American Journal of Transplantation 24 (2024) 1930–1940

Contents lists available at ScienceDirect

American Journal of Transplantation

journal homepage: www.amjtransplant.org

Minireview

Murine orthotopic lung transplant models: A comprehensive overview of genetic mismatch degrees and histopathological insights into chronic lung allograft dysfunction

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ARTICLE INFO

Keywords:
lung transplantation
mouse model
chronic lung allograft dysfunction

ABSTRACT

Currently, lung transplantation outcome remains inferior compared to other solid organ transplantations. A major cause for limited survival after lung transplantation is chronic lung allograft dysfunction. Numerous animal models have been developed to investigate chronic lung allograft dysfunction to discover adequate treatments. The murine orthotopic lung transplant model has been further optimized over the last years. However, different degrees of genetic mismatch between donor and recipient mice have been used, applying a single, minor, moderate, and major genetic mismatch. This review aims to reassess the existing murine mismatch models and provide a comprehensive overview, with a specific focus on

Abbreviations: BOS, bronchiolitis obliterans syndrome; CLAD, chronic lung allograft dysfunction; LTx, lung transplantation; MHC, major histocompatibility complex; OB, obliterative bronchiolitis; RAS, restrictive allograft syndrome.

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https://doi.org/10.1016/j.ajt.2024.07.033
Received 8 March 2024; Received in revised form 18 July 2024; Accepted 29 July 2024
Available online 2 August 2024

their eventual histopathological presentation. This will be crucial to leverage this model and tailor it according to specific research needs.

1. Introduction

Lung transplantation (LTx) is the last treatment option for well-selected patients with end-stage pulmonary disease. Although the 1-year survival is approximately 85%, long-term outcome remains poor with a median survival of 6 years,[1] mainly caused by chronic lung allograft dysfunction (CLAD).[1,2] CLAD is defined as persistent decline (≥20%) in forced expiratory volume in 1 second, compared with the baseline.[3-5] Within CLAD, different phenotypes have been described: an obstructive form (bronchiolitis obliterans syndrome, BOS), a restrictive form (restrictive allograft syndrome, RAS), a mixed form (combination of obstructive and restrictive patterns), and an undefined form (patients not meeting the criteria).[3-6] BOS is typically considered a small airway disease and has a prevalence of 65% to 75%. On histopathological examination, it is characterized by obliterative bronchiolitis (OB), a collagenous obliteration of the lumen of smaller sized bronchioles. In contrast, RAS is diagnosed in 15% to 25% of CLAD patients and is histologically defined as (sub) pleural fibrotic changes and alveolar fibroelastosis with OB lesions.[3,4,6,7] To unravel the complex pathophysiological processes leading to these CLAD phenotypes, preclinical animal models have been developed.[7-9]

Indeed, various animal models have been used, including heterotopic and orthotopic trachea transplantation and orthotopic LTx in both rodents and larger animals, each with advantages and limitations, which are comprehensively reviewed elsewhere.[7-9] To obtain a model that mimics the clinical (BOS/RAS) situation as much as possible, orthotopic LTx is considered the most relevant model. This model has the advantage of simulating the transplantation of a vascularized organ in its relevant physiological environment. We will focus on murine LTx models as they are easily genetically modifiable, and the reproducibility of rat LTx models has been questioned.[9-11]

In 2007, the first orthotopic LTx in mice was described.[12] In subsequent investigations, a wide variety of combinations of different mouse strains have been used as donor or recipient animals, leading to different end results. This review aims to provide an overview of murine orthotopic LTx models of chronic rejection with a particular emphasis on the different histocompatibility complex-mismatched strain combinations.

2. Comparison of mismatch models and strains

In human transplantation, the degree of genetic mismatch between donor and recipient represents an important risk factor contributing to graft rejection, determining long-term survival.[13] Molecules that are responsible for rejection of transplanted tissue and the recognition of self and nonself are encoded in a highly polymorphic genomic region, the major histocompatibility complex (MHC).[14,15] In mice, the MHC is located on chromosome 17 and is named 'H2'.[15] Due to inbreeding, laboratory mice within each strain are homozygous, and each strain carries a unique haplotype. This is the result of brother-sister mating over 20 generations or more, leading to fixation of specific sets of alleles and causing a lack of genetic variation at most loci.[16-18] However, it is important to note that recent research has shown that inbred mice are not entirely isogenic and thus may still display some degree of genetic variation.[19] Different degrees of genetic mismatch between donor and recipient animals in murine orthotopic LTx models have been used so far: a single, minor, moderate, and major genetic mismatch (Figure 1), yielding variation in the induced pathology. An overview of the discussed studies for each model and their histologic outcomes can be found in Tables 1,[12,20-22] 2,[21,23,24,25-28] 3,[29,30] and 4.[31] Of note, allografts were consistently compared with a control group involving syngeneic transplantation. However, as these controls never showed significant pathology, they are not further discussed.

2.1. Major mismatch model

In 2007, Okazaki et al[12] introduced the first murine orthotopic LTx model. A vascularized, aerated left LTx was achieved, using cuff techniques for anastomoses of the bronchus, pulmonary artery, and vein. As the allogeneic transplantations were performed using the C57BL/6 ($H2^b$) into CBA/Ca ($H2^k$) and BALB/c ($H2^d$) into C57BL/6 ($H2^b$) strain combinations, total mismatches in class I and II MHC antigens are obtained, inducing a full MHC mismatch. Within 7 days posttransplant, the recipient mice developed severe acute rejection together with apoptosis of airway epithelial cells. In addition, the adventitial zone of the pulmonary arteries and veins near the hilum showed edema.[12] As this leads to almost complete destruction of the graft, it is less suitable for the evaluation of later time points.[7,12] This problem was addressed by 2 research groups suggesting alternate models.[20,23] A first approach used a minor histocompatibility complex-mismatched strain combination (see below).[23] Shortly thereafter, the issue of severe rejection was circumvented by using the major mismatch model [BALB/c ($H2^d$) into C57BL/6 ($H2^b$)] with daily immunosuppression (steroids and cyclosporine). They succeeded in mildly suppressing acute rejection and prolonging graft life, allowing investigation of OB-like pathology. Initially, 2 weeks posttransplant, acute rejection could be observed in all allografts, accompanied with an enlargement of the bronchovascular axes. However, this decreased in the following weeks and was absent at week 12. From 4 weeks on, 20% to 25% of the allografts showed distinct OB lesions. Over time, these evolved from immature and mainly fibrinous without cells to more organized lesions containing cells and angiogenesis to end-stage, dense collagenous lesions. The authors speculated that the variation in the model might be due to variability in cyclosporine serum levels because lower levels were

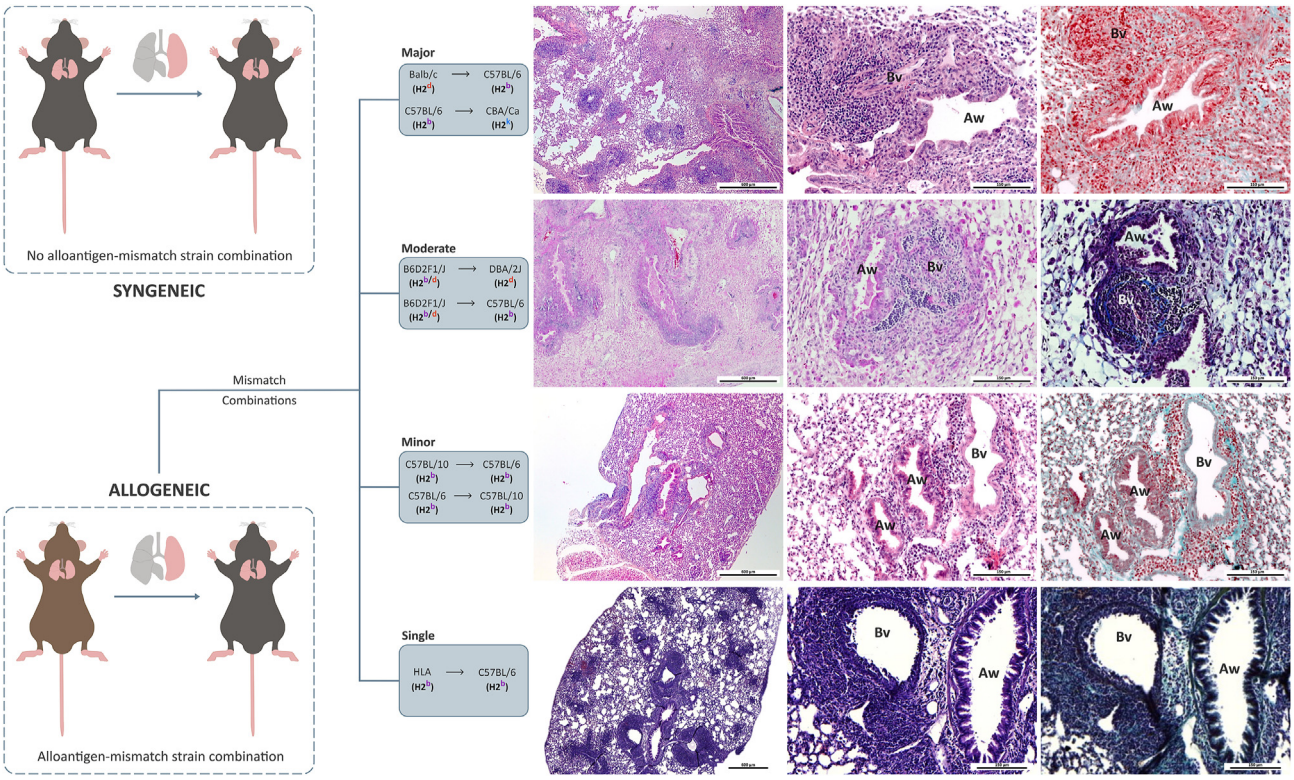

Figure. In this figure, both syngeneic and allogeneic orthotopic LTx are presented. The syngeneic orthotopic LTx is shown at the top left, where left lungs are transplanted between animals of the same inbred strain. At the bottom left, an allogeneic orthotopic LTx is depicted. Left lungs are transplanted between animals of the same species, which are genetically disparate individuals. Depending on the strains that are used, different degrees of genetic mismatch between donor and recipient animals have been defined, resulting in a major mismatch model, a moderate mismatch mode, a minor mismatch model, and a single mismatch model. Within the boxes, examples of strain combinations used in literature are provided along with their MHC haplotypes (indicated with a color). Additionally, for each model, an histologic overview image is shown, stained with HE (left), followed by a magnified view of the bronchovascular bundle stained with HE (middle) and Masson's trichrome (right). Major and moderate mismatch models (8 and 6 weeks post-LTx, respectively): Grafts show severe lymphocytic infiltration surrounding airways (Aw) and blood vessels (Bv). Additionally, mild perivascular and peribronchiolar fibrosis can be found around the bronchovascular bundle. In both instances, the vascular lumen is almost entirely obliterated. In the lung parenchyma, areas of fibrosis are noted. Minor mismatch model (8 weeks post-LTx): Graft shows mild to moderate lymphocytic infiltration discontinuously arranged around airways (Aw) and vessels (Bv). No remarkable fibrosis is seen. Single mismatch model (2 weeks post-LTx): Graft shows severe infiltration surrounding blood vessels (Bv) and moderate infiltration around the airways (Aw). HE, hematoxylin and eosin; LTx, lung transplantation; MHC, major histocompatibility complex.

seen in mice with OB lesions.[20] Yamada and colleagues,[21] who applied similar immunosuppressive treatment, subsequently observed obliterated airways in 83% of their major mismatch allografts [BALB/c ($H2^d$) into C57BL/6 ($H2^b$)] which manifested as intraluminal chronic airway fibrosis. Furthermore, all allografts demonstrated a high degree of peribronchiolar and perivascular lymphocytic infiltration (A4), as well as significant accumulation of fibrotic tissue around the airways and in the parenchyma, significant pleural fibrosis, and thicker airway walls, reminiscent of RAS.[21] Moreover, there are studies applying this major mismatch model using transgenic mice as donor.[22,32] By using transgenic mice, the role of specific key players can be further examined to better understand underlying mechanisms. In 2019, Liu et al[22] utilized triple-transgenic mice as donor [3T-FVB ($H2^q$) into C57BL/6 ($H2^b$)] to investigate whether the loss of club cells promotes the development of OB lesions. By day 16 post-LTx, high grade airway inflammation and severe OB lesions were observed in the allografts with club cell depletion after transient doxycycline ingestion, whereas syngeneic recipients showed, at most, mild inflammation without OB or peribronchial lesions as the club cell depletion-mediated bronchiolar injury was repaired.[22]

2.2. Minor mismatch model

As it appeared that the major mismatch model induced almost complete destruction of the graft, Fan et al[23] used a minor histocompatibility antigen mismatched model by transplanting the left lungs of C57BL/10 ($H2^b$) into C57BL/6 ($H2^b$) mice, leaving MHC class I and II antigens matched. In this minor mismatch model, mild acute rejection manifested 7 days posttransplant, which progressed to moderate acute rejection by day 14 and severe acute rejection by days 21, 28, and 35. In addition, by day 14, OB lesions could be observed in 33% of grafts. At day 21, OB was detected in 55% of the recipients and by day 28, in 44%. These lesions showed progression over time, evolving from subepithelial or polypoid fibrotic formations affecting the bronchiolar lumen on day 14, to larger lesions that almost completely

Table 1
Overview of published studies using the major mismatch orthotopic LTx model.

Major mismatch model								
Author	Strains		Time point (post-LTx)	Results				
	Donor	Recipient		Airways	Blood vessels	Pleura	Parenchyma	Epithelial cells
Okazaki et al[12] (2007)	BALB/c C57BL/6	C57BL/6 CBA/Ca	Day 7	N/A	- Acute rejection grade A3 - Edema in adventitial zone of pulmonary arteries and veins	N/A	Interstitial mononuclear infiltrates	- Apoptosis - Subepithelial lymphocytic infiltration in distal airways
De Vleeschauwer et al[20] (2012)	BALB/c	C57BL/6	Day 28	- Lymphocytic bronchiolitis in >75% of the allografts - OB in 25%-50% of the allografts	Acute rejection	N/A	Endogenous lipid pneumonia with foamy macrophages and interstitial inflammation	Damaged in areas where fibrotic plugs grow into the airway lumen
Yamada et al[21] (2018)	BALB/c	C57BL/6J	Day 56	- Severe lymphocytic bronchiolitis - Intraluminal airway fibrosis in 83% of the allografts - Peribronchiolar fibrosis	Acute rejection grade A4	Pleural fibrosis	Parenchymal fibrosis	N/A
Liu et al[22] (2019)	3T-FVB	C57BL/6 exposed to doxycycline	Day 16	- High grade lymphocytic bronchiolitis - Severe OB lesions	N/A	N/A	N/A	Club cell depletion

For each study, results of the allograft group on day 28 post-LTx are shown. In the event day 28 was not examined, the last time point post-LTx is discussed.
LTx, lung transplantation; N/A, not applicable; OB, obliterative bronchiolitis.

Table 2
Overview of published studies using the minor mismatch orthotopic LTx model.

Minor mismatch model								
Author	Strains		Time point	Results				
	Donor	Recipient	(post-LTx)	Airways	Blood vessels	Pleura	Parenchyma	Epithelial cells
Fan et al[23] (2011)	C57BL/10	C57BL/6	Day 28	- Mild lymphocytic bronchiolitis - OB in 44% of the allografts together with peribronchiolar fibrosis	Acute rejection grade A4	N/A	N/A	N/A
Suzuki et al[24] (2012)	C57BL/10	C57BL/6	Day 28	OB in 42.1% of the allografts	Acute rejection grade A3	N/A	N/A	N/A
	C57BL/6	C57BL/10		OB in 12.5% of the allografts	Acute rejection grade A3	N/A	N/A	N/A
Yamada et al[21] (2018)	C57BL/10J	C57BL/6J	Day 56	- Moderate lymphocytic bronchiolitis - Mild fibrosis in peribronchiolar areas	Moderate acute rejection	Pleural fibrosis	No significant fibrosis	N/A
	C57BL/10J	C57BL/6N		Moderate lymphocytic bronchiolitis	Moderate perivascular inflammation	Pleural fibrosis	No significant fibrosis	N/A
Martinu et al[25] (2019)	C57BL/10J	C57BL/6J	Day 28	*33% of the grafts are severely affected:* - Lymphocytic bronchiolitis - OB in $\geq$50% of the airways - Peri-airway fibrosis	- Moderate to severe acute rejection - Severe vascular fibrosis - Endothelialitis	Significant pleural thickening and fibrosis	Parenchymal fibrosis with fibroelastosis	Epithelial hyperplasia and flattening
				22% of the grafts are mildly affected: - Lymphocytic bronchiolitis - OB in $\leq$15% of the airways - Minimal peri-airway fibrosis	- Minimal acute rejection - Endothelialitis - Occasional vascular fibrosis	- Minimal pleural fibrosis - Mildly thickened pleura	Rare areas of patchy parenchymal fibrosis	Minimal epithelial hyperplasia and flattening

(*continued on next page*)

	C57BL/6J	C57BL/10J		- Lymphocytic bronchiolitis - OB in 30% of the allografts - Peri-airway fibrosis	Vascular fibrosis	Pleural fibrosis	Parenchymal fibrosis with fibroelastosis	Epithelial hyperplasia and flattening
Watanabe et al[26] (2019)	C57BL/10	C57BL/6 with prolonged storage	Day 28	- Lymphocytic bronchiolitis - OB - Peribronchial fibrosis	Acute rejection	No significant pleural thickening	Parenchymal fibrosis	N/A
Watanabe et al[27] (2023)	C57BL/10	C57BL/6 exposed to LPS	Day 28	- Lymphocytic bronchiolitis - Peri-airway fibrosis - OB	Acute rejection grade A3	N/A	Parenchymal fibrosis	Epithelial destruction and hyperplasia
Hata et al[28] (2022)	C57BL/6J$^{Thy\text{-}1\ -/-}$	C57BL/10J	Day 30	- OB - Collagen deposition	N/A	N/A	- Parenchymal fibrosis - Collagen deposition	N/A

For each study, results of the allograft group on day 28 post-LTx are shown. In the event day 28 was not examined, the last time point post-LTx is discussed.
LPS, lipopolysaccharide; LTx, lung transplantation; N/A, not applicable; OB, obliterative bronchiolitis.

Table 3
Overview of published studies using the moderate mismatch orthotopic LTx model.

Moderate mismatch model								
Author	Strains		Time point	Results				
	Donor	Recipient	(post-LTx)	Airways	Blood vessels	Pleura	Parenchyma	Epithelial cells
Mimura et al[29] (2015)	B6D2F1/J	DBA/2J	Day 28	- OB in all allografts - Peribronchial fibrosis	Perivascular fibrosis	N/A	N/A	- Epithelial denudation - Subepithelial fibrosis
Misumi et al[30] (2020)	B6D2F1/J	C57BL/6J	Day 28	- Lymphocytic bronchiolitis - Peribronchial fibrosis - Occasional OB-like fibroblastic plugs	- Acute rejection - Perivascular fibrosis - Endothelialitis and endothelial cell damage	Pleural fibrosis	Foamy macrophages in alveoli	N/A

For each study, results of the allograft group on day 28 post-LTx are shown.
LTx, lung transplantation; N/A, not applicable; OB, obliterative bronchiolitis.

blocked the airways on days 21 and 28, but were absent at day 35.[23] This variability was hypothesized to be due to the variable expression of minor histocompatibility antigens.[23,33] Therefore, if OB is minor antigen-dependent, this can lead to a nonuniform occurrence of OB in the allografts.[23] Similarly, Suzuki et al[24] did not observe OB uniformly in all recipient mice. At days 21 and 28 posttransplant, OB was observed in 42.1% of the allografts accompanied by moderate acute rejection (A3). In addition, transplantation was also performed with C57BL/6 as donor while C57BL/10 served as recipient. Now, OB was only found in 12.5% of the recipient mice at days 21 and 28 posttransplant.[24] This difference was also observed by Martinu et al.[25] At day 28 posttransplant, OB was observed in 66% of the recipient C57BL/6J grafts with C57BL/10J donors, whereas it was only present in 30% of the recipient C57BL/10J grafts with C57BL/6J donors. In the C57BL/10J to C57BL/6J group, 44% were affected with severe obliterative airway fibrosis, 22% showed mild obliterative airway fibrosis, and 33% demonstrated no OB. In recipients with severe obliterative airway fibrosis, >50% of the airways were obliterated and showed peribronchial fibrosis, extensive parenchymal fibrosis with a fibroelastosis pattern, thickened fibrotic pleura, severe vascular fibrosis, moderate to severe acute rejection, endothelialitis, and epithelial hyperplasia and flattening. The allografts that were mildly affected showed fewer lesions (<15% of the airways were obliterated) and contained relatively normal lung tissue in the other areas.[25] Once more, this indicates the variability in airway fibrosis in this minor mismatch model. Yamada et al[21] further investigated 2 minor antigen mismatched combinations: C57BL/10J ($H2^b$) into C57BL/6J ($H2^b$) and C57BL/10J ($H2^b$) into C57BL/6N ($H2^b$). Both recipient animals are substrains derived from C57BL/6 and were obtained from 2 different suppliers. With this approach, they aimed to assess whether genetic drift in these substrains could affect their susceptibility to airway fibrosis and whether diverse origins of the mice could impact phenotype expression. However, neither C57BL/6J nor C57BL/6N recipients showed airway obliteration at 8 weeks posttransplant, wheras both groups showed the same degree of moderate perivascular and peribronchiolar inflammation.[21] However, it later became clear that only performing minor mismatch transplants might not be sufficient, and an additional trigger seems needed. Indeed, Watanabe et al[26] showed that only grafts C57BL/10 ($H2^b$) into C57BL/6 ($H2^b$) with prolonged cold and warm ischemia leading to ischemia-reperfusion injury exhibited OB, peribronchial thickening, and parenchymal fibrosis, whereas grafts with minimal storage showed no pathology. This was later confirmed as repeated intratracheal administrations of lipopolysaccharides posttransplant led to significant airway obliteration, peribronchial fibrosis, and parenchymal fibrosis 28 days posttransplant, as well as significant perivascular and peribronchiolar acute rejection (A3), but not in phosphate-buffered saline-instilled controls, which only showed minimal fibrosis and acute rejection.[27] This was also corroborated by the study of Hata et al[28] where repeated lipopolysaccharide administration contributed to a significantly increased OB and fibrosis score compared to phosphate-buffered saline-treated recipients. Moreover, LTx was

Table 4
Published study using the single mismatch orthotopic LTx model.

Single mismatch model								
Author	Strains		*Time point*	Results				
	Donor	Recipient	*(post-LTx)*	Airways	Blood vessels	Pleura	Parenchyma	Epithelial cells
Smirnova et al[31] (2019)	C57BL/6-Tg(HLA-A2.1)$^{1Enge/J}$	C57BL/6	2 mo	- Lymphocytic bronchiolitis - ECM deposition around bronchi	- Acute rejection - ECM deposition around vessels	N/A	N/A	Subepithelial fibrosis

For this study, results of the allograft group 2 months post-LTx are shown.
ECM, extracellular matrix; LTx, lung transplantation; N/A, not applicable; OB, obliterative bronchiolitis.

not only performed from C57BL/6J into C57BL/10J mice. They also used Thy-1 knockout mice as donors to investigate the role of Thy-1 in CLAD [C57BL/6J$^{Thy\text{-}1\ -/-}$ ($H2^b$) into C57BL/10J ($H2^b$)], which is a surface glycoprotein that controls fibroblast differentiation and activation. Compared to LTx with C57BL/6J wild type grafts, loss of Thy-1 resulted in increased fibrosis, OB lesions, parenchymal fibrosis, and collagen deposition.[28] Additionally, Kawashima and colleagues[34] conducted a retrospective review of their minor model [C57BL/10 ($H2^b$) into C57BL/6 ($H2^b$)] to explore important determinants of the observed pathologic variability. They found that pathologic outcomes significantly differed within and across surgeons.

2.3. Moderate mismatch model

All the above studies indicate that there is a lot of variability and a very delicate balance between either futile rejection or too severe rejection. More recently, a moderate MHC mismatch model has been developed, transplanting from a F1 hybrid (cross between 2 inbred strains) into the parent strain. As a result, donor and recipient differ in 50% of MHC class I and II antigens. Mimura et al[29] used the B6D2F1/J ($H2^{b/d}$) strain (cross between C57BL/6J and DBA/2J) as donor mice and DBA/2J ($H2^d$) as recipients. In these grafts, an evolution was noticed from mild to moderate perivascular and peribronchial immune cell infiltration (lymphocytes, neutrophils, and eosinophils) and evidence of endothelial damage to the development of OB lesions and graft fibrosis in all mice by day 28. Intraluminal fibrinous exudate or fibrotic plugs were observed along with peribronchial and perivascular fibrosis. In addition, further progression was observed by day 40, with smooth muscle hypertrophy and an increase in collagen leading to luminal narrowing. As all allografts developed airway remodeling and fibrotic changes were observed consistently, this moderate mismatch combination was suggested as a reproducible model to investigate chronic graft failure.[29] Several years later, the same research group transplanted the lungs of F1 mice (B6D2F1/J) into the other parent strain (C57BL/6J). One week posttransplant, the allografts revealed moderate acute rejection together with mild cellular infiltration in the pleura. The latter showed progression by day 14 with plasma cell infiltration and evolving fibrosis, along with the presence of patchy fibrinous exudates in the alveoli. At day 28, acute cellular rejection persisted, and increasing fibrosis was observed in the pleura and along the bronchovascular bundles, with some airway lumina presenting with fibroblast plugs. Key observations on day 40 posttransplant included severe pleural fibrosis and thickening, along with peribronchial fibrosis and interlobular septal thickening and fibrosis. Moreover, in some allografts, pleuroparenchymal fibroelastosis was observed. As shown, histopathological features of RAS were demonstrated by transplanting B6D2F1/J into C57BL/6J, whereas a more OB-like pathology is observed when transplanting into DBA/2J recipients.[30]

2.4. Single mismatch model

In 2019, Smirnova et al[31] transplanted left lungs from HLA-A2–knockin mice in a C57BL/6J background (HLA mice) into C57BL/6J recipients. By using transgenic mice expressing the human HLA-A2 transgene, a single MHC class I mismatch occurred between donor and recipient.[35] Unlike the major mismatch model with total mismatches in class I and II MHC antigens, driving direct allorecognition, an indirect allorecognition response is triggered within this single mismatch model.[31,36] This is important because indirect allorecognition has been associated with chronic rejection and BOS[31,37,38] as the recipient's antigen-presenting cells can process and present donor MHC molecules to CD4+ T cells. In this model, large mononuclear infiltrates could be observed in the perivascular and peribronchial areas 1 month after transplantation. These infiltrates appeared more organized 2 months post-LTx. In addition, large amounts of extracellular matrix deposition could be observed around the bronchi and vessels as well as subepithelial fibrosis.[31]

2.5. Beyond histology: immunology and therapy

Although histologic analysis remains a fundamental component in assessing orthotopic LTx in mice, these models have also proven to be of great value in gaining deeper immunologic insights. For instance, using a major mismatch model, Kaes et al[39] investigated the alloimmune-related mechanism in rejection and showed that a classic immune response occurs involving both innate and adaptive immunity. At day 7 post-LTx, an increase in most of the measured immune cells including monocytes, interstitial macrophages, dendritic cells, natural killer cells, natural killer T cells, CD4+ T and CD8+ T cells, and B cells was

observed. By day 35, only dendritic and CD4+ T cells remained elevated.[39] Although the adaptive response in this study primarily demonstrated cell-mediated immunity, there is also mounting evidence indicating the significant role of humoral immunity in CLAD. Using a single mismatch model, Smirnova et al[31] demonstrated that B cells are an important driver of chronic rejection, as they were necessary for lymphoid follicle formation. This was also confirmed by Misumi et al[30] using the moderate mismatch model, who observed significant B cell and plasma cell infiltration in the allografts. Moreover, they indicated the crucial role of antibody secretion by B cells in mediating RAS-like pathology.

Additionally, these models are also relevant for testing potential therapeutic interventions. For instance, using the minor mismatch model, the potential therapeutic treatment with halofuginone was investigated. This plant derivative has been shown to have antifibrotic activities and inhibit Th17. Treatment with halofuginone reduced the percentage of obliterated airways as well as parenchymal fibrosis. It also decreased the expression of Th17- and fibrosis-associated genes.[40] In addition to the minor mismatch model, the other models have shown implications of findings for clinical care and therapeutic applications.[41,42] Translational studies confirming murine findings are crucial in further translating these findings to the clinic.

3. Critical remarks

Although the murine orthotopic LTx model is a major development to gain more insight into the pathogenesis of CLAD, it is also important to approach this model critically. A first important aspect is that mice lack small airways. Terminal bronchioles terminate directly into the alveoli because no respiratory bronchioles are present.[43,44] This anatomical difference poses a challenge in translating findings to the human context, particularly in terms of clinical similarity. Consequently, the extent to which human pathology is mirrored in this murine model is questionable. Although BOS and RAS-like pathology has been observed, no research has addressed the mixed and undefined phenotypes. The futility of lung function measurements in mice makes it difficult to fully correlate the findings to human CLAD. Therefore, to further increase the value of the model, it could be important to link histopathological findings to functional outcomes such as Flexi-vent measurements and in vivo imaging such as micro-computed tomography or positron emission tomography/computed tomography. Additionally, given that BOS is typically considered a small airway disease, it remains important to be aware of potential histopathological differences between human and murine lesions, and a careful comparison of these clinical manifestations is essential. Moreover, in humans, different presentations of this pathology can be defined, such as proliferative vs constrictive bronchiolitis, which thus far has not been addressed in the murine model. Finally, the lack of bronchial artery circulation in this murine model could also be considered a limitation. However, it is important to note that the significance of this circulation is debated in the human setting as well.[45,46]

In addition, it is important to consider the purpose of the study when choosing a model, as differences in outcome and reproducibility have been observed. Depending on the degree of genetic mismatch, a different allorecognition pathway (direct or indirect) will be activated. The major mismatch model primarily drives direct allorecognition because both MHC class I and II differ between donor and recipient. Although this model represents the clinical situation most closely (ie, full MHC mismatch and use of immunosuppression), variability in outcome was observed, possibly due to variability in immunosuppression serum levels,[20] questioning the reproducibility of this model. As such, one could consider whether an immunosuppressed model is ideal when investigating the mechanisms leading to CLAD or testing possible therapeutic applications. Unlike in patients, regular titration to adjust immunosuppression dosage is not performed in this murine model, and daily administration of immunosuppression may be considered a disadvantage due to its labor-intensive nature. Therefore, this model may be more suitable for studying acute rejection or immunologic responses rather than broader mechanistic studies.

In contrast, the single mismatch model is based on the indirect allorecognition response. For studying CLAD, this is particularly interesting given that indirect allorecognition is a commonly accepted mechanism driving chronic rejection. Despite its promise, not much research has been conducted using this model. However, it is an appealing option to use transgenic mice when examining the specific key players that might be involved.

In addition to the single mismatch model, the minor mismatch model also activates the indirect allorecognition pathway. However, this model demonstrated significant variability in airway fibrosis, and an additional 'insult' seems to be needed to induce CLAD.[21,26-28] Therefore, this model might seem less optimal in this context.

Finally, the moderate mismatch model seems to display an adequate level of rejection, neither too weak nor too severe. Because donor and recipient differ in 50% of MHC class I and II antigens, direct allorecognition will be activated, which will be followed by contribution of indirect allorecognition. Here, however, no immunosuppression is needed. Although the number of studies using this model is limited, it has already yielded promising results and seems to be reproducible, which can be interesting to test therapeutic applications. Moreover, as histopathological features of both BOS and RAS have been demonstrated,[29,30] it has potential as a model to study CLAD.

A final important reflection is the need for consistent time points for examination and a more uniform pathologic diagnosis and terminology across studies.[47] For example, OB lesions have been denominated OB, obliterative airway fibrosis, intraluminal chronic airway fibrosis, intraluminal fibrotic airway obliteration, and intraluminal fibrosis, likely reflecting the same pathology.

The murine orthotopic LTx model could be crucial for enhancing human LTx outcomes, but variations in model outcomes highlight the importance of understanding each model's background, advantages, and limitations. Greater insights and a deeper understanding of these models will be essential for progress in this field.

Funding

This research project was funded by Bijzonder Onderzoeksfonds (BOF) of the University of Antwerp under grant n°46818.

Declaration of competing interest

The authors of this review have no conflicts of interest to disclose as described by the *American Journal of Transplantation.*

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References

1. Chambers DC, Perch M, Zuckermann A, et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: thirty-eighth adult lung transplantation report - 2021; focus on recipient characteristics. *J Heart Lung Transplant*. 2021; 40(10):1060–1072. https://doi.org/10.1016/j.healun.2021.07.021.
2. Bos S, Vos R, Van Raemdonck DE, Verleden GM. Survival in adult lung transplantation: where are we in 2020? *Curr Opin Organ Transplant*. 2020;25(3):268–273. https://doi.org/10.1097/MOT.0000000000000753.
3. Verleden SE, Vos R, Verleden GM. Chronic lung allograft dysfunction: light at the end of the tunnel? *Curr Opin Organ Transplant*. 2019;24(3): 318–323. https://doi.org/10.1097/MOT.0000000000000632.
4. Byrne D, Nador RG, English JC, et al. Chronic lung allograft dysfunction: review of CT and pathologic findings. *Radiol Cardiothorac Imaging*. 2021;3(1):e200314. https://doi.org/10.1148/ryct.2021200314.
5. Verleden GM, Glanville AR, Lease ED, et al. Chronic lung allograft dysfunction: definition, diagnostic criteria, and approaches to treatment-a consensus report from the Pulmonary Council of the ISHLT. *J Heart Lung Transplant*. 2019;38(5):493–503. https://doi.org/10.1016/j.healun.2019.03.009.
6. Verleden SE, Von der Thüsen J, Roux A, et al. When tissue is the issue: a histological review of chronic lung allograft dysfunction. *Am J Transplant*. 2020;20(10):2644–2651. https://doi.org/10.1111/ajt.15864.
7. Lama VN, Belperio JA, Christie JD, et al. Models of lung transplant research: a consensus statement from the National Heart, Lung, and Blood Institute workshop. *JCI Insight*. 2017;2(9):e93121. https://doi.org/10.1172/jci.insight.93121.
8. Jin X, Kaes J, Van Slambrouck J, et al. A comprehensive review on the surgical aspect of lung transplant models in mice and rats. *Cells*. 2022; 11(3):480. https://doi.org/10.3390/cells11030480.
9. Sato M, Keshavjee S, Liu M. Translational research: animal models of obliterative bronchiolitis after lung transplantation. *Am J Transplant*. 2009;9(9):1981–1987. https://doi.org/10.1111/j.1600-6143.2009.02770.x.
10. 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(11):1509–1517.
11. 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(4): 390–398. https://doi.org/10.1016/j.healun.2007.01.018.
12. Okazaki M, Krupnick AS, Kornfeld CG, et al. A mouse model of orthotopic vascularized aerated lung transplantation. *Am J Transplant*. 2007;7(6): 1672–1679. https://doi.org/10.1111/j.1600-6143.2007.01819.x.
13. Zanoni F, Kiryluk K. Genetic background and transplantation outcomes: insights from genome-wide association studies. *Curr Opin Organ Transplant*. 2020;25(1):35–41. https://doi.org/10.1097/MOT.0000000000000718.
14. Shiina T, Blancher A, Inoko H, Kulski JK. Comparative genomics of the human, macaque and mouse major histocompatibility complex. *Immunology*. 2017;150(2):127–138. https://doi.org/10.1111/imm.12624.
15. Ohtsuka M, Inoko H, Kulski JK, Yoshimura S. Major histocompatibility complex (Mhc) class Ib gene duplications, organization and expression patterns in mouse strain C57BL/6. *BMC Genomics*. 2008;9:178. https://doi.org/10.1186/1471-2164-9-178.
16. Maloy S, Hughes K, eds. *Brenner's Encyclopedia of Genetics*. 2nd ed. Academic Press; 2013:482–485.
17. Silver L. Inbred strain. In: Maloy S, Hughes K, eds. *Brenner's Encyclopedia of Genetics*. 2nd ed. Academic Press; 2001.
18. Linder CC, Davisson MT. Chapter 3. Strains, stocks, and mutant mice. In: Hedrich HJ, Bullock G, eds. *The Laboratory Mouse*. Academic Press; 2004:25–46.
19. Chebib J, Jackson BC, López-Cortegano E, Tautz D, Keightley PD. Inbred lab mice are not isogenic: genetic variation within inbred strains used to infer the mutation rate per nucleotide site. *Heredity*. 2021; 126(1):107–116. https://doi.org/10.1038/s41437-020-00361-1.
20. De Vleeschauwer S, Jungraithmayr W, Wauters S, et al. Chronic rejection pathology after orthotopic lung transplantation in mice: the development of a murine BOS model and its drawbacks. *PLoS One*. 2012;7(1):e29802. https://doi.org/10.1371/journal.pone.0029802.
21. Yamada Y, Windirsch K, Dubs L, et al. Chronic airway fibrosis in orthotopic mouse lung transplantation models-an experimental reappraisal. *Transplantation*. 2018;102(2):e49–e58. https://doi.org/10.1097/TP.0000000000001917.
22. Liu Z, Liao F, Scozzi D, et al. An obligatory role for club cells in preventing obliterative bronchiolitis in lung transplants. *JCI Insight*. 2019;5(9):e124732. https://doi.org/10.1172/jci.insight.124732.
23. Fan L, Benson HL, Vittal R, et al. Neutralizing IL-17 prevents obliterative bronchiolitis in murine orthotopic lung transplantation. *Am J Transplant*. 2011;11(5):911–922. https://doi.org/10.1111/j.1600-6143.2011.03482.x.
24. Suzuki H, Fan L, Wilkes DS. Development of obliterative bronchiolitis in a murine model of orthotopic lung transplantation. *J Vis Exp*. 2012;(65): 3947. https://doi.org/10.3791/3947.
25. Martinu T, Oishi H, Juvet SC, et al. Spectrum of chronic lung allograft pathology in a mouse minor-mismatched orthotopic lung transplant model. *Am J Transplant*. 2019;19(1):247–258. https://doi.org/10.1111/ajt.15167.
26. Watanabe T, Martinu T, Chruscinski A, et al. A B cell-dependent pathway drives chronic lung allograft rejection after ischemia-reperfusion injury in mice. *Am J Transplant*. 2019;19(12):3377–3389. https://doi.org/10.1111/ajt.15550.
27. Watanabe T, Juvet SC, Berra G, et al. Donor IL-17 receptor A regulates LPS-potentiated acute and chronic murine lung allograft rejection. *JCI Insight*. 2023;8(21):e158002. https://doi.org/10.1172/jci.insight.158002.
28. Hata A, Guo Y, Miller AE, et al. Loss of stromal cell Thy-1 plays a critical role in lipopolysaccharide induced chronic lung allograft dysfunction. *J Heart Lung Transplant*. 2022;41(8):1044–1054. https://doi.org/10.1016/j.healun.2022.05.009.
29. Mimura T, Walker N, Aoki Y, et al. Local origin of mesenchymal cells in a murine orthotopic lung transplantation model of bronchiolitis obliterans. *Am J Pathol*. 2015;185(6):1564–1574. https://doi.org/10.1016/j.ajpath.2015.03.002.
30. Misumi K, Wheeler DS, Aoki Y, et al. Humoral immune responses mediate the development of a restrictive phenotype of chronic lung

allograft dysfunction. *JCI Insight*. 2020;5(23):e136533. https://doi.org/10.1172/jci.insight.136533.
31. Smirnova NF, Conlon TM, Morrone C, et al. Inhibition of B cell-dependent lymphoid follicle formation prevents lymphocytic bronchiolitis after lung transplantation. *JCI Insight*. 2019;4(3):e123971. https://doi.org/10.1172/jci.insight.123971.
32. Liu Z, Liao F, Zhu J, et al. Reprogramming alveolar macrophage responses to TGF-β reveals $CCR2^{+}$ monocyte activity that promotes bronchiolitis obliterans syndrome. *J Clin Invest*. 2022;132(19):e159229. https://doi.org/10.1172/JCI159229.
33. Slingsby JH, Hogarth MB, Simpson E, Walport MJ, Morley BJ. New microsatellite polymorphisms identified between C57BL/6, C57BL/10, and C57BL/KsJ inbred mouse strains. *Immunogenetics*. 1996;43(1-2):72–75. https://doi.org/10.1007/BF00186607.
34. Kawashima M, Oliver JD, Watanabe T, et al. Surgeon-dependent histopathological variations in minor alloantigen-mismatched mouse lung transplantation. *JHLT Open*. 2024;4:100050. https://doi.org/10.1016/j.jhlto.2023.100050.
35. Smith MA, Jaramillo A, SivaSai KSR, et al. Indirect recognition and antibody production against a single mismatched HLA-A2-transgenic molecule precede the development of obliterative airway disease in murine heterotopic tracheal allografts. *Transplantation*. 2002;73(2):186–193.
36. Smirnova NF, Riemondy K, Bueno M, et al. Single-cell transcriptome mapping identifies a local, innate B cell population driving chronic rejection after lung transplantation. *JCI Insight*. 2022;7(18):e156648. https://doi.org/10.1172/jci.insight.156648.
37. Reznik SI, Jaramillo A, SivaSai KS, et al. Indirect allorecognition of mismatched donor HLA class II peptides in lung transplant recipients with bronchiolitis obliterans syndrome. *Am J Transplant*. 2001;1(3):228–235. https://doi.org/10.1034/j.1600-6143.2001.001003228.x.
38. Stanford RE, Ahmed S, Hodson M, Banner NR, Rose ML. A role for indirect allorecognition in lung transplant recipients with obliterative bronchiolitis. *Am J Transplant*. 2003;3(6):736–742. https://doi.org/10.1034/j.1600-6143.2003.00142.x.
39. Kaes J, Pollenus E, Hooft C, et al. The immunopathology of pulmonary rejection after murine lung transplantation. *Cells*. 2024;13(3):241. https://doi.org/10.3390/cells13030241.
40. Oishi H, Martinu T, Sato M, et al. Halofuginone treatment reduces interleukin-17A and ameliorates features of chronic lung allograft dysfunction in a mouse orthotopic lung transplant model. *J Heart Lung Transplant*. 2016;35(4):518–527. https://doi.org/10.1016/j.healun.2015.12.003.
41. Cao P, Aoki Y, Badri L, et al. Autocrine lysophosphatidic acid signaling activates β-catenin and promotes lung allograft fibrosis. *J Clin Invest*. 2017;127(4):1517–1530. https://doi.org/10.1172/JCI88896.
42. Matsumoto H, Suzuki H, Yamanaka T, et al. Anti-CD20 antibody and calcineurin inhibitor combination therapy effectively suppresses antibody-mediated rejection in murine orthotopic lung transplantation. *Life (Basel)*. 2023;13(10):2042. https://doi.org/10.3390/life13102042.
43. Basil MC, Morrisey EE. Lung regeneration: a tale of mice and men. *Semin Cell Dev Biol*. 2020;100:88–100. https://doi.org/10.1016/j.semcdb.2019.11.006.
44. Chapman DG, Tully JE, Nolin JD, Janssen-Heininger YM, Irvin CG. Animal models of allergic airways disease: where are we and where to next? *J Cell Biochem*. 2014;115(12):2055–2064. https://doi.org/10.1002/jcb.24881.
45. Andreasson ASI, Dark JH. Revascularisation of the bronchial arteries in pulmonary transplant; does it ever have a role? *J Thorac Dis*. 2022;14(12):4578–4581. https://doi.org/10.21037/jtd-22-1368.
46. Mohanka M, Banga A. Alterations in pulmonary physiology with lung transplantation. *Compr Physiol*. 2023;13(1):4269–4293. https://doi.org/10.1002/cphy.c220008.
47. Cravedi P, Riella LV, Ford ML, et al. Advancing mouse models for transplantation research. Am J Transplant. Published online January 12, 2024. doi:10.1016/j.ajt.2024.01.006.