords:

Transplantation

Mouse model

Experimental

Lung

ABSTRACT

In recent years, the number of lung transplantations performed as the last option for many respiratory diseases has grown considerably, both in adults and children. However, the causes for the relatively short survival of lungs compared to other organ transplants still need to be studied.

Techniques have improved since the 1950s when experimental lung transplantation began, and the different animal species used now include rodents. The advantage of using these small species is that the surgical model has been expanded and standardized, and different respiratory problems can be studied.

In this review we examine the different technical strategies used in experimental transplantation in rats and mice, focusing on surgical techniques and anesthesia and monitoring methods, and highlighting the major contributions of mouse lung transplantation to the field.

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Aspectos técnicos y utilidades del trasplante pulmonar experimental murino

RESUMEN

A lo largo de las últimas décadas, el número de trasplantes pulmonares realizados como terapia final de muchas enfermedades respiratorias ha ido creciendo considerablemente, tanto en la población adulta como a nivel pediátrico. Sin embargo, se hace muy necesario estudiar las causas por las que su supervivencia es relativamente baja en comparación con otros trasplantes de órganos.

Por ello, desde mediados del siglo pasado comenzaron a realizarse trasplantes pulmonares experimentales, cuya técnica ha ido mejorando, y se ha ampliado a distintas especies animales hasta llegar a los roedores. La ventaja que presentan estas especies pequeñas ha facilitado que el modelo quirúrgico se haya extendido y estandarizado, permitiendo estudiar diferentes aspectos relacionados con las enfermedades respiratorias.

En esta revisión se analizan las distintas modalidades técnicas disponibles de trasplante experimental en rata y ratón, destacando tanto la técnica quirúrgica como la anestésica o la monitorización, así como las principales aportaciones generadas por el trasplante pulmonar murino.

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Palabras clave:

Trasplante

Modelo murino

Experimental

Pulmón

Introduction

Lung transplantation is occasionally the only therapeutic option available in the final stages of respiratory diseases such as chronic

obstructive pulmonary disease, diffuse interstitial pulmonary disease, bronchiectasis associated with cystic fibrosis, bronchiolitis obliterans, sarcoidosis or arterial pulmonary hypertension.^{1–3}

Since the first transplantations were performed in animals in 1940,^{4,5} these experimental models have contributed to the development of surgical procedures and the prevention of associated complications, such as rejection or ischemia–reperfusion injury. Although the first mouse transplantation took place in 1971,⁶ in the 20th century the procedure was generally performed in dogs,^{4,5} pigs,⁷ and monkeys,^{8–10} since larger animals offer

[☆] Please cite this article as: Ruiz-Pérez D. Aspectos técnicos y utilidades del trasplante pulmonar experimental murino. Arch Bronconeumol. 2016;52:596–604.

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advantages in the development of surgical techniques and transfer of results to humans in the clinical setting. These species continue to be used today, particularly for surgical training, but smaller species, such as rabbits,¹¹ rats and mice, which have considerable advantages in terms of expense, management and logistics, and are less constrained by ethical considerations, have acquired greater prominence.

Orthotopic transplantation of the left lung was initially developed in rat models,⁶ while heart-lung transplant is less common due to high mortality rates.¹² Improvements in surgical and anesthetic techniques, mechanical ventilation and preservation and reperfusion of organs have improved the success rate and reproducibility of the model. Heterotopic transplantation models have also been developed, although ventilation and good graft perfusion have not been achieved.^{13,14} Finally, the mouse model is enhanced by the availability of a wide range of strains that allow the study of genetic factors that may affect the transplant.^{15,16}

The aim of this review is to describe the different procedures used in mouse lung transplantation, paying special attention to surgical techniques and perioperative management, including anesthesia-analgesia, mechanical ventilation, post-operative care, and prevention of complications. We also review the main clinical contributions of lung transplantation in rodents.

Anesthesia and Perioperative Care

Anesthetic protocols must provide sufficient anesthesia and analgesia to maintain the physiological stability of the animal, and they must be easily reversible after surgery is completed. Although induction and recovery from inhalation anesthesia¹⁶ (1.5%–2% isoflurane or 2.5%–3% sevoflurane, both known to protect the lung¹⁷) is rapid, this technique can be difficult to apply in the absence of proper facilities. Specifically, considerable economic resources are called for, as inhalation anesthetics are costly, and a team specializing in mechanical ventilation must be available to administer the anesthetic gases. When inhalation anesthesia is not an option, parenteral methods can be used instead (Table 1).

Ketamine is one of the most widely used drugs. It provides an analgesic effect which is enhanced when administered together with an α_2 -adrenergic receptor antagonist, such as xylazine or medetomidine, which simultaneously reduces muscle rigidity. This combination of anesthetics can be easily reversed with the administration of an α_2 -adrenergic receptor antagonist, such as atipamezole. Other combinations include ketamine and the benzodiazepine diazepam, or fentanyl combined with medetomidine.

Diuresis caused by α_2 -adrenergic receptor antagonists must be compensated with subcutaneous fluid replacement. Ophthalmic ointment must also be applied to prevent corneal dryness.

Thoracotomy is a potent nociceptive stimulus, so appropriate analgesia is essential. Analgesia is generally provided in the form of preoperative subcutaneous administration of opioids (e.g., buprenorphine, 10–50 $\mu\text{g}/\text{kg}/8\text{ h}$ in rats or 50–100 $\mu\text{g}/\text{kg}/8\text{ h}$ in mice) combined with non-steroidal anti-inflammatory drugs (meloxicam 2 mg/kg/day). Finally antibiotic prophylaxis with cefazolin (30 mg/kg) or ceftriaxone (70 mg/kg) is recommended.

Ventilation

Once anesthetized, the animals must be intubated with 14 G catheters for rats >300 g, 16 G for rats 200–300 g or 22 G for mice weighing around 25 g, and mechanically ventilated. For the recipient, the pressure-controlled mode is usually recommended, since the left lung will not be functional during a large part of the surgery.

The ventilator should be set to a tidal volume of 7–10 ml/kg (if volume-controlled ventilation is used), or a maximum pressure of 10–12 cm H₂O (in the case of pressure-controlled ventilation), with a respiratory rate of 60–100 breaths/minute. Moreover, a positive end-expiratory pressure of 2–5 cm H₂O is highly recommended for avoiding the formation of atelectasis during expiration.

Intraoperative Monitoring

Equipment specifically designed for rodents is available for recording pulse oximetry, capnography, non-invasive arterial pressure or electrocardiogram. Devices designed for humans or large animals are generally unsuitable for rodents.

It is also important to monitor body temperature using a thermometer or rectal probe and to maintain it between 37.5 and 38.5 °C. Hypothermia is a very common complication in rodents and adverse events can occur if temperatures fall below 32 °C, including delayed recovery from anesthesia. An electric blanket or hot water bottle can be used to maintain body temperature, although the animal must be monitored to avoid possible hyperthermia. Oxygen saturation tends to remain at levels above 99% when the inspired fraction of oxygen is increased to between 30% and 60%, compared to 21% ambient air.

Surgical Aspects of Orthotopic Lung Transplantation in Mice and Rats

The left lung orthotopic transplantation model is the most widely used, thanks to its viability and greater simplicity, since the left lung is formed of a single lobe, while the right lung is divided in 4. However, orthotopic transplantation of the right lung has also been performed in mice.

Preparation of the Donor

Extraction of the complete heart-lung block is generally recommended. The animal is anesthetized and placed in a supine

Table 1
Anesthetic Combinations Used in Rats and Mice During Surgical Procedures, Including Lung Transplantation.

Protocol	Dose (mg/kg)		Duration (min)
	Rat	Mouse	
Ketamine + medetomidine	75–100 + 0.25–0.5	75–150 + 0.5–1	40–50
Ketamine + xylazine	75–100 + 12	75–150 + 12–20	40–50
Ketamine + diazepam	80 + 20	75–100 + 5	30–40
Medetomidine + fentanyl	0.3 + 0.3	–	50–60
Atipamezole ^a (α_2 -adrenergic antagonist)	0.5–1	0.5–1	–

^a Medetomidine and xylazine can be blocked with atipamezole.

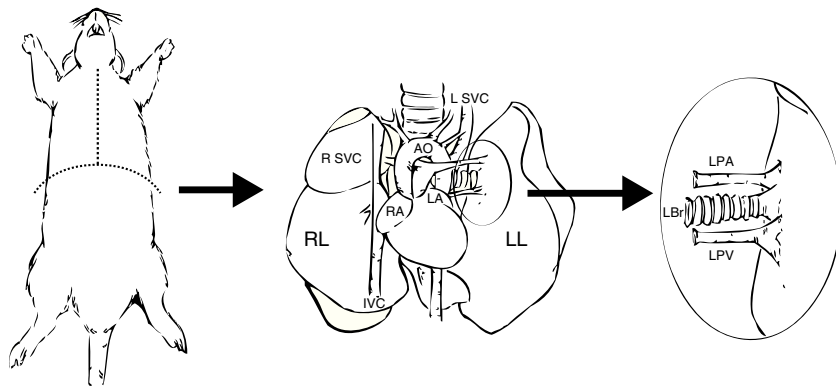


Fig. 1. Extraction and preparation of the donor lung. Median sternotomy and dissection and extraction of the heart–lung block. Dissection of the left pulmonary artery (LPA), left pulmonary veins (LPV), and the left bronchus (LBr). AO: thoracic aorta; IVC: inferior vena cava; LA: left atrium; L SVC: left superior vena cava; LL: left left; RA: right atrium; R SVC: right superior vena cava.

position, and the operative field is prepared by shaving the skin of the chest and applying povidone iodine or chlorhexidine in an outward movement from the center of the chest. A median sternotomy is then performed with inferior vena cava, subcutaneous or intracoronary heparin delivery (100 IU/100 g) to prevent clots forming in the lung for transplant. Using an optical microscope, the lung hilum is dissected to locate the aortic arch, which is then clamped. Incisions are simultaneously made in the atria and the inferior vena cava.

In most models, antegrade perfusion is performed via the right ventricle^{19,20} or the pulmonary trunk^{15,21–23} although it is advisable to combine this procedure with retrograde perfusion from the left atrium²² to simulate physiological conditions. The lungs may be perfused at a constant, uniform pressure (10–15 cm H₂O) with cold preservation fluid (4 °C), consisting of low potassium dextran glucose (LPDG).

The trachea is then ligated and dissected for extraction of the entire heart–lung block, with the lung inflated to prevent the formation of atelectasis and to improve oxygenation and the presence of surfactant in order to reduce ischemia–reperfusion injury.^{24,25} Recommendations for inflation range between 50%^{22,25} and 75%–100%²⁶ of total lung capacity, taking care to avoid barotrauma. Inflation before preservation also reduces the expression of proinflammatory markers after reperfusion.²⁶

For preservation, the donor lung is generally maintained at 4 °C to minimize ischemic injury to the graft.²⁷ The most commonly used preservation solution is low potassium dextran (Perfadex),^{16,28–30} although others are available, including Celsior,³¹ Euro-Collins or Wisconsin.²⁴

Preparation of the Graft

When the lung block has been extracted, the left lung must be separated, clamping the bronchus to keep it inflated. The bronchus, the artery and the pulmonary veins are dissected as distally as possible to the lung to assist anastomosis (Fig. 1).

Preparation of the left lung will vary depending on the anastomosis method used: vascular and bronchial anastomosis with sutures,^{6,19,21,32} bronchial anastomosis with sutures and vascular anastomosis with cuffs,^{30,33–36} and vascular and bronchial anastomosis with cuffs^{20,22,23,35–39} (Fig. 2). In the first case, the lung hilum is dissected, separating each structure for anastomosis, while maintaining the lung in the low temperature preservation medium. In the latter 2 cases, rather than using sutures, the vascular and bronchial anastomoses are simplified by using cuffs designed for transplantation of lungs³³ and other organs.^{40–42} These are ring-shaped devices made of polyvinyl chloride or Teflon®, prepared from cannulae or catheters. Sizes for rats range from 18 G to 14 G,

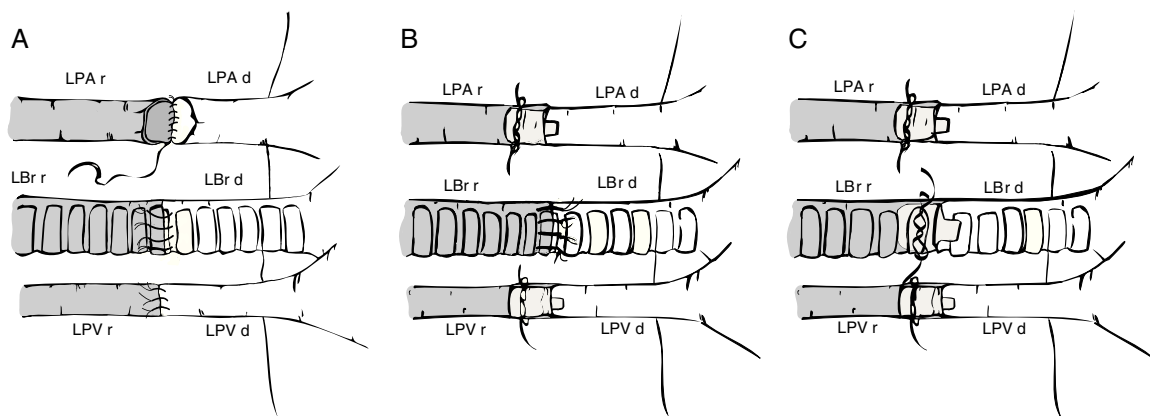


Fig. 2. Surgical techniques in mouse model lung transplantation. (A) Vascular and bronchial anastomosis with sutures. (B) Bronchial anastomosis with sutures and use of vascular cuffs in the pulmonary vein and artery. (C) Vascular and bronchial anastomosis using cuffs. LBr d: left bronchus of donor animal; LBr r: left bronchus of recipient animal; LPA d: left pulmonary artery of donor animal; LPA r: left pulmonary artery of recipient animal; LPV d: left pulmonary vein of donor animal; LPV r: left pulmonary vein of recipient animal.

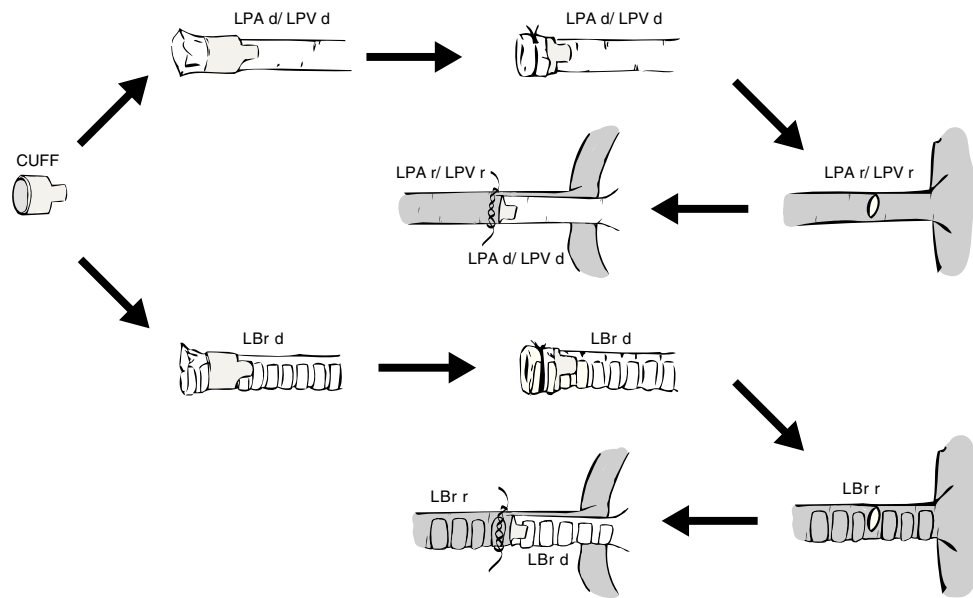


Fig. 3. Placement of a cuff in the donor lung and anastomosis with the recipient lung. LBr d: left bronchus of donor animal; LBr r: left bronchus of recipient animal; LPA d: left pulmonary artery of donor animal; LPA r: left pulmonary artery of recipient animal; LPV d: left pulmonary vein of donor animal; LPV r: left pulmonary vein of recipient animal.

the larger ones being used in the bronchus and the smaller ones in the pulmonary artery.²² Conventional cuffs consist of a main body, on which the vessels or the bronchus are joined and an extension tab to facilitate manipulation (Fig. 3). The cuff is 2–3 mm in length (body 1, 1.5 or 2 mm and the tab 1 or 1.5 mm). A modified, tabless cuff model has been developed,^{32,36,42} but this makes it more complicated to manipulate. For this reason, some groups use the standard cuff, and remove the tab after anastomosis has been performed.³³

Irrespective of the type of cuff used, the procedure is conducted by introducing the vein, artery or bronchus of the donor lung through the hole of the cuff. It is then everted over the cuff and fixed to the cuff with a polypropylene ligature, 7/0 or 8/0 in rats^{22,23} and 10/0 in mice.¹⁸ The bronchus is occasionally prepared at the moment of implantation to prevent preservation solution from infiltrating the airway.^{18,20}

Recipient Preparation

The animal is anesthetized, given appropriate analgesia and placed in right lateral decubitus position. The surgical field is prepared in the same way as for the donor animal and a lateral thoractomy is made through the fourth intercostal space. The left lung is extracted and the principal structures are isolated, clamping the left pulmonary artery, vein and bronchus, as distally as possible to the lung. These structures are dissected as close as possible to the pulmonary parenchyma. To avoid the formation of thrombi, heparinized saline (0.5–1 IU/ml) is administered to the artery and vein. All structures must be handled carefully to avoid vascular tears.

The vascular structures and the bronchus are then anastomosed. Cuff anastomosis is shown in the photograph in Fig. 4.

When the bronchus has been anastomosed, the donor lung must be ventilated to avoid the formation of atelectasis and to enhance alveolar recruitment. Rapid lung reperfusion indicates that the anastomosis is patent. Finally, the native lung is extracted from the recipient animal, leaving only the donor lung.

A drainage tube must be inserted so that remains of blood, fluid or air around the lungs can be eliminated and the lungs can expand

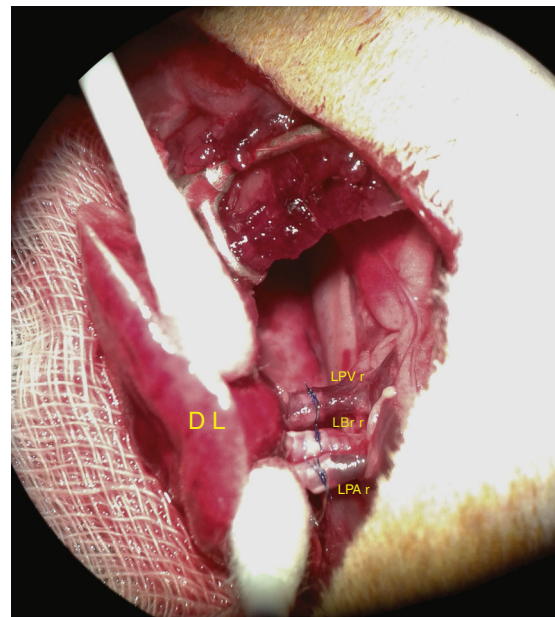


Fig. 4. Photograph of lung transplantation performed using the cuff technique in blood vessels and bronchus. DL: donor lung; LBr r: left bronchus of recipient animal; LPA r: left bronchus of recipient animal; LPV r: left pulmonary vein of recipient animal.

normally. The chest cavity is then closed by layers, and the drainage tube is withdrawn when the animal recovers from anesthesia and begins to breathe spontaneously.

The time to complete surgery varies, depending on the group of professionals and the technique used. In general, the procedure is faster with the cuff technique than with conventional sutures, particularly when sutures are applied to vessels and the bronchus. Similarly, the survival rate is higher when anastomosis is performed with cuffs, compared to sutures (Table 2).

Table 2
Comparison of Different Mouse Model Lung Transplantation Protocols.

Technique	Surgery Time (min)	Type of Anastomosis	Order of Anastomosis	Cuff Length (mm)	Cuff Diameter (mm)	Survival (%)
<i>Suture</i>						
Asimacopoulos, ⁶ 1971	Not described	Suture	B-A-V	–	–	50
Marck, ³² 1982	240	Suture	V-A-B	–	–	80 ^a 50 ^b
Prop, ¹⁹ 1984	210 ± 31 and 238 ± 19	Suture	V-A-B	–	–	89 and 75
7521 + 2008	78.4 ± 5.2	Suture	B-A-V	–	–	89 ^a 50 ^b
<i>Vascular cuffs and bronchial suture</i>						
Mizuta, ³³ 1989	101 ± 4.8	Conventional cuff	V-A-B	2 (body: 1; tab: 1)	2 and 1.65	88.8
Kubisa, ³⁴ 2003	120	Conventional cuff	V-A-B	Not described	0.8	96.57
Mizobuchi, ³⁵ 2004	116 ± 1.6	Conventional cuff	V-A-B	4 (body: 2.5; tab: 1.5)	1.65	95.6
Zhai, ³⁶ 2008	Not described	Modified cuff	A-B-V	2.5 (body: 1; tab: 1.5)	1.65	Not described
Habertheuer, ³⁰ 2013	130 ± 9	Conventional cuff	B-A-V	Not described	Not described	100 (70–90–100)
<i>Vascular and bronchial cuffs</i>						
Mizuta, ³⁷ 1991	64.1 ± 3.6	Not described	Not described	Not described	Not described	90 ^b
Reis, ³⁸ 1995	108.6 ± 4.2	Conventional cuff	A-V-B	3 (body: 2; tab: 1)	1.65	Not described
Santana Rodríguez, ²³ 2004	110 ± 12.7	Conventional cuff	V-B-A	3 (body: 1.5; tab: 1.5)	2.2 and 1.65	Not described
Mizobuchi, ³⁵ 2004	84.8 ± 0.6	Conventional cuff	V-B-A	4 (body: 2.5; tab: 1.5)	2.1 and 1.65	91.8
Zhai ³⁶ , 2008	Not described	Modified cuff	A-B-V	2.5 (body: 1; tab: 1.5)	1.65	Not described
Sugimoto, ³⁹ 2009	110 approx.	Not described	A-B-V	Not described	Not described	93.6
Jungraithmayr, ¹⁶ 2009	95.6 ± 8.5	Conventional cuff	A-B-V	Different sizes	0.64–0.51–0.4	80
Santana Rodríguez, ²² 2011	107.7 ± 6.2	Conventional cuff	A-B-V	3 (body: 1.5; tab: 1.5)	1.65	87.6
Zheng, ⁴³ 2013	96.8 ± 6.2	Conventional cuff	A-V-B	Different sizes	0.81–0.64–0.51	96.7
Zheng, ⁴³ 2013	85.3 ± 6.9	Modified cuff	A-V-B	Not described	0.81–0.64–0.51	80

Order of anastomosis: A: artery; B: bronchus; V: vein.

^a Perioperative mortality.

^b Postoperative mortality.

Postoperative Care

After surgery, analgesic and antibiotic treatment must be administered for at least 5–6 days, and analgesia can be increased in case of pain. The surgical wound must be cleaned and disinfected to aid healing. Protocols for monitoring and assessment of endpoint criteria must be established, including aspects such as loss of weight, physical appearance, ventilatory pattern, presence of cyanosis, or the behavior of the animal. If any of these variables changes, corrective actions must be immediately implemented.

Perioperative Complications and Management

The most significant complications occur as a result of the surgical procedure, in the preparation of both the donor and the recipient.⁴⁴ A relatively long learning curve is required before skills are acquired in the use of microsurgical equipment and success in the technique is achieved, and mortality can be high during the training period.¹⁵ Some authors set 50 transplantations as the threshold for perfecting the procedure.²⁰ The duration of the surgery and time spent performing anastomoses impact on the appearance of complications and on the animal's survival.^{5,16,30,32,44}

In general, the use of cuffs requires less skill and experience, and the procedure is faster than with sutures.^{38,45} Vascular cuffs reduce the risk of bleeding and with it the formation of granulation tissue, reducing the long-term risk of thrombosis or stenosis in the anastomosis sites.³⁸ Bronchial leaks, a factor causing pneumothorax which compromises the functionality of the graft, are rarer with cuff anastomosis.³⁵ However, the diameter of the cuff must be adequate to avoid reducing the size of the lumen, and moreover, a foreign-body reaction may occur.^{35,38,45} Modified cuffs, without extension handles or tabs, reduce the risk of twisting of the

vessels or bronchi after anastomosis and injury caused by these extensions themselves. They are also smaller, so less foreign-body reaction is produced.³⁶ Other perioperative complications include vena cava rupture, pleural empyema,³⁴ respiratory arrest,³⁸ and venous thrombosis.⁴³

The most significant postoperative complications include respiratory failure, hemoptysis, thrombosis of a bronchial vein or artery, pneumothorax, respiratory infections, pleural effusion, pulmonary edema, and graft rejection.^{19,46}

Main Scientific Contributions

Despite the complexity of the surgery, rodent lung transplantation models are sufficiently standardized and reproducible to be useful in the evaluation of different surgical techniques and the mechanisms of the main transplantation complications in humans. To date, mouse lung transplantation has helped generate knowledge on donor characteristics,^{24,47} lung conservation and preservation models,^{26,48,49} ischemia–reperfusion injury,^{48,50–52} and particularly, acute and chronic rejection (Table 3).^{48,53–66}

Primary Graft Dysfunction due to Ischemia–Reperfusion Injury

Primary graft dysfunction occurs in the first 72 h and severity depends on the PaO₂/FiO₂ ratio and the presence of radiological infiltrates. It is a significant cause of morbidity and mortality in the immediate postoperative period.

Analysis of this phenomenon in mouse models has led to a better characterization of the causative mechanisms, in which the primary lesion of the neutrophils caused by ischemia–reperfusion appears to play a fundamental role. Thus, neutrophils isolated from the airways of transplant recipient have been seen to

Table 3
Summary of Principal Aspects Evaluated in Mouse Model Lung Transplantation.

Author and Year	Species	Sex	Strains	Model	Objective
Aeba et al., ⁴⁹ 1992	Rat	Male	Lewis (donor and recipient)	Left orthotopic lung transplantation after 6 or 12 h of cold ischemia	Comparison of different lung preservation solutions (Wisconsin and Euro-Collins)
Zweers et al., ⁴⁷ 2004	Rat	Male	Fisher 344 (donor); Wistar Kyoto WKY (recipient)	Left orthotopic lung transplantation with conventional suture technique and induced brain death in the donor for 6 h	Study of effect of induced brain death on chronic rejection after lung transplantation
Okazaki et al., ⁵⁸ 2007	Mouse	Male	BALB/c, C57BL/6 (B6) ^a (donor); B6 (recipient)	Left orthotopic lung transplantation with cuff technique and heterotopic trachea transplantation	Comparison of trachea transplantation and lung transplantation in acute rejection study
Greschus et al., ⁵³ 2009	Rat	Male	F344, Dark Agouti, WKY (donors); WKY, Lewis, F344	Left orthotopic lung transplantation with vascular cuff technique and bronchial suture	Volumetric tomography monitoring and evaluation of transplanted lung
Santana-Rodríguez et al., ⁵² 2011	Rat	Male	Sprague-Dawley (donor and recipient)	<i>Left orthotopic lung transplantation with cuff technique</i>	Evaluation of the effect of estradiol on ischemia–reperfusion lung injury
Santana Rodríguez et al., ²² 2011	Rat	Male	Sprague-Dawley (donor and recipient)	Left orthotopic lung transplantation with cuff technique and induced brain death in the donor	Evaluation of anastomosis patency and signs of ischemia–reperfusion injury similar to acute rejection in 2 technical models
Kreisel et al., ⁵⁰ 2011	Mouse	Male	Balb/c, B6, B10.BR CD11c-EYFP (donor); B6 LysM-GFP, B6 TNF- $\alpha^{-/-}$ (recipient)	Left orthotopic lung transplantation with cuff technique after 1 h and 18 h of cold ischemia	Study of primary graft dysfunction due to ischemia–reperfusion injury
Kreisel et al., ⁵¹ 2011	Mouse	Male	B6 (donor)-B6 (B6), B6 (Bcl3)	<i>Left orthotopic lung transplantation with cuff technique</i>	Study of transcription factor Bcl3 and its association with acute rejection after lung transplantation
Dodd-o et al., ⁵⁹ 2011	Mouse	Male	B6, BALB/c (donor); B6 (recipient)	<i>Left orthotopic lung transplantation with cuff technique</i>	Study of reduction of acute rejection with use of anti-CD-154 antibodies
Jungraithmayr et al., ⁵⁵ 2012	Mouse	Male	BALB/c (H-2Kd) (donor); B6 (recipient)	<i>Left orthotopic lung transplantation with cuff technique</i>	Evaluation of acute rejection using scanning electron microscopy
Santana-Rodríguez et al., ⁶⁶ 2012	Rat	Male	Sprague-Dawley (donor and recipient)	<i>Left orthotopic lung transplantation with cuff technique</i>	Evaluation of lung transplantation using DNA microarrays
Chen et al., ⁵⁷ 2013	Mouse	Male	B6, BALB/c (donor); B6 (recipient)	<i>Left orthotopic lung transplantation with cuff technique</i>	Identification of acute rejection by studying T cell metabolism with imaging techniques (micro-positron emission tomography).
Erne et al., ⁶² 2013	Rat	Male	Brown-Norway (donor); Lewis (recipient)	<i>Left orthotopic lung transplantation with cuff technique</i>	Reduction of acute lung rejection with the administration of N-acetylcysteine
Tanaka et al., ²⁶ 2014	Rat	Male	Lewis (donor and recipient)	Left orthotopic lung transplantation with cuff technique after 6 h of preservation at different percentages of inflation	Evaluation of effect of inflation of donor lungs during pulmonary preservation.
Takahashi et al., ⁵⁶ 2014	Rat	Male	Brown-Norway (donor); Lewis (recipient)	<i>Left orthotopic lung transplantation with cuff technique</i>	Study of lung function in acute rejection using noninvasive techniques such as forced oscillation
Kreisel et al., ⁶¹ 2010	Mouse	Male	B6 ^{CD45.1} B6II ⁻ (donor); B6, B6Rag ^{-/-} , B6/B6II ⁻ F1 (recipient)	Left orthotopic lung transplantation with cuff technique and bone marrow transplantation	Study of effect of pulmonary nonhematopoietic cells and their effect on CD4+ T cells
Zhou et al., ⁶⁰ 2015	Mouse	Male	B6, BALB/c (donor); B6 (recipient)	<i>Left orthotopic lung transplantation with cuff technique</i>	Relationship between acute rejection and IL-17 production
Raissadati et al., ⁶³ 2015	Mouse	Male	BALB/c (donor); B6 (recipient)	Heterotopic trachea transplantation	Study of the effect of CCN1 protein in a bronchiolitis obliterans model
Wu et al., ⁶⁴ 2015	Mouse	Male	C57Bl/10, B6 (donor); B6 (recipient)	<i>Left orthotopic lung transplantation with cuff technique</i>	Study of the influence of CD4+ T cells and IL-17 production in a bronchiolitis obliterans model
Chuck et al., ⁵⁴ 2016	Mouse	Male	B6, BALB/c (H-2Kd) (donor); B6 (recipient)	<i>Left orthotopic lung transplantation with cuff technique</i>	Evaluation of changes in the transplanted lung due to acute rejection and ischemia–reperfusion injury using ultra-short magnetic resonance.

^a Mouse strain C57BL/6 indicated as B6.

stimulate the donor's dendritic cells by a contact-dependent mechanism to induce IL-12 production and to expand alloantigen-specific T cells.⁵⁰ This phenomenon, in turn, has been shown to be regulated by the degranulation induced by TNF- α associated with the neutrophil plasma membrane.⁵⁰ Furthermore, some factors which modulate this cell-mediated response have been identified, which may be of potential interest in the design of future therapeutic approaches. The transcriptional factor Bc13 has been seen to limit granulopoiesis via a dependent NF- κ B pathway.⁵¹

Acute Cell-mediated Rejection

Mouse models provide an ideal medium for the study of acute cell-mediated rejection, as the grade of concordance or discordance of the major histocompatibility antigen can be evaluated in pure strains. This is of particular interest in the development of specific monitoring systems for this transplantation model. In addition to using bronchoalveolar lavages for the study of airway cellularity and measuring oxygenation capacity with arterial blood gases, specific imaging techniques have been developed, such as volumetric tomography⁵³ or ultra-short magnetic resonance.⁵⁴ These, together with the histological evaluation of the ultrastructural changes in acute rejection using scanning electron microscopy,⁵⁵ improve the monitoring of the transplanted lung.

Complementary procedures have also been developed for the evaluation of lung function. The forced oscillation technique is very useful in mouse models, since dynamic elastance and its frequency dependence appear to have the ability to predict the grade of acute rejection,⁵⁶ and can be used as non-invasive indices for detecting and monitoring disease progression. In an image-function setting, micro-positron emission tomography can be used to identify increased ¹⁸F-fluorodeoxyglucose uptake by neutrophils and CD8 lymphocytes,⁵⁷ providing a more precise picture of acute rejection from the evaluation of T cell metabolism.

The evaluation of the pathogenic mechanisms of acute rejection in mouse models has fostered the development of interventions for prolonging graft survival, for example, by blocking anti-CD28-B7 costimulators and the CD40-CD40 ligand.⁵⁸ Other authors have shown in an MHC-mismatched orthotopic mouse lung transplantation model that monotherapy with anti-CD154 antibodies may be sufficient to attenuate acute rejection⁵⁹ by blocking CD154/CD40 costimulation, which fosters a shift in the graft environment toward a predominance of CD4(+) CD25(+) Foxp3(+) regulatory T cells.⁶⁰ Some pulmonary nonhematopoietic cells have also been reported to play an important role in the *in vivo* suppression of CD4(+) T cell immune-mediated responses.⁶¹

Although it is still early, these findings may be transferred to clinical practice in the form of N-acetylcysteine therapy. The administration of this compound in mouse models has been shown to diminish acute lung rejection by reducing the macrophage and T cell infiltration that is intimately related with reduction of the NF- κ B-mediated inflammatory signaling pathway.

Chronic Rejection

Chronic rejection usually manifests as the development of bronchiolitis obliterans, caused by lymphocyte and neutrophils cell infiltration attributable to a series of factors, such as excessive activation of the innate immune system, abnormal angiogenesis, or inadequate epithelial regeneration and remodeling of the lymphoproliferative tissue.⁴⁸

Although each animal model has its advantages and limitations in the study of bronchiolitis obliterans, the mouse model of bronchiolitis obliterans associated with lung transplantation mimics more closely chronic rejection in humans than other models of bronchiolitis obliterans induced by chemical agents. Moreover, it can be used to make serial evaluations of both lung function and multiple morphological changes or altered cell function, and for testing the efficacy of different drugs. Another important advantage is the availability of models with knock-out and transgenic strains of multiple human diseases, that can be used to reproduce situations of chronic rejection relatively similar to those that occur in different end-stage respiratory diseases, and for evaluating the contribution of potential signaling pathways.

However, some anatomical and physiological factors of the mouse lung cause translational difficulties. Compared to human lungs, mouse bronchioles have fewer submucosal glands and a greater number of Clara cells. This environment protects against bronchiolar epithelial injury, primarily due the production of Clara cell secretory protein and a solution similar to lung surfactant, so more vascular injury than epithelial injury is generated in these rejection models.⁵⁸ Nevertheless, significant advances have been made in recent years in the development of different bronchiolitis obliterans mouse models^{63–65} and DNA microarrays for evaluating chronic rejection after transplantation.⁶⁶

In conclusion, techniques in mouse lung transplantation have developed significantly, contributing to a range of different surgical alternatives with excellent viability of transplanted animals. These experimental surgical models that have demonstrated their utility in generating knowledge on the pathogenic mechanisms of ischemia-reperfusion injury and rejection are now more widely accessible, and may be translated to humans in the medium term.

Funding

This project received assistance with grant FPU2013-01638, awarded to Daniel Ruiz-Pérez by the Spanish Ministry of Education, Culture and Sport. The grant provider played no part in study design, data collection and analysis, or the preparation of the manuscript.

Conflict of Interests

The authors state that they have no conflict of interests.

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