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Diagnosis, Pathophysiology and Experimental Models of Chronic Lung Allograft Rejection

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Abstract

Chronic rejection is the Achilles' heel of modern lung transplantation, characterized by a slow, progressive decline in allograft function. Clinically, this manifests as obstructive disease, restrictive disease, or a mixture of the 2 depending on the underlying pathology. The 2 major phenotypes of chronic rejection include bronchiolitis obliterans syndrome and restrictive allograft syndrome. The last decade of research has revealed that each of these phenotypes has a unique underlying pathophysiology which may require a distinct treatment regimen for optimal control. Insights into the intricate alloimmune pathways contributing to chronic rejection have been gained from both large and small animal models, suggesting directions for future research. In this review, we explore the pathological hallmarks of chronic rejection, recent insights gained from both clinical and basic science research, and the current state of animal models of chronic lung rejection.

Introduction

Lung transplantation outcomes have improved over the last 2 decades. Advances in operative technique, donor selection, and postoperative care have improved short-term outcomes [1, 2]. Long-term outcomes, however, remain disappointing, and the median survival is 5.3 years [3]. This comparatively poor long-term survival is predominantly a result of chronic rejection, which affects approximately 50% of patients at 5 years and is the leading cause of mortality beyond the first year after transplantation [3,4]. Traditionally the mainstay of treatment for chronic rejection has entailed a change in the patient's immunosuppressive

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regimen [5]. While some studies have shown marginal benefits with this approach, chronic rejection typically progresses inexorably to respiratory failure and death.

Research over the last decade has revealed that chronic rejection is a heterogeneous process with a variety of immune responses contributing to it, in part explaining why some patients respond to medical interventions while others do not [6,7]. Innate and adaptive immunity, both humoral and cellular arms, contribute to chronic rejection, which can present clinically in a variety of phenotypes, as discussed below. Experimental models of chronic rejection in small and large animals have provided a great deal of insight into these complex and interwoven immune pathways [8,9]. Technical barriers initially limited these experimental models to large animal models of orthotopic lung transplants, which lack immunological tools to conduct mechanistic studies, and heterotopic tracheal transplants in rodents, which may not adequately model basic physiological processes of clinical lung transplantation [10,11]. Two decades of perfecting animal transplantation techniques has created models that are now more applicable to human subjects, and recent findings have directed novel targets of therapy and suggest future areas of investigation.

Chronic Lung Allograft Dysfunction

The term "**chronic lung allograft dysfunction**" (CLAD) has emerged over the last decade as an umbrella term for the diagnosis for patients with chronic lung dysfunction after transplantation [12]. CLAD comprises 2 phenotypes with some overlap. Obliterative bronchiolitis (OB) is the prototypic form of chronic rejection and CLAD. OB results in an obstructive ventilatory defect, and therefore, is sometimes referred to as **obstructive CLAD**. Histologically this is characterized by luminal obliteration of respiratory and terminal bronchioles, but because histologic confirmation is difficult with transbronchial lung biopsy, bronchiolitis obliterans syndrome (BOS) is the clinical surrogate [13]. BOS is defined according to changes in spirometry. Baseline pulmonary function is defined as the mean of the 2 best posttransplant FEV1 or FVC values, and BOS is defined as a 20% decline in FEV_1 [14]. Inherent in this definition is the exclusion of other potential causes of allograft dysfunction such as bronchostenosis, respiratory infection, and acute rejection. More recently, a unique form of CLAD has been recognized that is characterized by a restrictive ventilatory defect and fibrotic pulmonary opacities on imaging. **Restrictive CLAD** (FVC ≤ 80%) or restrictive allograft syndrome (RAS or rCLAD) portends a significantly worse prognosis than BOS. Importantly, some clinical cases demonstrate mixed features, especially over time. The International Society for Heart and Lung Transplantation (ISHLT) has recently convened a working group to propose a new definition for CLAD and standardize the nomenclature, and this work is currently in progress.

Regardless of the cause, patients with chronic rejection often present clinically in a nonspecific manner with worsening dyspnea. A nonproductive cough often develops in the presence of rejection due to bronchiolitis [15]. Chest radiographs are often unchanged from previous films, but may show patchy hyperinflation, segmental atelectasis, and/or bronchiectasis. Chest CT scans may reveal bronchiectasis, bronchitis, architectural distortion, air trapping, and mixed hypo- and hyper-attenuation known as "mosaicism"; however, these findings are typically seen in advanced cases and chest CT may be normal in

early cases of BOS [16]. Diagnosis based on tissue pathology is not sensitive because of the patchy nature of disease. In fact, the majority of transbronchial biopsies obtained from patients diagnosed clinically with chronic rejection do not show evidence of rejection at all [17]. While other methods of detection, such as high-resolution chest CT scans, have been advocated for, the most common and reliable method of diagnosis remains via pulmonary function testing.

The vast majority of clinical research on chronic lung rejection has been performed on patients diagnosed with BOS, as this was historically thought to be the sole process responsible for chronic lung rejection. Over the last decade, RAS and neutrophilic reversible allograft dysfunction (NRAD) have emerged as clinically related but pathologically distinct phenotypes of chronic rejection manifesting in restrictive and obstructive CLAD, respectively. Certainly, BOS remains the most prevalent form of chronic rejection and much of what is known about OB can be applied to the other phenotypes. However, patients with NRAD and RAS have been found to have distinctly different pathologies and respond to several nontraditional therapies [18, 19], highlighting their differences and the need for tailored therapeutic approaches.

Obliterative bronchiolitis

Clinical presentation

Obliterative bronchiolitis (OB) is the most common form of chronic lung allograft rejection, with >50% of patients meeting the clinical diagnosis at 5 years [4]. Once diagnosed, the median survival after onset is approximately 2.5 years, with substantially worse outcomes among those diagnosed with high grade disease or onset within 2 years posttransplant [20]. The hallmark of this disease is obliteration of small airways that obstructs the flow of air. Tissue pathology will reveal “dense eosinophilic hyaline fibrosis in the submucosa of membranous and respiratory bronchioles, resulting in partial or complete luminal occlusion” [21]. The small airways may be either partially or completely obstructed, depending on whether or not the fibrous proliferation is concentric or eccentric, and the destruction may extend past the epithelium and into the submucosa and/or adventitia [22,21]. Thus, the diagnosis of OB is dependent on an airway biopsy, usually transbronchial, which creates a problem due to the insensitive and invasive nature of this test [13,16]. Thus, the need for a clinical diagnosis was appreciated nearly 2 decades ago, prompting the creation of the term **bronchiolitis obliterans syndrome** (BOS). BOS is now widely accepted as the clinical manifestation of OB, defined as pulmonary function testing revealing FEV1 ≤ 80% baseline function for ≥ 3 weeks in the absence of other lung pathology [14,22]. A scoring system has been devised to grade BOS based on severity, distinguishing between a best FEV1 of 66-80% (BOS 1), 51-65% (BOS 2), and <50% (BOS 3) [23].

Immune mechanisms

A wide of array of lung allograft insults predispose patients to OB, likely because any damage to the airway epithelium causes inflammation and facilitates allorecognition. For example, acute rejection, PGD, posttransplant infection, and gastroesophageal reflux disease all have been shown to increase the risk of BOS. Notably, OB can be seen independent of

lung transplantation, where airway pathology results from airway injury, persistent inflammation, and aberrant repair. For example, alloimmune mediated injury to airway epithelial cells may trigger OB following bone marrow transplantation [24]. Nonalloimmune insults alone may precipitate OB as well, as evidenced by a case series published in 2002 describing clinical OB among workers at a popcorn production plant [25]. The cause of OB in these workers was attributed to diacetyl exposure, a chemical present in the butter flavoring [26]. The capacity of diacetyl to induce OB has since been validated in animal models [27].

Investigations using both human tissues and animal models have suggested that BOS is associated with epithelial to mesenchymal transition (EMT), whereby damaged airway epithelial cells are lose their tight intercellular attachments and acquire a fibroblast phenotype [28]. Interestingly, there also exists evidence that cyclosporine, azathioprine, and mycophenolate, all commonly used following lung transplantation, may contribute to EMT, further supporting the notation that immunosuppressive regimens tailored towards lung transplantation are needed [29].

In addition to the small airway changes that hallmark OB, microvascular changes have been observed in chronically rejected human. In 2010, Luckraz at Papworth Hospital analyzed postmortem specimens from 99 lung transplant patients, focusing on the microvasculature of the small airways. In patients with OB, nonoccluded airways had a significant reduction in the number of blood vessels supplying them [30]. These results raised the possibility damage to the microvasculature of small airways, perhaps mediated through alloimmunity, precedes the development of airway fibrosis during chronic rejection. Bronchial artery disruption during the transplant procedure itself may lead to microvascular insufficiency, small airway hypoxia, and ultimately impair epithelial regeneration [31]. Indeed, lungs are the only solid organ transplant that do not have a systemic arterial reconstruction. Based on this theory, bronchial artery revascularization has been studied clinically, showing a modest increase in survival in small studies [32,33].

Cell-mediated immunity is one source of allorecognition that leads to chronic rejection and is perhaps the best understood alloimmune pathway. In the process of cell-mediated immunity, CD8+ and CD4+ T cells recognize MHC class I and II, respectively, thus initiating allorecognition. In this context, it is noteworthy that airway epithelial cells have been shown to express MHC II, in part explain why they appear to be the predominant target of chronic rejection [34]. Once activated, alloimmune CD4+ T cells release cytokines that stimulate CD8+ T cell and B cell expansion, which can form aggregates of immune cells along the small airways [35]. The role of CD8+ T cells in chronic rejection is poorly understood, though they certainly play a role in acute rejection in experimental models [36]. Interestingly, subsets of both CD4+ and CD8+ T cells, known as Foxp3+ regulatory T cells and central memory CD8+ T cells, respectively, appear to be required for lung tolerance in animal models [37,38].

It has long been known that **humoral immunity** plays a significant role in the pathogenesis of BOS. In 1998, Sundaresan demonstrated that the presence of donor-recipient HLA-A mismatch and anti-HLA antibodies are significant risk factors for BOS development [39].

Several studies since then have shown that the development of donor-specific antibodies (DSA) is a strong risk factor for the development of BOS [40,41,42]. When airway epithelial cells are treated in vivo with anti-HLA class I antibodies they proliferate substantially and then undergo apoptosis [43]. Interestingly, Bharat showed that PGD induces proinflammatory cytokines and upregulation of donor HLA-II antigens, providing a potential mechanistic explanation for the link between PGD and development of BOS [44]. Recently, evidence has emerged that antibody-mediated immunity is not only directed against donor antigens, but also self-antigens. The prevailing hypothesis is that airway inflammation causes the exposure of sequestered self-antigens and facilitates **autoimmunity**. The 2 most prominent self-antigens in this setting include collagen V (col V) and K-α1 tubulin, both of which are components of small airways and not exposed to the host immune system in normal states [45]. Both of these molecules and their respective autoantibodies, distinct from anti-HLA antibodies, have been detected in BOS patients [46,47]. This may explain why OB can been seen in autoimmune disorders outside of transplantation [15,48].

The contribution of **innate immunity** in developing OB has been yet another topic of recent investigation. Several pathways of the innate immune system have been shown to facilitate recruitment of inflammatory cells into lung allografts and contribute to PGD or acute rejection, including DAP12, TLR 4 and MyD88 [49,50,51]. In 2010 Kastelijn analyzed the genotype of 110 lung transplant recipients and found that polymorphisms of TLR 2, TLR 4 and TLR 9 are associated with BOS [52]. Given the broad and relatively nonspecific triggers for the innate immune system, more basic science studies are called for to elucidate these mechanisms and direct preclinical studies.

Current and evolving treatments

When BOS is diagnosed the mainstay of therapy is optimization of the patient's **immunosuppressive regimen**, though data supporting specific changes are lacking. Acute decline is lung function is often treated with a steroid boost followed by a taper [5]. When lung function fails to respond, changes in maintenance immunosuppression are needed, such as changing a patient's calcineurin inhibitor and/or antimetabolite to another drug in the same class [53]. When patients fail to respond to these conservative measures, a cytolytic agent is often indicated (eg, antithymocyte globulin). Several studies have shown an improvement in lung function or reduction in the rate of decline after these therapies are given [54,55]. However, these treatments should be cautiously implemented, as studies examining cytolytic therapy are limited by small sample sizes and the lack of appropriate control groups. More intensive immunosuppression carries substantial risks, such as infection and malignancy. Several other **targeted therapies**, such as anti-CD52 and anti-IL2 antibodies, have been studied in patients with refractory BOS with variable success and require further clinical investigation before being widely accepted [56,57]. Small, uncontrolled studies evaluating therapeutic interventions in patients with BOS need to be interpreted with caution given that the rate of decline in lung function is not linear and occurs predominantly in the first 6 months [58]. Thus, prospective, randomized controlled trials are needed in this population to determine the efficacy of new interventions.

Unfortunately, some patients have little or no response to medical therapy, and additional strategies are necessary; one such approach is **extracorporeal photopheresis** (ECP). ECP involves removing leukocytes from the blood, treating them with ultraviolet radiation in the presence of 8-methoxypsoralen (8-MOP), and returning the leukocytes to circulation. While the exact mechanisms are unknown, the process appears to preferentially induce apoptosis of activated immune cells, which are phagocytosed after reinfusion, create tolerogenic antigen presenting cells, and expand regulatory T cells [59]. A recent meta-analysis of available, noninvasive BOS therapies found evidence that ECP in combination with established immunosuppression may stabilize lung function compared to standard therapy [60]. **Total lymphoid irradiation** (TLI) is another means of intensive immunosuppression generally reserved for those with rapidly progressive disease, as it carries substantial side effects including pancytopenia and infection. The procedure entails irradiation of the major lymphatic beds of the body, thus providing profound and nonspecific immunosuppression. Initial experience with this technology in cardiac and renal transplant patients showed promising results [61,62]. Some reports suggest that TLI reduces the rate of decline in lung function in patients with progressive BOS [63].

For patients who fail the therapies discussed above, **retransplantation** remains the only effective therapy. Given the paucity of suitable lung donors, retransplantation is a limited therapy for chronic rejection. The most recent analysis from the ISHLT shows that 4.1% of all lung transplants performed are retransplants, for which the most common indication is BOS [64]. While retransplantation generally portends a significantly worse survival compared to primary transplantation (53% vs. 37% at 5 years), recent studies suggests that retransplant outcomes for BOS are substantially better than those for RAS [2,65]. Nevertheless, these suboptimal outcomes following retransplantation highlight the need for more effective means of treating BOS.

Neutrophilic Reversible Allograft Dysfunction

In the early 2000s, several authors reported a dichotomous response to azithromycin (AZM) among BOS patients, with some patients experiencing significant improvements in lung function and others showing no response [66,67]. AZM was initially trialed in the lung transplant population due to the potential anti-inflammatory effects seen in asthma patients [66]. Following these reports, Verleden examined bronchoalveolar lavage (BAL) specimens among “responders” (6/14 or 4.82%) and “nonresponders” (8/14 or 57.1%) of AZM. They found increased airway neutrophilia and levels of the neutrophil chemotactic factor, IL-8, among responders; this neutrophilic predominance led to the development of the term **neutrophilic reversible allograft dysfunction** (NRAD) [18].

NRAD is felt to be a variant of BOS rather than a distinct phenotype of chronic rejection, though some controversy exists regarding this issue. It is diagnosed when patients meeting criteria for BOS respond to AZM therapy (FEV1 increase of ≥10%). BAL will reveal excess neutrophils (≥15%) in the absence of an infection [7]. The exact mechanisms underlying NRAD remain poorly understood, but are likely a result of neutrophilic graft infiltration. To this end, 2 recent studies have demonstrated a link between neutrophilic graft infiltration and potentiation of alloimmunity after experimental lung transplantation. Neutrophils that

infiltrate murine lungs following reperfusion can activate antigen presenting cells, triggering their production of IL-12 thereby augmenting Th1-driven alloimmune responses [68]. Also, *Pseudomonas aeruginosa* infection can trigger the rejection of murine lung allografts through enhancement of T cell activation within the graft that is mediated by costimulatory molecules on the surface of neutrophils [69]. However, the majority of research on NRAD specifically has resolved around the effect of AZM. AZM has well-studied antibacterial and antiviral properties, which may carry benefit given that the lungs are the only solid organ transplant that is not sterile and constantly exposed to environmental pathogens [70]. However, the efficacy of AZM cannot merely be attributed to these antimicrobial properties, as AZM directly alters several different human tissues in the absence of infection. In 2007 it was shown that AZM reduces fibroblast growth factors in human airway smooth muscle cells [71]. Furthermore, Vanaudenaerde has shown that macrolides (eg, AZM), but not steroids, attenuate IL-8 production in activated human airway smooth muscle cells. In this study the authors also found intracellular changes in airway smooth muscle cells treated with AZM, as evidenced by a decrease in mitogen-activated-protein kinase (MAPK) activation and markers of oxidative stress [72].

The prognosis of NRAD is relatively good compared to that of traditional BOS [7]. The treatment is initially the same for both conditions, with the exception of AZM therapy. While AZM is generally well tolerated and appears to be the most benign drug in the macrolide class, some patients do experience side effects such as gastrointestinal symptoms, drug interactions, and cardiovascular complications. The timeline for initiating AZM and whether or not this drug should be given to all lung transplant patients remains a subject of debate. Should NRAD fail to improve with conservative therapy, ECP, TLI and retransplantation are currently being used as salvage therapy just as they are for traditional BOS [73].

Restrictive Allograft Dysfunction

In 2001 Sato demonstrated that 30% of lung transplant patients diagnosed with CLAD had a restrictive ventilatory defect, rather than the obstructive pattern seen in traditional BOS, and predominant upper lobe fibrotic infiltrates seen on chest imaging. The authors proposed **restrictive allograft syndrome** as a distinct phenotype of chronic lung rejection, which has since been reaffirmed as a distinct pattern of rejection [65,74].

Clinical presentation

A small case series of RAS patients has shown that the disease manifests through a series of acute exacerbations, in contrast to the slow and progressive decline in lung function seen in BOS patients. These patients most often present with shortness of breath or respiratory distress, and in 21 of the 25 patients the last episode of acute exacerbation led to death or urgent retransplantation [75]. Groups have used different definitions of RAS to report their experiences over the past 10 years. In general, definitions have been based on restrictive ventilatory defects and fibrotic infiltrates on chest imaging. Some have used a definition of FVC ≤ 80% baseline function for ≥ 3 weeks in absence of other allograft pathology [12,7]. An ISHLT working group is currently developing a standardized definition for RAS to

facilitate clinical research across centers. Biopsies frequently reveal diffuse alveolar damage and fibrosis of the alveolar interstitium. One report demonstrated pleuroparenchymal fibroelastosis as a common histologic finding among patients with RAS. Importantly, a significant proportion of histologic cases in this report had concomitant OB, illustrating that some patients have mixed features of RAS and OB [76]. Indeed, the original reports of OB as a manifestation of chronic lung rejection after heart-lung transplantation described concomitant restrictive ventilatory abnormalities, interstitial and pleural fibrosis, and OB [77,78]. Chest CT scans may reveal diffuse ground-glass opacities, which is uncharacteristic of traditional OB. The ensuing prognosis is poor and significantly worse than that of BOS, with a mean time from diagnosis to death or retransplantation being 490 days [65,79].

Immune mechanisms

The pathogenesis of RAS appears to be similar to pulmonary fibrosis in nontransplant patients, particularly when compared with idiopathic pleuroparenchymal fibroelastosis (IPPFE). A blinded study of tissue histology from RAS, IPPFE and interstitial pneumonia revealed substantial similarities among RAS and IPPFE that were distinctly different from interstitial pneumonia. In this study both RAS and IPPFE tissue showed marked intra-alveolar fibrosis and elastosis, and RAS allografts also had signs of OB and marked vascular inflammation [80]. Other authors have shown similar histological results, reinforcing this hypothesis [76].

The application of what is known about pulmonary fibrosis to RAS research is needed, as the exact immune mechanisms have yet to be elucidated. For example, idiopathic pulmonary fibrosis (IPF) has been extensively studied and is known to largely be a result of fibroblast proliferation, with TGF-β playing a central role [81]. TGF-β has also been shown to be a marker of chronic rejection, though it has yet to be investigated in the RAS population [82]. Notably, in vitro treatment of human pleural mesothelial cells (PMCs) with TGF-β is capable of inducing EMT-like changes in PMCs (mesothelial mesenchymal transition) [83]. The same group found PMCs in human lungs with IPF and showed in mice that PMCs are capable of migrating through lung parenchyma into the airways [84]. These observations may provide the impetus for future investigations into the pathophysiology of RAS after lung transplantation.

Finally, compared to the BOS population multiple alarmins are upregulated in RAS patients. Alarmins are endogenous molecules that trigger the innate immune system and are released upon tissue injury or immune activation. Characterization of alarmin profiles may lead to more precise diagnosis and earlier treatment, thus allowing for intervention prior to the rapid decline in graft function often seen with RAS [85].

Current and evolving treatments

No consensus guidelines currently exist for the treatment of RAS given its relatively recent discovery. At this time a patient diagnosed with RAS is treated in a similar manner to one newly diagnosed with BOS. Targeted therapies are yet to be discovered, and no animal models are available at this time to guide investigation. As such, further investigations of RAS are greatly needed, both on a clinical and basic science level.

Experimental Models of Chronic Lung Rejection

Since the inception of pulmonary transplantation various small and large animal models have been established, many of which have been used to study chronic lung rejection. Large animals were used in the early models, including dogs [86], pigs [87], and sheep [88]. Compared to other large animals, the orthotopic lung transplantation model in miniature swine has the advantage of defined MHC loci, allowing for the design of mechanistic studies of alloimmune responses to pulmonary grafts. Transplantation of lungs from MHC matched, minor antigen mismatched donors in the presence of a brief course of postoperative immunosuppression results in the development of airway changes reminiscent of chronic rejection in humans [87]. However, the high associated costs, ethical considerations, and difficulties managing large animals in the postoperative period have led many investigators to pursue small animal models to model chronic lung rejection.

Several species of small animals, both mammals and rodents, have been used to study chronic rejection, including ferrets, rabbits, mice and rats. Due to technical difficulties associated with orthotopic lung transplantation in small animals, many investigators have used tracheal allografts to model chronic rejection. Hertz and colleagues first devised a heterotopic tracheal transplantation model in 1993, whereby a segment of donor trachea is placed subcutaneously into a recipient. Allogeneic grafts develop fibro-obliterative changes in the airways, which are reminiscent of obliterative bronchiolitis lesions in human lung allografts [11]. This model has since been adopted by many other investigators and has yielded important insights into immune mechanisms that result in airway epithelial destruction [89]. Subsequently, variations of the tracheal transplant model have been utilized, including orthotopic [90] and intrapulmonary grafts [91]. Using an orthotopic model, in which allogeneic grafts develop subepithelial fibrosis, Babu provided experimental evidence that airway ischemia may contribute to chronic rejection after human lung transplantation [92]. Their study raised the possibility that bronchial artery revascularization at the time of transplantation may be an avenue to decrease the incidence of OB. The intrapulmonary tracheal transplant model was introduced in 2009. The authors suggested that this may represent a more physiologic model, given that the graft is exposed to the pulmonary milieu [93]. Despite their many advantages such as technical ease, tracheal transplant models have several limitations including development of pathological changes in large (rather than small) airways, absence of direct revascularization, and – in the case of heterotopic and intrapulmonary models – lack of exposure to the external environment.

Orthotopic vascularized, aerated lung transplant models, which more closely resemble the human lung transplant procedure, have been used in ferrets, rats, and mice. Sui and colleagues have described a ferret model of orthotopic lower lobe transplantation that develops airway changes consistent with OB when tailored immunosuppression, consisting of azathioprine and methylprednisone, is administered post-operatively. Histological examination of lungs from the ferret model are strikingly similar to human OB, with predominant findings of small airway occlusion, extensive collagen deposition, and lymphocytic infiltrates [94]. Using this model, the authors have recently shown that OB is associated with a depletion of airway-residing stem cells, revealing a potential mechanism of airway fibrosis [95]. While some controversy exists in the literature as to whether the

orthotopic rat lung transplant model is suitable to study chronic rejection, some investigators have reported the use of the minor MHC-mismatched Fischer 344→Wistar Kyoto strain combination for this purpose [96,97]. In 2007 Okazaki developed an orthotopic vascularized lung transplant model in the mouse [98]. Using this model and an immunosuppression regimen consisting of cyclosporine and steroids, OB lesions have been described in a fully MHC mismatched strain combination (Balb/c→C57BL/6) [99]. Others have shown that lung grafts in major MHC matched, minor MHC mismatched strain combinations develop fibrotic airway lesions that partially obstruct the airway lumen. Importantly, neutralization of IL-17 prevents the formation of these lesions in mice, extending previous findings that IL-17 dependent immunity to autoantigens is associated with chronic lung rejection in humans [47,100]. More recently, accumulation of fibrotic airway lesions has also been observed in F1 → parent strain combinations, where signaling pathways in donor mesenchymal cells have been described that contribute to fibrotic responses in lung allografts [101,102].

Finally, several types of human lung tissue have been used in vitro to study the cellular and molecular causes of chronic rejection. For example, Vanaudenaerde has cultured airway smooth muscle cells and found that when stimulated with IL-17 these cells release the neutrophil chemoattractant IL-8 [103]. Additionally, Borthwick was able to culture airway epithelial cells and induce their transition to mesenchymal cells after treatment with TGF-β, pointing to one potential pathway of airway remodeling in chronic rejection [104].

Given the wide array of animal models currently being used, results from these studies must be carefully interpreted with their specific limitations in mind. To this end, the National Heart, Lung, and Blood Institute (NHLBI) recently published a consensus statement on the state of animal models of lung transplantation [105]. The authors made recommendations on where future studies exploring mechanisms of CLAD should be directed. The report highlights the need for the development of new animal models of RAS, as treatment for this severe form of graft rejection is hampered by our limited mechanistic understanding of its underlying pathology. The authors also called for collaboration between laboratories using various experimental models of CLAD.

Conclusion

Lung transplant research over the last 2 decades has substantially increased our understanding of allograft rejection. Much of this knowledge, specifically with regard to surgical technique and organ perseveration, has been translated clinically. However, chronic rejection has proven to be a formidable challenge that the transplant community has yet to overcome. The complex and multifactorial nature of BOS and RAS have made the pathophysiology difficult to discern. The 2 phenotypes appear to share common pathways of allorecognition, however, their unique clinical presentations highlight differences that we have yet to understand. In particular, little is known about the pathogenesis of RAS and better animal models are needed to elucidate this process. Given that the predominant cause of long-term morbidity and mortality after lung transplantation is chronic rejection, timely and intensive research on this topic is critically needed [64]. As a community we must embrace this challenge and continue rigorous investigation of chronic rejection, both clinically and experimentally.

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Abbreviations

CLAD	chronic lung allograft dysfunction
BOS	bronchiolitis obliterans syndrome
OB	obliterative bronchiolitis
NRAD	neutrophilic reversible allograft dysfunction
RAS	restrictive allograft syndrome
FVC	forced vital capacity
FEV	forced expiratory volume
PGD	primary graft dysfunction
ECP	extracorporeal photopheresis
TLI	total lymphoid irradiation
AZM	azithromycin
ISHLT	International Society for Heart & Lung Transplantation
IPPFE	idiopathic pleuroparenchymal fibroelastosis
IPF	idiopathic pulmonary fibrosis

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