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LUNG TRANSPLANTATION MODELS FOR PRECLINICAL TRIAL (LITERATURE REVIEW)

N.S. Bunenkov[1–3], A.L. Akopov[2], S.V. Popov[3–5], A.A. Karpov[1, 7], S.M. Minasyan[1, 2], R.G. Gusejnov[3, 4, 6], V.V. Perepelitsa[3, 4], M.M. Galagudza[1, 2]

[1] Almazov National Medical Research Centre, St. Petersburg, Russian Federation
[2] Pavlov University, St. Petersburg, Russian Federation
[3] St. Luke's Clinical Hospital, St. Petersburg, Russian Federation
[4] St. Petersburg Medico-Social Institute, St. Petersburg, Russian Federation
[5] Kirov Military Medical Academy, St. Petersburg, Russian Federation
[6] St. Petersburg State University, St. Petersburg, Russian Federation
[7] Saint Petersburg Electrotechnical University "LETI", St. Petersburg, Russian Federation

Lung transplantation (LT) is the only treatment for many end-stage lung diseases. Despite significant progress in transplantology and surgery, LT remains a high-tech surgical procedure performed at select research centers. Primary graft dysfunction, acute rejection, and chronic lung allograft dysfunction are serious problems that can worsen lung transplant outcomes significantly. Using animal models in experimental studies to investigate these pathologic conditions is one of the more rational approaches. A literature review was conducted in order to select a suitable model that reproduces pathologic processes developing after LT. The literature was searched and analyzed in MEDLINE and Elibrary databases, and the US National Institute of Health guidelines for the period up to December 2023 were reviewed. It was found that the most frequently used models are small laboratory animal models (without LT) and large animal models (with LT).

Keywords: lung transplantation model, primary graft dysfunction, acute lung rejection, chronic lung rejection.

INTRODUCTION

Lung transplantation (LT) is the only method of treatment for many end-stage lung diseases [7]. Although transplantology and surgery have advanced significantly, LT remains a high-tech surgical procedure performed at select research centers. In 2022, 14 lung transplants were performed in Russia [17]. Primary graft dysfunction, acute rejection, chronic lung allograft dysfunction are major problems that can significantly worsen LT outcomes. One of the rational approaches is to study these pathologic conditions in experimental animal studies [16, 26, 27, 37].In this context, summarizing the available data on the choice of an appropriate model that replicates the pathological processes that develop following LT appears to be useful.

A model that substantially approximates the clinical situation in preclinical studies of LT is one in which LT is reproduced, i.e., organ harvesting from a donor animal, preservation and surgical implantation into a recipient (Fig. 1) [4, 23, 24, 28, 40].

However, this model has significant drawbacks, such as extreme technical complexity (e.g., suturing mouse or rat pulmonary vessels) and high cost (operating microscope and/or use of a heart-lung machine and oxygenators for dual pulmonary transplantation) [4, 15, 24, 28, 30].

It should also be noted that the surgical technique of LT in experiments on large and small animals differs significantly.

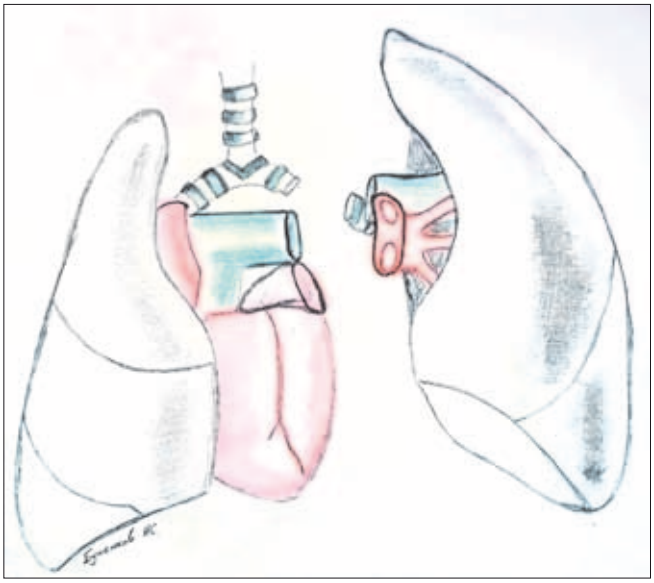

Fig. 1. Simplified diagram of single lung transplantation

Corresponding author: Nikolay Bunenkov. Address: 6–8, L'va Tolstogo str., St. Petersburg, 197022, Russian Federation. Phone: (812) 338-62-79. E-mail: bunenkov2006@gmail.com

We were able to gather data on the best models for LT preclinical investigations by analyzing the MEDLINE database, searching for publications in the Russian National Library and the scientific electronic library eLibrary from 2013 to December 2023.

The following models are used to study LT outcomes in the experiment:

1. Primary graft dysfunction:
   a. hilar occlusion;
   b. *ex vivo* perfusion of isolated lungs;
   c. working with cell cultures.
2. Acute rejection:
   a. heterotopic tracheal transplantation;
   b. orthotopic tracheal transplantation;
   c. orthotopic lung transplantation.
3. Chronic rejection:
   a. heterotopic tracheal transplantation;
   b. orthotopic tracheal transplantation;
   c. intrapulmonary tracheal transplantation;
   d. bone marrow transplantation;
   e. orthotopic lung transplantation.

Primary graft dysfunction (PGD) is an acute lung allograft injury of varying severity, ranging from mild capillary leak in alveoli to severe diffuse alveolar damage occurring within the first 72 hours after LT. PGD is characterized by radiographic evidence of pulmonary edema and a progressive hypoxemia for no apparent reason [12, 30].

***In vitro* modeling of PGD** involves exposure of relevant pulmonary cells in cultures (or cocultures) to acute hypoxia followed by reoxygenation [5, 10, 41, 43]. A model for storing cell cultures in a preservation solution (Perfadex) at 4 °C before allowing the cells to warm to room temperature, followed by reoxygenation in 37 °C culture media is also characterized. *In vitro* models have demonstrated that longer cold aerobic times enhance apoptosis, cytoskeletal remodeling, permeability, as well as upregulation of innate and adaptive immune pathways. *In vitro* studies allow rational use of resources, but they must be validated by *in vivo* experiments [6, 30, 51].

***In vivo* modeling of PGD** is possible using unilateral hilar occlusion followed by reperfusion (Fig. 2).

Under mechanical ventilation, a thoracotomy is performed, and a clamp or ligature is applied to the hilum. This model is widely used in small rodents [30, 35]. The sham-operated group includes animals with access to the hilum without clamping it. The disadvantage of this model is that it requires attention to the mechanical ventilation aspect, as this may lead to ventilation-induced lung injury that can add to ischemia-reperfusion injury (IRI). However, using the combination of ventilation-induced injury and IRI may parallel what can occur during human lung transplantation IRI [30, 50].

Another variation of the hilar clamp model involves isolating and clamping the pulmonary artery alone to preserve gas exchange, and is referred to as the non-hypoxic lung ischemia model [30].

Additionally, IRI has been studied using isolated, perfused rodent lung models in which the lungs are manipulated either *ex vivo* or *in situ*, and continuously perfused with synthetic media and ventilated in a temperature-controlled chamber [14, 18, 19, 30, 38].

Orthotopic single lung, autologous or allogeneic LT is also used to study PGD. In this approach, the cold ischemic time of the donor lung is intentionally prolonged up to 18 hours [25, 30]. In the case of allogeneic transplantation, in addition to PGD, the model allows studying antigen-independent processes preceding acute rejection.

The key criteria for evaluating the outcome of preservation are: 1) determination of the degree of lung edema (weighing the organ before/after reperfusion); 2) translocation of exogenously administered Evan's blue dye or radiolabeled or fluorescently labeled proteins, or by measuring the accumulation of endogenous proteins (total protein, albumin, IgM) in the bronchoalveolar lavage fluid [43]. Testing for cardiopulmonary hemodynamics, lung function, and oxygenation using indwelling devices, surface probes, and arterial blood gases can also be performed [29].

Lung IRI is accompanied by lipid peroxidation and has been associated with reduced arterial oxygenation, decreased compliance and increased pulmonary vascular resistance [30]. Fibrin deposition, elevated expression of plasminogen activator inhibitor-1 (PAI-1), extravasation and recruitment of immune cells, increased levels

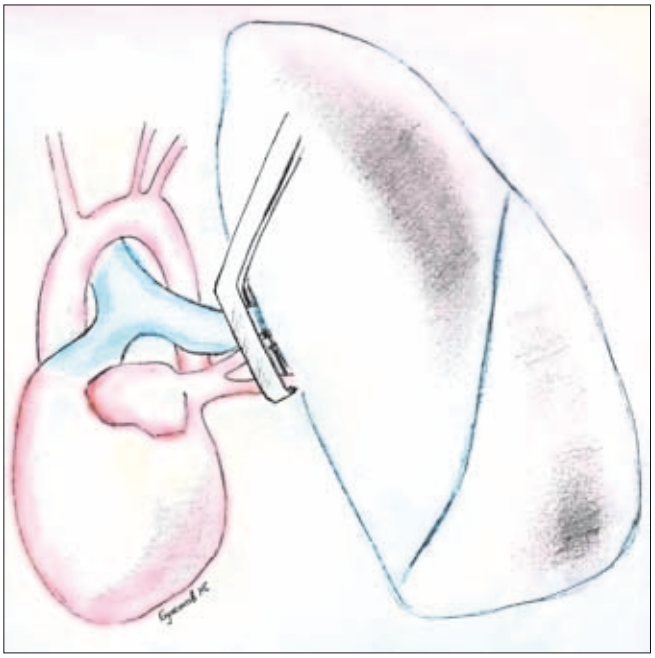

Fig. 2. Hilar occlusion is one way to study pathophysiologic changes after lung transplantation

of alarmins (particularly HMGB1), myeloperoxidase (a marker of neutrophil/mononuclear phagocyte activation and infiltration), and proinflammatory cytokines and chemokines are also noted in the lungs [29, 30, 39].

An advantage of the hilar transplant model is that it is less technically demanding than the single-lung transplant model. The model is a suitable platform for testing therapeutic delivery, lung rehabilitative potential, and biomarkers predictive of PG.

The orthotopic LT model is more informative when surgical technique needs to be developed and for studying post-transplant immune responses [30, 47].

The technique of allograft optimization through *ex vivo* lung perfusion (EVLP) has generated significant interest [19, 30, 38]. Most models using EVLP have utilized human lungs or large animal (mainly porcine) models with or without subsequent transplantation [30].

Imaging in PGD. Solid organ transplantation is accompanied by an immune response due to the presence of foreign antigens. Methods to image mobilization and activation of the immune system during this period are currently limited. However, SPECT (single-photon emission computed tomography) and PET (positron emission tomography) can detect and monitor a variety of pathophysiological processes, such as T cell activity by glucose uptake and neutrophil activation by binding to the formyl peptide receptor 1 (FPR1). The use of multiphoton intravital microscopy for imaging the PGD has also been reported [30].

Modeling of acute rejection. There are two forms of acute rejection (AR): acute cellular rejection (ACR) and antibody-mediated rejection (AMR) [30]. ACR is well characterized and is classified into two subtypes: 1) type A is a lymphocytic inflammatory cellular infiltrate that ranges from a mild perivascular infiltrate with no obvious tissue injury, to infiltrates that also involve the interstitium and air-spaces with prominent acute lung injury (ALI) with vasculitis; type B airway inflammation, namely lymphocytic bronchitis, is currently classified as either low-grade, with no tissue injury, or high-grade, in which there is a more extensive infiltrate associated with injury to the airway [3, 30]. Both A and B types of ACR are thought to increase the risk of development of bronchiolitis obliterans. The mechanisms of AMR are not as detailed, but are known to include C4d deposition in capillaries, neutrophilic capillaritis, intravascular macrophages, and ALI. Preclinical models are suitable for studying both ACR and AMR [30, 36].

Acute lung rejection in rats and mice. Since the 1960s, experiments using canine and rat orthotopic lung transplant models have examined various aspects of AR [30, 36]. For example, in a rat model, the onset of AR was more rapid in lungs when compared with heart grafts. The most commonly used mouse model to study lung rejection in the 1990s and early 2000s was heterotopic tracheal transplantation. In this model, allograft rejection demonstrates early inflammation, epithelial necrosis, and fibroproliferation in the airway lumen, which were not observed in the isografts. This model is valuable for studying ACR as well as chronic rejection.

Later, the orthotopic tracheal transplantation model was developed to study early changes in AR. The intrapulmonary tracheal transplant model as a model of AR has been useful to show the importance of intrapulmonary *de novo* lymphoid tissue [30].

Orthotopic LT in mice, while highly technically challenging, allows to observe histological changes as early as 3 days after transplantation from an MHC-incompatible donor, which are accompanied by perivascular and peribronchial mononuclear infiltration. These changes are similar to those observed in transbronchial biopsies of lung transplant patients suffering from AR [8, 30, 34]. The mouse model of orthotopic LT allows for the design of experiments to evaluate the role of respiratory pathogens on alloimmunity and AR responses. Pseudomonas aeruginosa respiratory infections can break immunosuppression-mediated lung allograft tolerance. This model is a suitable platform to evaluate new diagnostic modalities for AR. For example, fluorodeoxyglucose PET (FDG-PET) can be used to monitor acute lung allograft rejection owing to a high rate of metabolism of graft-infiltrating T cells [30]. Thus, the orthotopic mouse lung transplant model is an effective experimental platform to study mechanisms that contribute to AR, test noninvasive diagnostic modalities as well as to study immune tolerance, and evaluate strategies to prevent or treat this complication.

Antibody-mediated rejection. Elicitation of immune responses to the mismatched donor human leukocyte antigen (HLA) and breakdown of tolerance to tissue-restricted self-antigens pose a significant challenge to acceptance and continued graft function following organ transplantation. While the mechanisms of AMR are not firmly established, *de novo* donor-specific antibodies against HLA have been shown to predispose to the development of immune responses to lung self-antigens and bronchiolitis obliterans syndrome [22, 30]. To define mechanisms leading to anti-MHC–mediated development of rejection, a preclinical murine model was developed in which exogenous anti-MHC was administered into the native lungs and elicited production of antibodies and T cell responses specific for lung-associated self-antigens, type V collagen [col(V)], and K-α 1 tubulin, culminating in fibrotic pathology [22, 30]. Human lung transplant recipients develop antibodies against col(V), a protein predominantly found in the interstitium and not ordinarily exposed to the immune system [30]. In the rat LT model, allografts in minor histocompatibility

complex–mismatched recipients induce col(V)-specific T and B immunity after transplantation and appear to be an important source of autoantigen that autoantibodies ligate [30]. To study the exact roles of allo- and autoantibodies in acute and chronic rejection, orthotopic LT mouse models are more preferable [30, 33].

Microvascular injury and large airway disease. Autopsy results of patients who died with bronchiolitis obliterans syndrome showed that chronic rejection correlated with microvascular injury of the airway [30]. Such a relationship between microvascular destruction during AR and subsequent chronic rejection has been suggested with all solid-organ transplants.

The orthotopic tracheal transplantation (OTT) model is useful for the study of airway microvessels because the tracheal vasculature can be easily visualized in one tissue plane [9, 30, 44]. Tracheal transplantation is performed by simply cutting off the donor trachea and suturing it into the recipient in place of the excised section of the native trachea; alternatively, the donor trachea can be sewn in parallel to the native airway. During AR, this model has revealed that the airways undergo a transient loss of a functional microcirculation accompanied by localized tissue hypoxia and ischemia; although a functional microcirculation returns, these grafts cannot be rescued with immunosuppression once the vascular bed is transiently lost [30, 33]. Thus, the development of chronic rejection is also based on non-immune mechanisms. The proposed OTT model can incorporate fiber optic bioprobes that detect tissue oxygenation and perfusion over time. Another facet of a compromised circulation occurs at the time of transplantation in the airway anastomosis that does not include a restored bronchial circulation and is susceptible to dehiscence and infectio. The relative ischemia of the airway anastomosis is associated with a proclivity to infection, especially with *Aspergillus* and *Pseudomonas* [30].

The tracheal allograft model is a recognized and effective platform for preclinical studies, but it is less relevant nowadays because in clinical practice it can be decellularized and repopulated with recipient-derived cells prior to surgical implantation, an approach that should prevent AR and limit the need for chronic immunosuppression [1, 20, 30]. A more limited repopulation of donor-derived cells can be observed *in vivo* using the mouse OTT model. This line of study may have value in determining how both destructive and reparative processes occur through the migration of cell populations. OTT also facilitates lineage fate mapping studies to track the movement and transformation of various cell types in the allograft recipient. The mouse orthotopic lung transplant model also holds promise as an effective platform for evaluating the relative contribution of recipient cells in both the disease and repair of small airways and pulmonary parenchyma [30].

Lymphatic contribution to acute rejection. In a healthy lung, there is a highly complex network of lymphatics consisting of subpleural lymphatics largely over the lower lobes, and a deeper lymphatic network running along the major airways and the blood vasculature in the interstitial spaces [30, 45]. The visceral pleura and the neighboring lung tissue are drained through the superficial network into the hilar area of the lung where they connect with the deeper plexus of lymphatics. At the time of transplant, the bronchus, bronchial artery, pulmonary artery, and vein are severed at the level of the hilum. However, only the bronchus, pulmonary artery, and vein are re-anastomosed. A recent clinical study has revealed that, unlike chronic organ failure in kidney transplantation, lymphangiogenesis is not altered in patients with chronic lung allograft dysfunction [48]. In a canine lung transplant model, functional lymphatic drainage was restored at seven days after transplantation in isografts [30]. In allografts treated with immunosuppression, a functional lymphatic bed is observed between 2 and 4 weeks after transplantation. Lymphatic biology can also be effectively studied in the mouse orthotopic lung transplantation model, which revealed a marked decline in the density of lymphatic vessels, accompanied by accumulation of low-molecular-weight hyaluronan in mouse orthotopic allografts undergoing AR [34]. Work in this model has suggested a protective role for the promotion of lymphangiogenesis in the posttransplant period. In general, the role of the lymphatic vessels in acute and chronic rejection in lung transplants is poorly understood and requires more serious research.

CHRONIC LUNG ALLOGRAFT DYSFUNCTION (CLAD)

A high rate of chronic graft failure continues to be the most significant hurdle in improving long-term survival after lung transplant. In the 1980s, obliterative bronchiolitis (OB) was identified as a common pathology in chronically failing lung transplants; [30, 48]. OB was subsequently discovered to also be a complication of bone marrow transplant recipients. Histologic features of lung transplant–associated OB include anatomic restriction to membranous and respiratory bronchioles, vasculopathy with progressive myointimal thickening of the pulmonary arteries and veins [48]. Clinically, OB presents as a progressive obstructive decline in lung function termed bronchiolitis obliterans syndrome (BOS) [45, 48]. Approximately 50% of patients demonstrate this syndrome by five years after transplant [21, 30]. Clinical studies demonstrate a strong link between AR, specifically airway involvement with lymphocytic bronchitis, and BOS [13, 30].Other complications in the

posttransplant period such as PGD have also been linked to BOS. While BOS remains the predominant cause of CLAD, more recently a restrictive allograft syndrome (RAS) has been described. Patients presenting with RAS have been demonstrated to exhibit distinct histologic phenotypes such as pleural and subpleural fibrotic changes, intra-alveolar fibrinous exudate, and acute fibrinous pneumonia [30, 48]. A recent model has been developed utilizing fully MHC-mismatched orthotopic lung transplants treated with chronic immunosuppression and evaluated ten weeks after transplant; these mice develop some features of RAS [30].

***In vitro* modeling of obliterative bronchiolitis** involves the use of individual cell populations, such as bronchial epithelial cells, mesenchymal stromal cells, and airway smooth muscle cells [30]. Animal models involve allogeneic tissue transplantation to reproduce fibrotic airway remodeling by heterotopic or orthotopic transplantation of the trachea, as well as orthotopic transplantation of a single lung [30, 40].

Tracheal transplant models of chronic rejection. Initial discoveries in posttransplantation OB have been fueled primarily by the heterotopic tracheal transplantation (HTT) model in which a harvested donor trachea is transplanted into a subcutaneous pouch in the dorsal surface of the neck or omentum. [30, 46]. Following the IRI and AR phases of injury, these transplants undergo a fibroproliferative phase with partial denuding of the epithelium at day 14 and fibro-obliteration of the allograft trachea at day 21 [30, 46]. Conversely, the isografts have a healing airway graft at day 7; however, this is followed by essentially normal isografts by days 14 and 21 [30]. This model has been utilized in both rats and mice, although more consistent fibrosis is noted in rat versus mouse tracheas and is suitable for studying alloantigen-mediated airway fibrosis. Airway luminal obliteration can be quantified at various time points after transplantation. Fibrosis can be evaluated by staining with picrosirius or trichrome. In addition, the trachea can be treated with enzymes followed by flow cytometry of the cell suspension to assess cellular composition. Also, this model is convenient for evaluation of the microvasculature and lymphatics, providing an avenue to study their role in OB development.

The major criticism of the tracheal transplantation model is that fibrotic obliteration is being modeled in a large cartilaginous airway that is histologically distinct from the small airways that are the site of human OB [30, 49]. Its relevance is also somewhat limited by the absence of a normal air interface and native mediastinal lymphatic drainage. Most importantly, human OB develops in a complex *in vivo* environment with distinct cellular niches that cannot be reproduced in a tracheal transplant placed in an extrapulmonary environment [30, 42]. Thus, the HTT model is useful as a high-throughput screen for alloimmunity-induced fibrosis, but findings obtained with this procedure must be interpreted with caution [30, 49].

In the OTT model, epithelial regeneration from migration of recipient-derived epithelial cells limits the development of fibrotic occlusion or OB. Although obliterative lesions are not observed, OTTs develop lymphocytic bronchitis (a large airway precursor of BOS) as well as subepithelial fibrosis [11, 30].

The intrapulmonary tracheal transplant model via thoracotomy has also been used to attempt to simulate airway fibrosis. Another good model involves transplantation of human trachea together with peripheral blood mononuclear cells into an immunodeficient mouse [30].

Orthotopic lung transplant models of chronic rejection. Orthotopic single-lung transplantation in rats is successfully used to study acute rejection. However, it significantly limits the possibilities of modeling chronic rejection, because it does not allow to induce OB. LT across MHC mismatch and across minor histocompatibility complex–mismatched combinations has been shown to recapitulate some aspects of OB pathology in allografts. However, there is some disagreement in the transplant community about how closely OB-like lesions generated in orthotopic lung transplants recapitulate the human lesion [30, 42]. Other donor-recipient combinations have also been demonstrated to develop OB-like lesions at late time points of 2 to 3 months after transplantation. However, there appears to be a difference in animals obtained from different vendors as well as concerns of reproducibility across centers [30, 49]. Therefore, at present, a consensus on a definitive rat lung transplant model to study OB pathogenesis has not emerged [30, 42]. Attempts to approximate the rat model to the clinical situation via intratracheal gastric fluid challenge after allogeneic LT have been reported [31].

The use of MHC-mismatched mice allows to obtain severe AR by day 7 after LT, and this nearly complete destruction of the lung prevents longer time-point evaluation for development of OB, so that the animal dies before it develops OB [30, 49]. This issue was circumvented and a transplant involving minor histocompatibility complex mismatch developed only mild rejection within 1 week. Peribronchial and intraluminal fibrotic lesions were described at days 21 and 28 [30]. However, these airway fibrotic lesions were noted in only 50% of the transplanted mice and were limited to a small number of airways in the allografts [31]. Use of immunosuppression (cyclosporine + steroids) to prolong graft life in MHC mismatch has also allowed for investigation of the development of OB pathology. This model only generates OB-like lesions in 25–50% of the mice, with many mice demonstrating no evidence of OB or regaining normal histology after 12 weeks; the remaining

animals showed no OB signs or a histological picture of a healthy lung [30, 49].

Fibrotic remodeling of the allograft is the predominant cause of CLAD; hence, a relevant animal model for investigating CLAD must recapitulate allograft fibrogenesis and allow for meaningful targeting of specific pathways. Mesenchymal cells act as the first link in the development of fibrosis [30, 32]. These donor graft-derived cells appear to be the predominant contributors to OB lesions [30, 32]. In a heterotopic tracheal transplantation model, mesenchymal cells contributing to fibrosis originated from recipient cells [30, 49]. In an allogeneic orthotopic LT model in mice, it was shown that collagen I–expressing cells were of donor origin [30]. Thus, in the study of the role of mesenchymal cells in chronic rejection, the whole-lung transplant model holds an advantage over tracheal transplantation because it is more reflective of the clinical situation [30, 32]. The choice of models for studying the mechanisms of post-LT rejection is presented as a scheme in Fig. 3.

ASSESSMENT OF THE DEGREE OF GRAFT INJURY IN EXPERIMENTAL MODELS

The American Thoracic Society in 2022 released an official document with guidelines for the assessment of ALI in animal experiments [29]. The document is based on the results of a survey of 50 experts working in the field of clinical (pulmonology, intensive care, pediatrics, immunology, etc.) and basic medicine (cell biology, physiology) studying ALI [29].

In clinical practice, ALI is described using the term "acute respiratory distress syndrome (ARDS)", but small animal models lack some of the ARDS manifestations and therefore cannot fully reproduce the clinical situation [2, 29]. Large animals are more likely to reproduce the clinical manifestations of ARDS, so the results of experiments in large animals are important for clinical application [29].

The American Thoracic Society recommends the use of an ALI model that demonstrates the following four domains: 1) histological evidence of tissue injury, 2) alteration of the alveolar–capillary barrier, 3) presence of an inflammatory response, and 4) physiologic dysfunction (Table) [29].

According to the American Thoracic Society guidelines, a preclinical model of acute lung injury should demonstrate at least three of the above four domains for lung injury [29]. It was proposed that demonstrating ALI requires measurement of at least one parameter for each of the four domains. In the case of preclinical drug testing or translation into clinical practice, demonstration of all four ALI domains with the presentation of at least one indicator for each domain is recommended [29].

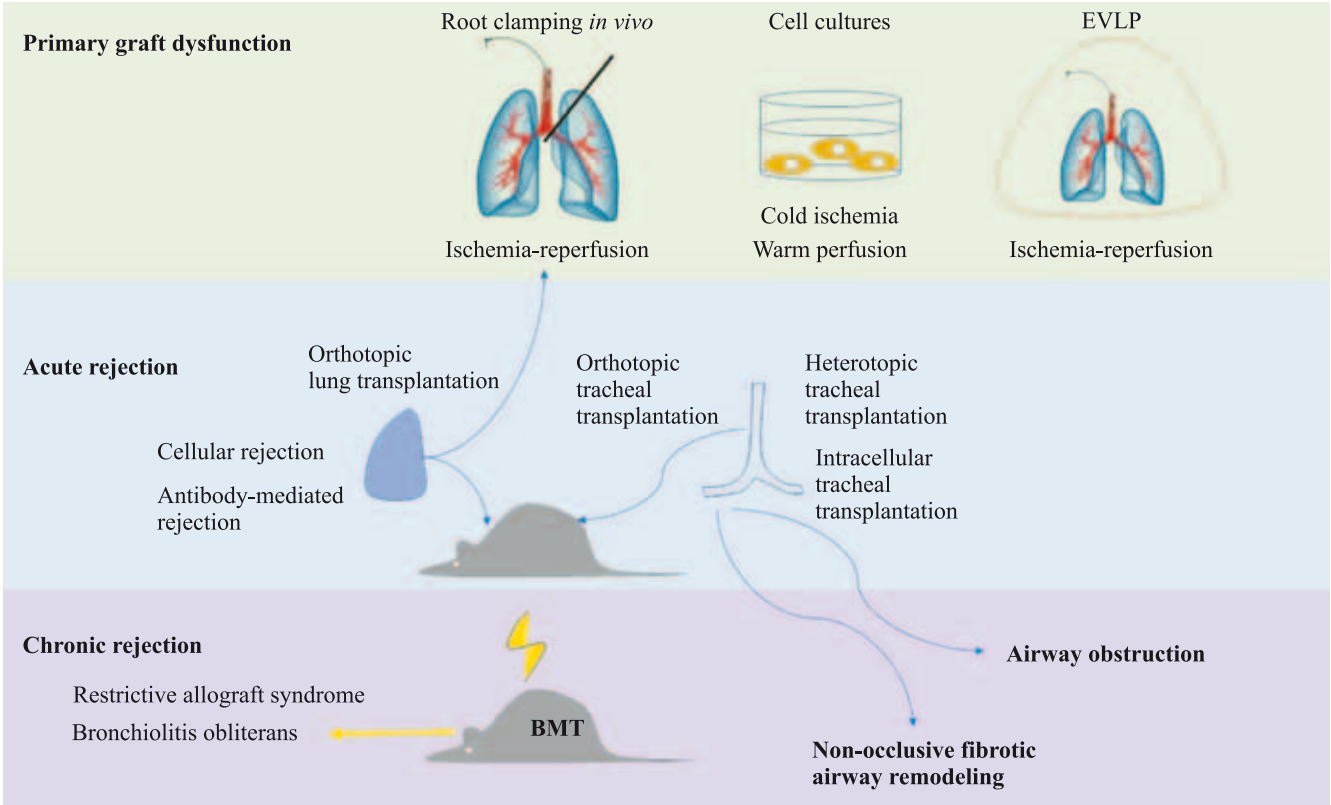

Fig. 3. Modeling of pathological processes after lung transplantation. Note: BMT, bone marrow transplantation; EVLP, *ex vivo* lung perfusion

Table

Parameters reflecting the degree of lung injury in animal experiments

Domains	Domain recommendations
Histological evidence of tissue injury	– Pulmonary alveolar proteinosis – Evidence of alveolar epithelial injury (cell death, epithelial denudation, ATII proliferation) – Neutrophil infiltration of the alveolar space – Thickening of alveolar septae and/or interstitial edema – Diffuse alveolar damage pattern – Respiratory distress syndrome – RBCs in the airways or extravasated red cells – Neutrophil infiltration of alveolar septae or interstitium – Perivascular inflammation, including intravascular accumulation of neutrophils – Perivascular edema – Hepatization – Weakening or loss of tight intercellular junctions – Presence of microthrombi
Alteration of the alveolar-capillary barrier	– Elevated BAL albumin, IgM, or other large circulating protein – Increased lung wet-to-dry weight ratio – Elevated BAL total protein – Evan's blue dye accumulation in lung homogenate – Increased Pulmonary vascular permeability index and/or filtration coefficient – Rate of accumulation of tagged marker (fluorescent probe, I-131 albumin, etc.) in the airspace – Transport of large-molecular-weight substance (≥70 kD, e.g. Dextran) – Accumulation of airspace-injected tracers into the circulation – Circulating markers of epithelial and/or airway injury (RAGE, SP-D, KL-6) – Hemorrhage and/or RBCs in airspace – Elevated BAL RAGE – Transport of a very large (~300 kD) tracer across barrier – Decreased surfactant function
Presence of an inflammatory response	– Increase in chemokines or cytokines in BAL or lung tissue – Increased neutrophil count in BAL or lung tissue (absolute count or by neutrophil elastase or myeloperoxidase content) – Increased pro-inflammatory monocyte and macrophage (and/or lymphocytes) subpopulations in BAL or lung tissue – Endothelial cell adhesion molecule expression or mediator release – Changes in acute response genes – Inflammasome activation – Mitochondrial dysfunction – Neutrophil extracellular traps
Evidence of physiological dysfunction	– Arterial blood gas measurements of oxygenation – Decreased compliance (distensibility) – Changes in alveolar fluid clearance – Decreased oxygenation measured by non-invasive methods – Respiratory deterioration – Lung changes in lung imaging – Dead space and/or partial pressure of carbon dioxide – Weight loss – Systemic organ dysfunction – Impaired systemic hemodynamics

Note: ATII, Type II alveolar epithelial cell; RBCs, red blood cells; KL-6, Krebs von den Lungen-6; RAGE, receptors for advanced glycation end products; SP-D, surfactant protein D; BAL, bronchoalveolar lavage; MCP, monocyte chemotactic protein.

The term "acute injury" implies the establishment of a certain time frame from the moment of exposure to the damaging factor to the manifestation of the above-described injury signs. However, at present, the exact time interval is not defined [29, 45]. Some authors indicate that an injury should be considered acute if the signs of injury occurred within 24 hours after exposure to an unfavorable factor [29]. Others accept 72 hours, up to 7 days, and even up to 10 days [29]. Thus, according to the American Thoracic Society guidelines, the time interval can be chosen depending on the goals of the study, believing that an interval of 24 hours is too strict [29].

CONCLUSION

In vitro and *in vivo* models may be recommended for studying PGD, specifically hilar transection surgery without LT, or orthotopic LT. The use of EVLP is also possible.

An HTT model and the more technically demanding OTT are recommended for studying acute rejection. Orthotopic LT is also suitable for investigating immunological tolerance.

Obliterative bronchiolitis in terminal bronchioles can be modeled in experiments with HTT. OTT allows the study of large airway lesions in chronic rejection, but occlusive lesions are not observed. Orthotopic allogeneic LT in mice is an extremely technically demanding model for studying chronic rejection, and is characterized by instability and heterogeneity in the results obtained. It should also be noted that the need for a model that reproduces restrictive allograft syndrome has not yet been met.

At least three or four criteria are used in experimental models to determine the severity of graft injury after LT, including histological evidence of tissue injury, alteration of the alveolar–capillary barrier, inflammatory response, and physiologic dysfunction. It is necessary to continue developing models that replicate the pathogenic processes that patients experience following LT.

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The authors declare no conflict of interest.

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