

## Experimental models of lung transplantation

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## 1. ABSTRACT

Lung transplantation is a life saving treatment for end stage pulmonary diseases. The development and refinement of this therapy required the utilization of various animal models, without which this procedure would not have become a clinical reality. Canine models were critical in the initial breakthroughs in surgical technique and immunosuppressive regimens, which directly led to the first successful human lung transplantation. Orthotopic lung transplant models in the rat provided a platform for more detailed investigation of immune responses to pulmonary grafts. Investigation of chronic rejection of lungs has significantly been advanced through the use of mouse tracheal transplant experiments. And finally, the advent of orthotopic, vascularized lung transplantation in the mouse opened the door to the use of genetic and molecular tools that are necessary for the rigorous mechanistic study of alloimmune and non-alloimmune factors contributing to lung graft failure. Taken together, animal models will continue to be a cornerstone in the advancement of clinical success in lung transplantation.

## 2. INTRODUCTION

Early attempts at transplantation of lungs in humans were faced with technical challenges. Specifically, the airway anastomosis proved to be a hurdle to successful completion of this procedure. The refinement of surgical techniques in large animals has been pivotal to establish lung transplantation as a routine clinical procedure. The development of experimental lung transplant models in genetically defined small animals has allowed investigators to define non-immunological and immunological facets of lung transplantation that contribute to persistently inferior outcomes when compared to other organ transplants. In this review we will provide a historical overview of animal models of experimental lung transplantation that have been and will continue to be instrumental in improving the health of human pulmonary transplant patients (Table 1).

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**Table 1.** Milestones in lung transplantation

Year	Animal Model	Human Landmark
1946	<b>Dog:</b> First LTx in any mammal(3)	
1949	<b>Dog:</b> First published account of single LTx in dogs (4)	
1954	<b>Dog:</b> First allogeneic LTx in U.S.(6)	
1963		First human single LTx(1)
1971	<b>Large Rat:</b> Orthotopic LTx with sutured anastomoses(16)	
1981	<b>Dog:</b> LTx studies of bronchial anastomotic healing and steroids (10-12)	
1982	<b>Small Rat:</b> Orthotopic LTx with sutured anastomoses(17)	
1983		First long term LTx survivor using surgical and immunosuppressive modifications(13)
1986	<b>Dog:</b> First en-bloc bilateral LTx(14)	
1988	<b>Monkey:</b> First en-bloc double LTx(15)	First en-bloc double LTx(15)
1989	<b>Rat:</b> Orthotopic LTx with cuff technique(56)	First sequential bilateral LTx(57)
1993	<b>Mouse:</b> Heterotopic tracheal Tx(35)	
2001	<b>Mini Swine:</b> Orthotopic LTx(29)	
2002	<b>Mouse:</b> Orthotopic tracheal Tx(39)	
2005	<b>Mouse:</b> Intrapulmonary tracheal Tx(41)	
2007	<b>Mouse:</b> Orthotopic left LTx(43)	
2010	<b>Mouse:</b> Orthotopic right LTx(51)	

### 3. CANINE LUNG TRANSPLANTATION MODELS AS A PLATFORM FOR DEVELOPMENT OF SURGICAL TECHNIQUES AND IMMUNOSUPPRESSION

The technical feasibility of lung transplantation was established in large animal models. Insights and principles gained particularly through experiments in the canine model were essential for James Hardy to be able to attempt the first human lung transplantation at The University of Mississippi in 1963 (1).

One individual merits special mention with regard to the history of animal models of lung transplantation. In the 1940's, Vladimir P. Demikhov, an innovative Russian surgeon-scientist performed the first intrathoracic lung transplantation in a dog, the first such procedure performed in a mammal (2). He also performed intrathoracic heart and combined heart-lung transplants. These procedures were performed without cardiopulmonary bypass and utilized a novel technique of closed circuit donor heart-lung autoperfusion. Because of the Cold War, news of Demikhov's pioneering experiments was limited in the Western world, with the provocative exception being his most infamously notable procedure: canine head transplantation in 1954. However, Demikhov has since gained significant scientific recognition including being considered the father of heart and lung transplantation by several well known surgeons such as Christiaan Barnard (2, 3).

Henri Metras of France reported single lung transplantation in dogs to The Academy of Sciences in Paris in 1949. This pioneering work was published in the French literature during the subsequent year (4). Notably, he established technical principles that have stood the test of time. To this end, Metras described the use of atrial cuffs for the pulmonary venous anastomosis and used the same sequence of anastomoses (first bronchial followed by arterial and finally venous) as routinely employed today. Interestingly, he also reestablished the bronchial arterial supply. While the vast majority of contemporary lung transplant surgeons do not routinely perform bronchial arterial revascularization, recent experimental work has

suggested that small airway hypoxemia due to lack of systemic arterial blood supply may contribute to chronic rejection (5). The first report of animal lung transplantation in North America was in 1952, when a group at the University of Kansas described the allotransplantation of left canine lungs (6). In these initial experiments, the donor lung was prepared by transecting the left auricle, left main pulmonary artery and left bronchus with anastomoses to the recipient left auricle, left main pulmonary artery and mainstem bronchus. Survival ranged from 1 to 12 days. The impact of the immune system was examined through the administration of anti-histamine (diphenhydramine), cortisone or total body radiation with some improvement in the group treated with cortisone. Another set of experiments involved performing splenectomy concomitantly with pulmonary transplantation or preceding the lung transplant. While these early experiments indicated that lung transplantation may be achievable from a technical perspective, the host immune system was quickly noted to be a significant barrier. To this end, the authors stated that "although the operative technique of transplantation of one entire lung has been demonstrated to be feasible, the limitations imposed by foreign protein implantation appear responsible for the present failure of these organs to survive. Further studies are in progress to investigate the antigen-antibody mechanism in these homologous lung transplants and possible methods of altering it" (6).

To circumvent the poorly understood detrimental effect of the host immune system, much work during this era involved autotransplants where a pneumonectomy was performed with immediate reimplantation in the same animal (7, 8). Experiments in the canine model of lung transplantation, carried out by Hardy's group, were published in 1963 (9). Studies involving 343 dogs were aimed at addressing four objectives: 1) to evaluate the functional capacity of immediately reimplanted lung autotransplants, 2) to examine the effect of periods of cold storage upon function of autologous implants, 3) to assess the function of a lung allograft and 4) to determine the efficacy of immunosuppressive agents in prolonging allograft survival. Notably, there was a significant survival benefit with the use of methotrexate (13.8 days),

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azathioprine (30.4 days) or azathioprine in combination with hydrocortisone (27.9 days) as compared with untreated animals (7.4 days) (8).

Although Hardy's monumental surgical milestone in 1963 indicated the feasibility of human pulmonary transplantation, it became quickly apparent that the integrity of the bronchial anastomosis in the setting of immunosuppression represented a major obstacle. Only approximately 40 lung transplants were performed worldwide during the subsequent two decades, which was in large part due to the feared complication of bronchial dehiscence. Cooper and colleagues at Toronto General Hospital employed a canine pulmonary transplant model to study and optimize bronchial wound healing in immunosuppressed lung recipients. An important observation from these experiments was that the negative effects of high dose corticosteroids on bronchial healing could be avoided by substituting cyclosporine for steroids, thereby improving anastomotic healing while suppressing alloimmune responses (10, 11). This canine model also served to generate a method of wrapping the bronchial suture line with a flap of well vascularized omentum, thereby protecting against dehiscence perhaps in part by providing increased vascular flow (12). The insights and experience gained from these animal studies were directly translated to the first human single lung transplant with long term success performed by the Toronto Lung Transplant Group on November 7<sup>th</sup>, 1983 (13). Similarly, techniques for bilateral lung transplantation were developed first in canines then validated in cynomolgus monkeys prior to successful translation to human beings (14, 15).

### 4. ORTHOTOPIC LUNG TRANSPLANTATION IN RATS

After these technical aspects had been developed and refined in canine models, the attention of the scientific community was turned to more rigorous investigation of the factors affecting short and long term survival of pulmonary grafts. Animal models that were genetically better defined were developed in attempts to mechanistically study processes that contribute to the failure of lung transplants.

The rat was the first genetically defined species to be widely utilized for study of lung transplantation. Microsurgical techniques that were developed for the transplantation of other solid organs in rats were adapted to the lung. In 1971, Asimacopoulos provided the first description of orthotopic lung transplantation in the rat involving sutured anastomoses of hilar structures in relatively large animals weighing between 400 and 600 grams (16). Approximately one decade later, Marck and colleagues refined analogous techniques for smaller rats (17). However, the technical difficulty of this procedure resulted in high complication rates, which hindered its widespread use. In an attempt to simplify and standardize the procedure, Mizuta and colleagues adapted cuff techniques that had been successfully used for vascular anastomoses in orthotopic rat liver transplantation (18, 19). This resulted in a significant shortening of the duration of

the procedure (half of previous reports) and ischemic time (one third of previous reports). Orthotopic rat lung transplantation using cuff techniques represented an important advance in experimental pulmonary transplantation, which is reflected by a surge in publications in this field in the early 1990's. This method was easily reproducible and, compared to work in large animals, the rat model allowed for more rapid accrual of experimental data.

The availability of well defined inbred strains and experimental tools enabled investigators to explore new biological questions. Studies employing rat orthotopic lung transplants have led to fresh insights into ischemia reperfusion injury and graft rejection. For example, Belperio and colleagues performed mechanistic studies into the role of neutrophil chemoattractants in mediating ischemia reperfusion injury (20). Importantly, expression patterns of chemokines in the rat model mirrored those seen in human lung recipients that suffered from primary graft dysfunction. In 1985, Prop's group reported a series of rat lung transplant studies, which provided a detailed description of allograft rejection in multiple strain combinations in this model (21-24). The authors defined four phases of acute rejection: latent, vascular, alveolar and destruction. Their experiments clearly demonstrated that rejection of pulmonary grafts proceed at a faster rate compared to other organs. The authors postulated that donor passenger leukocytes, present in bronchus-associated lymphoid tissue (BALT) within the lung graft, played a dominant role in accelerating the tempo of acute rejection. It is important to note that, while rat lungs express BALT constitutively, these lymphoid structures are not expressed at baseline in mouse and human lungs, but can be rather induced after inflammation (25, 26). Only few investigators have used the rat orthotopic lung transplant model to study chronic rejection, which is at least in part due to the morphological differences between fibrotic airway occlusion observed in humans and lesions seen in rat lung grafts. Furthermore, whether orthotopic rat lung transplantation represents a suitable model to study bronchiolitis obliterans has been controversial. For example, while some investigators have reported the development of obliterative bronchiolitis in non-immunosuppressed minor MHC-mismatched F344 → Wistar Kyoto lung transplants, others have cautioned against the use of pulmonary transplants in this strain combination to study chronic lung allograft rejection (27, 28).

### 5. ORTHOTOPIC PORCINE LUNG TRANSPLANTATION MODELS

Allan and associates described an orthotopic lung transplant model in MHC-inbred miniature swine. This is an important model as grafts transplanted into immunosuppressed MHC-matched, minor antigen-mismatched recipients develop airway lesions that reproduce changes associated with chronic rejection in humans (29). It has been used to study clinically relevant issues such as the role of gastric aspiration in promoting graft rejection (30). Notably, in part due to the availability

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of some relevant knockouts, such as galactosyl transferase gene-deficient animals, porcine lungs have also been used to evaluate xenogeneic responses both *ex vivo* and *in vivo* (31, 32). It is apparent that pulmonary transplantation experiments in large animals carry multiple complexities that limit the ability to perform high throughput mechanistic investigations. However, the great preclinical value of large animals in lung transplantation research was recently exemplified by Cypel and colleagues at Toronto General Hospital. Building on results in the porcine model they demonstrated the ability to recondition marginal human lungs for transplantation (33, 34).

### 6. TRACHEAL TRANSPLANTATION MODELS

Given the obvious benefits of an experimental model for lung transplantation in the mouse, Hertz and colleagues developed a murine heterotopic tracheal transplantation in 1993 (35). This procedure involved en bloc harvesting of the trachea and main bronchi with subsequent implantation into a subcutaneous pocket of a recipient mouse. When transplanted into a non-immunosuppressed allogeneic host, tracheal grafts develop apoptosis of airway epithelial cells with progressive fibrotic obliteration of the lumen. These lesions closely resemble bronchiolitis obliterans in human lung transplant recipients. Due to its technical ease, mastery of this procedure required little training. Given that numerous transgenic and knockout mouse strains were available, many laboratories started using heterotopic tracheal transplantation to conduct novel mechanistic studies. In fact, during the ensuing decade this model became the most commonly utilized experimental platform in lung transplantation and, arguably, to this date remains one of the best available models to study chronic pulmonary rejection. Numerous studies have addressed requirements for T cell activation and evaluated the effects of immunosuppressive strategies in this model. Potentially valuable insights have been gained about pathogenesis of chronic rejection in humans. Based on the observation that MCP-1 levels are elevated in the bronchoalveolar lavage fluid of human lung recipients, who suffer from bronchiolitis obliterans, Belperio and colleagues examined the role of the MCP-1 receptor CCR2 in the rejection of heterotopic tracheal transplants (36). They found that inhibition of MCP-1 / CCR2 signaling reduced infiltration of mononuclear cells into the tracheal grafts and attenuated fibrotic obliteration of the airways. In addition to mice, heterotopic tracheal or bronchial transplants have also been used in rats and larger animals (37, 38).

However, important differences exist between heterotopically transplanted tracheal grafts and orthotopic lung transplants, which may limit its clinical translation. Heterotopic tracheal grafts are not vascularized, not exposed to the external environment and involve large airways. In 2002, Genden and colleagues described orthotopic tracheal transplantation, which allowed for grafted airways to be exposed to the ambient environment (39). Despite inherent limitations of this technique, which include large airway physiology and rapid reepithelialization with recipient epithelium, this model has

expanded our understanding of events that contribute to airway fibrosis. To this end, a study by Babu and co-workers suggested that the lack of bronchial arterial revascularization may play a role in the development of chronic airway changes in lung transplants (40). Furthermore, concerns have been expressed about heterotopic tracheal transplants, because they are placed in an environment that does not mirror the clinical situation. To address this shortcoming, Keshavjee's group developed an intrapulmonary tracheal transplant model, where the tracheal graft is incorporated into the recipient's lung rather than placed in a subcutaneous pocket (41). Using this model in the rat, Sato and colleagues have observed that rejection is associated with lymphoid neogenesis in the lung graft (42). As chronically rejected human lung grafts also have evidence of *de novo* lymphoid tissue, this observation may have implications for immunological events that play a role in the development of bronchiolitis obliterans.

### 7. ORTHOTOPIC LUNG TRANSPLANTATION IN THE MOUSE

In 2007, our laboratory reported the first orthotopic vascularized lung transplantation in the mouse (43). Left lungs were transplanted using cuffs analogous to techniques established in the rat model. This model, while technically demanding, has been subsequently reproduced by several laboratories (44, 45). Importantly, changes associated with ischemia reperfusion injury and acute rejection in orthotopically transplanted mouse lungs resemble those observed in human pulmonary grafts. Recent work from our laboratory has highlighted requirements for the acute rejection of lung grafts that differ from those for other organs. For example, unlike the case for cardiac allografts the acute rejection of lungs is not dependent on CD4<sup>+</sup> T cells (46). We have extended the observations of several investigators, who have demonstrated that adaptive immune responses can be initiated within the lung, by demonstrating that T cells are activated within pulmonary grafts shortly after transplantation and that lung grafts can be rejected independent of secondary lymphoid organs (25, 47). This sets the lung apart from other tissue and organ grafts, where initiation of graft rejection depends on activation of T cells within secondary lymphoid tissue, and may provide an explanation for the comparatively rapid rejection of lung grafts that had been observed in humans (48, 49). The orthotopic lung transplant model has also yielded some new insights into mechanisms contributing to ischemia reperfusion injury-mediated graft dysfunction. To this end, using intravital two-photon microscopy we have described a previously unrecognized role for monocytes in the regulation of neutrophil trafficking through lung grafts (50). In 2010, our laboratory described a method for orthotopic transplantation of right lungs in the mouse (51). As mice can tolerate a left pneumonectomy, this provides a model, where survival of the recipient animal depends on the function of the transplanted lung.

Similar to the case in rats, it has been a matter of debate whether mouse lungs can develop fibrotic airway

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lesions that resemble human bronchiolitis obliterans. In humans, graft vs. host disease after bone marrow transplantation frequently affects the lungs, where it can result in the development of obliterative bronchiolitis (52). In a mouse model of allogeneic bone marrow transplantation across MHC barriers, Panoskaltis-Mortari observed pulmonary graft vs. host disease with fibrotic occlusion of small airways (53). In contrast, investigators at Duke University demonstrated that intratracheal administration of lipopolysaccharide in recipients of allogeneic bone marrow transplants, while exacerbating inflammation in their lungs, does not result in lesions that resemble obliterative bronchiolitis (54). We have reported that lung allografts develop intense lymphocytic bronchiolitis and extensive interstitial fibrosis, but not fibrotic occlusion of their airways after transplantation into nonimmunosuppressed MHC-mismatched hosts (55). In fact, airway epithelial cells upregulated anti-apoptotic proteins and remained intact in these otherwise destroyed grafts. Recently, Fan and coworkers reported the development of an obliterative bronchiolitis model in a MHC-matched and minor histocompatibility antigen incompatible strain combination, which could be prevented through neutralization of IL-17 (44). However, lesions in the mouse lungs were polypoid, differing in appearance from the concentric fibrotic changes observed in the small airways of chronically rejected human lungs.

## 8. CONCLUSIONS

Over the past 50 years the development and refinement of animal models of lung transplantation have significantly evolved and have been essential for making human lung transplantation not just a reality, but a widely accepted therapy for patients with end stage pulmonary disease. As evidenced from the rich history of experimental pulmonary transplantation beginning with the pioneering work performed by Demikhov, Metras and Kindle to the recent development of new mouse models, improvements in clinical lung transplantation will continue to depend on insights gained from experimental models.

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**Abbreviations:** LTx: lung transplantation, Tx: transplantation

**Key Words:** Lung transplantation, Experimental models, Animal models, Dogs, Rats, Swine, Mice, Heterotopic, Orthotopic, Review

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