Transplant International

REVIEW

Autoantibodies in lung transplantation

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SUMMARY

Chronic lung allograft dysfunction (CLAD) comprises both bronchiolitis obliterans syndrome and restrictive allograft syndrome as subtypes. After lung transplantation, CLAD remains a major limitation for long-term survival, and lung transplant recipients therefore have poorer outcomes compared with recipients of other solid organ transplants. Although the number of lung transplants continues to increase globally, the field demands detailed understanding of immunoregulatory mechanisms and more effective individualized therapies to combat CLAD. Emerging evidence suggests that CLAD is multifactorial and involves a complex, delicate interplay of multiple factors, including perioperative donor characteristics, inflammation induced immediately following transplant, post-transplant infection and interplay between allo- and autoimmunity directed to donor antigens. Recently, identification of stress-induced exosome release from the transplanted organ has emerged as an underlying mechanism in the development of chronic rejection and promises to prompt novel strategies for future therapeutic interventions. In this review, we will discuss recent studies and ongoing research into the mechanisms for the development of CLAD, with emphasis on immune responses to lung-associated self-antigens—that is, autoimmunity.

Transplant International 2020; 33: 41–49

Key words
alloimmunity, autoimmunity, chronic lung allograft dysfunction, collagen V, exosomes, K-alpha 1 tubulin

Received: 1 July 2019; Revision requested: 22 July 2019; Accepted: 4 August 2019; Published online: 27 August 2019

Introduction

Lung transplantation (LTx) is increasingly used as a life-saving treatment for patients diagnosed with end-stage lung diseases, including interstitial lung disease, chronic obstructive pulmonary disease and cystic fibrosis. Since 2012, the number of LTx has increased, and the Registry of the International Society of Heart and Lung Transplantation reported 67 260 LTx procedures worldwide as of 30 June 2017 [1]. As of 2017, roughly 14 000 people in the United States had undergone LTx. Of these, 9492 were 50 years or older, 4075 were between 18 and 49 years of age, and 408 were younger than 18 years [2]. However, the demand for donor lungs continues to outpace the availability for transplantation; as of 16 June 2019, 1439 patients were on the LTx waitlist, according to The Organ Procurement and Transplantation Network data (https://optn.transplant.hrsa.gov/).

Lung transplant recipients (LTxRs) experienced the highest rates of hospitalization for transplant complications as compared to recipients of other solid organ transplants. Furthermore, lung allografts have the poorest long-term outcomes compared with other solid organs. The main limitation of long-term survival after LTx is a condition known as chronic lung allograft dysfunction (CLAD), a term first introduced by Glanville

doi:10.1111/tri.13487

in 2010 [3]. CLAD is defined as a persistent decline in measured forced expiratory volume from the baseline value [4]. Bronchiolitis obliterans syndrome (BOS) is the most common clinical manifestation of CLAD, affecting approximately 50% of LTxRs within 5 years of transplant [5]. Besides this obstructive phenotype, the consensus definition of CLAD also includes restrictive allograft syndrome [6]. Both antigen-dependent and antigen-independent factors lead to the development of nonresolved inflammation and fibrotic responses in the allograft. This review will highlight the prominent role of antibodies (Abs) to lung self-antigens (SAgs), also known as autoantibodies, in the development of CLAD. We will describe work that has already been done in this arena and will also discuss future implications.

Antibodies to mismatched donor human leucocyte antigens: role in the pathogenesis of CLAD

Mounting evidence suggests that autoimmunity plays a prominent role in the pathogenesis of CLAD [7,8]. Recent studies from our group and from others have reported that both humoral- and cellular-mediated immune responses against mismatched donor histocompatibility antigens increase the risk of CLAD after LTx [9]. However, the role of humoral-mediated immunity against mismatched donor histocompatibility antigens has gained much attention in recent years. Human leucocyte antigens (HLAs), the polymorphic molecules used to present peptides to T-cell receptor, have a crucial role in immune surveillance. Recent lines of evidence have shown that allorecognition of mismatched donor anti-human leucocyte antigen plays an important role in chronic rejection after transplantation. Studies have shown that development of Abs to mismatched HLA class I and II is associated with BOS progression [10–13]. In our recently published studies, we demonstrated a strong correlation between the development of circulating Abs to a SAg, K-alpha 1 tubulin (Kα1T) and the development of BOS after human LTx [14,15]. Several studies have demonstrated a distinct correlation between the presence of HLA Abs and the development of chronic rejection in LTxRs [12,16]. Anti-HLA Abs arising *de novo* were found to be significant predictors of BOS development [17]. After LTx, donor-specific HLA Abs (DSA) development is common and plays a significant risk factor for BOS progression. Interestingly, few studies have demonstrated that DSA directed against class II HLAs was present in LTxRs at 1 year post-transplant [18]. Based on the findings that *de novo* DSA development following LTx correlates significantly with BOS, many LTx centres monitor for DSA prospectively using Luminex single HLA antigen coated beads in order to predict LTxRs at risk for developing CLAD, though the timings vary between centres. There is some evidence that DSA-targeting therapies can lower the risk of BOS [19,20].

Development of HLA Abs can trigger NF-κB, a transcription factor involved in the activation of various proinflammatory cytokines, that contributes to BOS development [21]. Furthermore, crosslinking of HLA Abs triggers cytoskeleton remodelling and activation of Rho GTP, Src and FAK, resulting in cell proliferation and survival [22–24]. Of interest, human airway epithelial cells subjected to anti-HLA class 1 lead to a BOS-like phenotype, including fibrosis and small airway obstruction [11,25]. Furthermore, anti-HLA class I can induce the release of fibrogenic growth factors such as platelet-derived growth factor, insulin-like growth factor-1 and TGF-β by binding to airway epithelial cells [11–13]. These events culminate in the activation of fibroblast and myofibroblasts along with proinflammatory cascade and regeneration of extracellular matrix. It is generally accepted that TGF-β is the master regulator of the fibrogenic process, and its effect can be largely modified by the inflammatory and tissue remodelling environment [26–28].

After LTx, donor HLA antigens in bronchoalveolar lavage fluid are presented to $CD4^+$ T helper cells. Such T helper cells are required for the growth and maturation of antigen-specific B cells, resulting in Abs to mismatched HLA antigens [29]. A murine model study demonstrated that $CD8^+CCR7^+$ T-cell-mediated immunosuppression in pulmonary allografts occur in an IFN-gamma-dependent mechanism [30]. In addition, recent studies show that regulatory T cells (Treg) play an important role in immunosuppression and negatively correlate with the development of CLAD [31,32]. Moreover, the balance between Treg and Th17 cell immunity will determine the fate of the lung allograft. Finally, approaches to maintain or expand antigen-specific Treg cell populations will be beneficial to decipher their impact on CLAD development.

Alloimmunity-induced autoimmunity: crosstalk between allo- and autoimmune responses

The interplay between various effector mechanisms of allo- and autoimmunity in the pathogenesis of chronic graft rejection is still emerging. Our previous studies have demonstrated a correlation between an

alloimmune response, donor-specific Abs and an autoimmune response to SAgs [K-α1T and collagen V (Col-V)]. We also demonstrated that the development of this autoimmune response is mediated through IL-17 [33]. A significant body of evidence suggests that persistent secretion of IL-17 mediates chronic inflammation and can contribute to various autoimmune diseases [34–36]. As discussed above, disruptive mechanisms of the inflammation and subsequent tissue remodelling in the post-transplant period causes exposure of cryptic SAgs that may lead to the development of chronic rejection. The presence of DSA increases the risk of graft damage, and donor HLA antigens in bronchoalveolar lavage fluid are presented to T helper cells after LTx. Furthermore, $CD4^+$ T helper cells can reportedly recognize processed forms of soluble HLA and are involved in the development of humoral immune responses to donor HLA [13,37]. These findings challenge the existence of complex interplay between the two facets of allo- and autoimmunity after LTx. Undoubtedly, a detailed understanding of the interplay between those facets of immunity will provide a conceptual framework to devise therapeutic strategies in LTx.

Pre-existing autoimmunity induces primary graft dysfunction after lung transplant

Our group has shown that pre-existing autoimmunity is one of the most predominant risk factors for the development of CLAD [38,39]. Pre-existing Abs to lung SAgs can increase the risk for primary graft dysfunction (PGD), as they increase the development of pro-inflammatory cytokines and increase the risk of development of Abs to HLA and non-HLA antigens, which are known risk factors for CLAD [38]. Recently, pre-existing Abs to lung-associated SAgs (in the absence of Abs to donor HLA) were shown to result in antibody-mediated acute rejection [39]. Since patients with primary lung disease are known to be susceptible for comorbid conditions, our studies might explain why some patients waiting for LTx have pre-existing autoantibodies. Analysis of a large cohort of patients awaiting LTx revealed that about 30% of patients have pre-existing Abs to lung SAgs, primarily in patients with idiopathic pulmonary fibrosis and cystic fibrosis [38,40]. Furthermore, we demonstrated that the loss of Treg cells combined with hydrochloric acid-induced lung injury resulted in the development of *de novo* lung-restricted autoimmunity [41]. Therefore, it is safe to say that both pre-existing and *de novo* lung-restricted autoantibodies lead to acute and chronic lung rejection.

A growing body of evidence suggests that graft rejection after LTx is an immunologically mediated process, resulting in fibroproliferative changes accompanied by epithelial and endothelial cell injury [42,43]. A significant proportion of transplant recipients undergo chronic rejection despite the absence of Abs to donor HLA. This prompted researchers to identify Abs against SAgs, including K-α1T (an airway epithelial cell surface antigen) and Col-V (an extracellular matrix protein) after LTx [14,43]. The exact molecular mechanisms of immune responses against lung transplant SAgs in transplant rejection are less well understood. It is generally accepted that stimuli including ischaemia reperfusion injury, PGD and respiratory viral infections may promote SAg expression. Furthermore, interstitial remodelling after LTx can enhance exposure of such cryptic SAgs, which may trigger aberrant immune responses and contribute to the development of fibrosis and primary lung graft rejection.

Recent studies from our laboratory have demonstrated that administration of Abs to MHC class I induced the development of Abs against SAgs (K-α1T, Col-V), which are Th17-dependent and resulted in an obliterative airway disease (OAD) of native lungs [25,44]. A rat LTx model study demonstrated that the immune responses to Col-V play a significant role in the development of OAD [45]. The relationship between DSA and SAgs was suggested in a retrospective study in which DSA preceded the development of Abs against lung SAgs [15]. Another study showed that self-reactive Abs can be found in the absence of DSA, suggesting a DSA-independent mechanism [40]. Taken together, these studies demonstrate that immune responses to lung SAgs (K-α1T, Col-V) can promote autoimmune responses and lead to the development of OAD.

Autoantibodies can induce complement activation and cause apoptosis in endothelial cells [46]. Complement activation of airway epithelial cells can lead to the expression of Col-V, exposing the SAg and resulting in autoimmune responses. Similarly, graft-reactive Abs induce complement cascade and contribute to lung tissue degradation. It is now widely recognized that complement activation is a potential marker for humoral rejection via the complement component 4. Prolonged autoimmune responses followed by aberrant remodelling process will cause tissue destruction and, ultimately, loss of graft function.

Autoantibodies in chronic lung graft rejection

The pathophysiology of BOS is not well understood. There are several risk factors for developing BOS,

including PGD, HLA Abs and respiratory viral infections. There is a definite role of the immune response in BOS. This is evident by anti-donor antigen (anti-HLA) Ab (alloimmunity) and immune response to SAgs (autoimmunity) in the pathogenesis of BOS. A significant body of evidence demonstrates that the development of chronic lung allograft rejection coincides with the presence of anti-HLA [11,15,47]. Strategies that decreased *de novo* development of anti-HLA were found to correlate with a decreased risk of BOS [15,48]. Several studies suggest a key role of autoimmunity in the pathogenesis of allograft rejection [49–51]. Tissue remodelling after LTx can expose cryptic SAgs that can trigger a Th-cellular immune response [32]. Our group [25] and others [52] demonstrated that human LTxRs developed an Ab response to Kα1T along with Col-V, and the presence of these Abs correlated with development of BOS.

Primary graft dysfunction occurs within 72 h post-transplantation and is the leading cause of early mortality in LTxRs [53,54]. PGD has been shown to be a major risk factor for BOS [55]. The presence of autoimmunity immediately after LTx in patients undergoing PGD suggests that autoimmunity may exist prior to LTx due to underlying lung disease [56,57]. Because PGD is a key risk factor for BOS, the role of autoimmune status in LTxRs before LTx needs to be further studied, to determine whether the pre-LTx autoimmune status affects CLAD after LTx.

The mechanism of autoantibody induction is poorly understood. Using serum samples from LTxRs before and after development of BOS, our laboratory [32] showed the presence of anti-Col-V Abs. After the diagnosis of BOS, the Abs were restricted to alpha-1chain. This shift in Abs occurs with a decrease in serum concentrations of IL-10 and an increase in serum IFN-gamma and IL-17. There was also an observed upregulation of matrix metalloprotease-2 (MMP2) and MMP9 [32]. These observations suggest that the activation of MMPs after LTx could result in the differential cleavage of Col-V and exposure of cryptic epitopes. In support of this hypothesis, the inhibition of MMPs in a model of ischaemia–reperfusion injury resulted in lower amounts of Col-V in the transplanted lung [43]. An influx of antigen-presenting cells to the lung in the presence of inflammation could lead to activation of T cells and priming autoreactive T cells, leading to the induction of autoreactive B cells [58,59].

These studies strongly suggest that alloimmune responses precede autoimmune responses, which lead to the pathology observed in chronic rejection. Therefore, DSA development and immunity to SAgs can be relevant biomarkers for predicting the development of chronic rejection after LTx [32]. Early treatment of patients who developed DSA with intravenous immunoglobulins and rituximab has been associated with a lower incidence of BOS [19].

Determination of Abs to lung SAgs is currently done in research laboratories using ELISA methods. Standardization and quality controls are yet to be established between laboratories. However, a few of the commercial companies dealing with HLA reagents are now preparing SAg coated beads for the Luminex platform so that it can be utilized in the clinical setting. These Luminex coated beads are now being analysed and compared with the ELISA results. It is likely that these reagents will be available in the near future to determine the presence of Abs to lung SAgs prior to and following transplantation.

Tregs, characterized by $CD4^+$ $CD25^+$ $Foxp3^+$ expression, have been shown to play an important role in maintaining tolerance towards SAgs [60]. A reduction in either the function or the number of Tregs can lead to autoimmune responses. Several reports have shown that loss of Tregs is associated with the development of chronic rejection [38,61,62]. Tregs have also been shown to promote the development of IL-10-secreting T cells that are protective against the development of autoimmunity and development of chronic rejection [8]. Immunosuppression can dramatically alter T-cell kinetics. Immunosuppressive drugs such as cyclosporine and tacrolimus can have a profound effect on Tregs, and this effect on Tregs—though beneficial in preventing acute rejection episodes—may lead to development of autoimmunity and set the stage for development of chronic rejection.

As therapeutic actions now provide survival of acute rejection, chronic rejection is increasingly recognized as an important problem in LTx. Alloimmunity and the resulting immune responses to SAgs during the post-transplant period are emerging as key risk factors for CLAD development [32]. This cascade, in which allograft immune responses trigger autoimmune responses and further activate alloimmune responses (i.e. crosstalk between allo- and autoimmunity), results in tissue inflammation and repairs processes that eventually lead to a pro-inflammatory, pro-fibrogenic environment that is the hallmark of chronic rejection.

In transplant immunology, the basis for alloimmunity is the recognition of polymorphisms present in donor MHC molecules. The impact of these polymorphisms is evident by studies showing that minimizing

polymorphisms between donor and recipient MHC molecules results in diminished rejection responses and improved outcomes for the allograft recipient. Data from several groups have shown that Col-V, a molecule highly conserved between individuals and species, is a key target of rejection in LTxRs [17,63]. This finding supports the concept that immune responses to a SAg, as well as alloimmunity, are involved in the process of lung allograft rejection. How does Col-V become an autoantigen during the rejection process? A study [64] demonstrated that lung allograft rejection is associated with upregulation of MMP-2 and MMP-9, and these metalloproteinases can cleave Col-V. A report by Yano *et al.* [65] showed that ischaemia–reperfusion injury in lung allografts is associated with MMP-9, and this could expose Col-V to infiltrating T cells. Therefore, the remodelling of the allograft may contribute to the rejection response by creating Col-V epitopes that can induce alloreactivity and autoimmunity [52].

Our own studies demonstrated the presence of Col-V-reactive T cells in human LTxRs [66]. We have also shown that BOS is associated with the expansion of IFN-gamma-producing, Col-V-specific Th1 cells with a reduction in IL-10-producing cells. These results support the hypothesis that an immune response to SAgs plays a role in the pathogenesis of BOS. These studies indicate that alloimmunity can expose SAgs to the immune system and can create an immune microenvironment that generates an immune response against the newly exposed SAgs. Another consideration for the development of immune responses to lung-associated SAgs after LTx is that the stressed organ releases nanovesicles (i.e. exosomes), which carry many of the antigens present in that organ. These nanovesicles, which measure between 30 and 200 nm in size, can be taken up by antigen-presenting cells effectively by all known pathways, including the semi-direct pathway, resulting in both cellular and humoral immune responses [67]. The role of exosomes in transplant rejection is being actively studied, and we will discuss the current understanding of the role of exosomes in allograft immunity below.

Exosomes in CLAD and development of autoimmunity

Exosomes are extracellular vesicles that are produced in the endosomal compartment of most eukaryotic cells. Exosomes are released from cells via fusion with the plasma membrane; they are present in tissues and can be found in biological fluids, including blood and urine [68]. Research has demonstrated that exosomes play a key role in cell-to-cell signalling, as released exosomes can bind to other cells, merge and release their contents into cells. Thus, exosomes serve as a conduit for cellular communication, including immune responses. Exosomes can contain RNA, protein and miRNAs, and these molecules can induce cellular changes upon binding to other cell types [69,70]. The impact of exosomes on the field of biology is highlighted by the 2013 Nobel Prize in Physiology or Medicine, where researchers who discovered exosomes and investigated their biological relevance were rewarded [69].

Recent reports from our laboratory [71–73] demonstrated exosomes in the sera and bronchoalveolar lavage fluid of human LTxRs diagnosed with acute and chronic rejection. These exosomes had distinct antigenic properties and contained mRNA and miRNA. The exosomes isolated from LTxRs diagnosed with rejection contained not only donor-mismatched HLA, but also from lung-restricted SAgs (Col-V, Kα1T), indicating that exosomes are secreted by transplanted lungs [71,72]. These exosomes also contained immunomodulatory miRNA involved in endothelial activation, inflammation and IL-17 upregulation [74]. Donor-derived exosomes were shown to induce T-cell-mediated allo-responses that result in allograft rejection [75,76]. These findings strongly support the hypothesis that the induction and continuous release of exosomes from the transplanted lung may play a key role in the pathogenesis of chronic rejection after LTx. Therefore, blocking exosome production and release may be a strategy for preventing CLAD.

Our group identified the presence of two lung SAgs (Col-V, Kα1T) on circulating exosomes that had been released from LTxRs diagnosed with acute or chronic rejection (i.e. BOS) [71]. The exosomes containing lung SAgs were released into the circulation prior to the development of Abs to the SAgs (Col-V, Kα1T). Donor HLA and SAgs were detected on the surfaces of donor-derived exosomes in LTxRs undergoing allograft rejection [74]. Donor-derived exosomes from stable LTxRs had no detectable levels of SAgs [74]. These findings suggest that circulating exosomes from the transplanted organ are released from the transplanted lung. These exosomes were shown to contain mismatched donor HLA and SAgs.

Other studies show that mice immunized with exosomes from LTxRs diagnosed with BOS developed both humoral and cellular immune responses to Col-V and Kα1T. Mice immunized with exosomes from stable LTxRs did not develop immune responses to SAgs.

These findings indicate a clear difference in exosome composition from BOS and stable LTxRs.

Our laboratory developed a murine model in which Abs to MHC were administered intrabronchially, and this induced OAD, analogous to chronic lung allograft rejection in humans. Importantly, Abs to lung SAgs (Col-V, Kα1T) were detected prior to the development of OAD. Using this model, we demonstrated that exosomes can be used to activate an immune response leading to Ab production to lung SAgs and leading to OAD [25,77]. These findings suggest a functional role of exosomes in the production of Abs against SAgs.

Our studies along with others [71,75,76,78] have demonstrated that circulating exosomes released from the transplanted organ play a key role in eliciting immune responses leading to rejection. The detection of mismatched donor HLA and lung SAgs (Col-V, Kα1T) was identified primarily in exosomes isolated from LTxRs diagnosed with ongoing rejection or in those experiencing clinical conditions that increase the risk for rejection. Circulating exosomes contained pro-inflammatory transcription factors (NFκβ, HIFα) as well as increased levels of MHC transactivator CIITA and 20S proteasome [74]. Furthermore, immunization of mice with exosomes isolated from LTxRs experiencing rejection induced Abs to HLA and lung SAgs, suggesting that circulating exosomes are highly immunogenic [71]. These results, although preliminary, strongly suggest that circulating exosomes originating from the transplanted organ can trigger the immune responses leading to chronic organ rejection. Understanding the role of exosomes in the development of immune responses to allo and SAgs, including the potential to alter exosome induction, will likely impact the overall success of transplanted organs.

Blocking exosome induction from the transplanted organ has the potential to prevent allograft immune responses. There are several small inhibitory molecules such as neutral sphingomyelinases (nsmase2) inhibitor (GW4869), imipramine hydrochloride (an inhibitor of acid sphingomyelinase), and dimethyl amiloride (an inhibitor of Na^+/H^+ and Na^+/Ca^{2+} exchangers) which can effectively block exosome induction from various cells and tissues. However, it is necessary to block only the exosomes induced from the transplanted organ, and not from other tissues, since exosome induction is a physiological process and is considered to be important for maintenance of normal physiological homoeostasis. One potential venue is to block exosome induction from the organ to be transplanted during *ex vivo* lung perfusion which is currently being investigated as a means to improve the functions of the organs and to use organs which are currently classified as marginal for various reasons.

Summary/conclusion

The pathophysiology of CLAD is multifactorial and irreversible. As presented here, the development of Abs against HLA antigens and tissue-associated SAgs (Kα1T, Col-V) precedes the clinical diagnosis of CLAD. Further work is needed to elucidate signalling pathways linking allo- and autoimmunity in the pathogenesis of CLAD. It is safe to say that patients on the LTx waiting list may have circulating Abs that not only contribute to PGD, but also to CLAD. Therefore, novel approaches are needed to help identify predictors and to identify patients at risk for developing CLAD, so that early intervention with personalized medicine can be carried out before irreversible damage is inflicted upon the transplanted lungs. Development of *de novo* autoimmunity to SAgs, induction of IL-17, levels of Treg cells and exosomes released by graft tissue all appears to be important factors in the development of CLAD and represent robust opportunities for development of novel therapeutic interventions. Investigation of exosome biology and their effect on autoimmunity in the pathogenesis of CLAD is ongoing. Further studies are warranted to characterize the mechanistic role of exosomes in the development of autoimmunity resulting in CLAD and the clinical utility of identifying tissue-associated autoantibodies as biomarkers for graft rejection.

Funding

This work was supported by NIH-HL056643, HL092514, St. Joseph's Foundation, and Dignity Health/ASU Strategic Initiative FP00013643 (TM).

Conflict of interest

The authors have declared no conflicts of interest.

REFERENCES

1. Khush KK, Cherikh WS, Chambers DC, *et al*. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: thirty-fifth adult heart transplantation report-2018; focus theme: multiorgan transplantation. *J Heart Lung Transplant* 2018; **37**: 1155.
2. Valapour M, Lehr CJ, Skeans MA, *et al*. OPTN/SRTR 2017 annual data report: lung. *Am J Transplant* 2019; **19** (Suppl. 2): 404.
3. Glanville AR. Bronchoscopic monitoring after lung transplantation. *Semin Respir Crit Care Med* 2010; **31**: 208.
4. 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**: 493.
5. Morrell MR, Despotis GJ, Lublin DM, Patterson GA, Trulock EP, Hachem RR. The efficacy of photopheresis for bronchiolitis obliterans syndrome after lung transplantation. *J Heart Lung Transplant* 2010; **29**: 424.
6. Sato M, Waddell TK, Wagnetz U, *et al*. Restrictive allograft syndrome (RAS): a novel form of chronic lung allograft dysfunction. *J Heart Lung Transplant* 2011; **30**: 735.
7. Weber DJ, Wilkes DS. The role of autoimmunity in obliterative bronchiolitis after lung transplantation. *Am J Physiol Lung Cell Mol Physiol* 2013; **304**: L307.
8. Bharat A, Fields RC, Steward N, Trulock EP, Patterson GA, Mohanakumar T. $CD4^{+}25^{+}$ regulatory T cells limit Th1-autoimmunity by inducing IL-10 producing T cells following human lung transplantation. *Am J Transplant* 2006; **6**: 1799.
9. Nath DS, Basha HI, Mohanakumar T. Antihuman leukocyte antigen antibody-induced autoimmunity: role in chronic rejection. *Curr Opin Organ Transplant* 2010; **15**: 16.
10. Jaramillo A, Smith MA, Phelan D, *et al*. Development of ELISA-detected anti-HLA antibodies precedes the development of bronchiolitis obliterans syndrome and correlates with progressive decline in pulmonary function after lung transplantation. *Transplantation* 1999; **67**: 1155.
11. Jaramillo A, Smith CR, Maruyama T, Zhang L, Patterson GA, Mohanakumar T. Anti-HLA class I antibody binding to airway epithelial cells induces production of fibrogenic growth factors and apoptotic cell death: a possible mechanism for bronchiolitis obliterans syndrome. *Hum Immunol* 2003; **64**: 521.
12. Palmer SM, Davis RD, Hadjiliadis D, *et al*. Development of an antibody specific to major histocompatibility antigens detectable by flow cytometry after lung transplant is associated with bronchiolitis obliterans syndrome. *Transplantation* 2002; **74**: 799.
13. 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**: 228.
14. Goers TA, Ramachandran S, Aloush A, Truloc E, Patterson GA, Mohanakumar T. De novo production of K-alpha 1 tubulin-specific antibodies: role in chronic lung allograft rejection. *J Immunol* 2008; **180**: 4487.
15. Hachem RR, Tiriveedhi V, Patterson GA, Aloush A, Trulock EP, Mohanakumar T. Antibodies to K-alpha 1 tubulin and collagen V are associated with chronic rejection after lung transplantation. *Am J Transplant* 2012; **12**: 2164.
16. Brugiere O, Suberbielle C, Thabut G, *et al*. Lung transplantation in patients with pretransplantation donor-specific antibodies detected by Luminex assay. *Transplantation* 2013; **95**: 761.
17. Sundaresan S, Mohanakumar T, Smith MA, *et al*. HLA-A locus mismatches and development of antibodies to HLA after lung transplantation correlate with the development of bronchiolitis obliterans syndrome. *Transplantation* 1998; **65**: 648.
18. Koutsokera A, Royer PJ, Antonietti JP, *et al*. Development of a multivariate prediction model for early-onset bronchiolitis obliterans syndrome and restrictive allograft syndrome in lung transplantation. *Front Med (Lausanne)* 2017; **4**: 109.
19. Hachem RR, Yusen RD, Meyers BF, *et al*. Anti-human leukocyte antigen antibodies and preemptive antibody-directed therapy after lung transplantation. *J Heart Lung Transplant* 2010; **29**: 973.
20. Baskaran G, Tiriveedhi V, Ramachandran S, *et al*. Efficacy of extracorporeal photopheresis in clearance of antibodies to donor-specific and lung-specific antigens in lung transplant recipients. *J Heart Lung Transplant* 2014; **33**: 950.
21. Smith JD, Lawson C, Yacoub MH, Rose ML. Activation of NF-[kappa]B in human endothelial cells induced by monoclonal and allospecific HLA antibodies. *Int Immunol* 2000; **12**: 563.
22. Zhang X, Valenzuela NM, Reed EF. HLA class I antibody-mediated endothelial and smooth muscle cell activation. *Curr Opin Organ Transplant* 2012; **17**: 446.
23. Ziegler ME, Souda P, Jin YP, Whitelegge JP, Reed EF. Characterization of the endothelial cell cytoskeleton following HLA class I ligation. *PLoS ONE* 2012; **7**: e29472.
24. Jin YP, Fishbein MC, Said JW, *et al*. Anti-HLA class I antibody-mediated activation of the P13K/Akt signaling pathway and induction of Bcl-2 and Bcl-xL expression in endothelial cells. *Hum Immunol* 2004; **65**: 291.
25. Fukami N, Ramachandran S, Saini D, *et al*. Antibodies to MHC class I induce autoimmunity: role in the pathogenesis of chronic rejection. *J Immunol* 2009; **182**: 309.
26. Borthwick LA, Corris PA, Mahida R, *et al*. TNFalpha from classically activated macrophages accentuates epithelial to mesenchymal transition in obliterative bronchiolitis. *Am J Transplant* 2013; **13**: 621.
27. Sureshbabu A, Muhsin SA, Choi ME. TGF-beta signaling in the kidney: profibrotic and protective effects. *Am J Physiol Renal Physiol* 2016; **310**: F596.
28. Sureshbabu A, Tonner E, Allan GJ, Flint DJ. Relative roles of TGF-beta and IGFBP-5 in idiopathic pulmonary fibrosis. *Pulm Med* 2011; **2011**: 517687.
29. Yamakuchi M, Kirkiles-Smith NC, Ferlito M, *et al*. Antibody to human leukocyte antigen triggers endothelial exocytosis. *Proc Natl Acad Sci USA* 2007; **104**: 1301.
30. Krupnick AS, Lin X, Li W, *et al*. Central memory CD8 + T lymphocytes mediate lung allograft acceptance. *J Clin Invest* 2014; **124**: 1130.
31. Gregson AL, Hoji A, Palchevskiy V, *et al*. Protection against bronchiolitis obliterans syndrome is associated with allograft CCR31+ CD45RA− T regulatory cells. *PLoS ONE* 2010; **5**: e11354.
32. Tiriveedhi V, Takenaka M, Ramachandran S, Gelman AE, Patterson GA, Mohanakumar T. T regulatory cells (CD4 + Foxp3 +) play a significant role in modulating the MHC class I antibody induced

obliterative airway disease. *Am J Transplant* 2012; **12**: 2663.
33. Banan B, Xu Z, Gunasekaran M, Mohanakumar T. Role of alloimmunity and autoimmunity in allograft rejection. *Clin Transpl* 2013: 325.
34. Shin MS, Lee N, Kang I. Effector T-cell subsets in systemic lupus erythematosus: update focusing on Th17 cells. *Curr Opin Rheumatol* 2011; **23**: 444.
35. Lin X, Rui K, Deng J, *et al*. Th17 cells play a critical role in the development of experimental Sjogren's syndrome. *Ann Rheum Dis* 2015; **74**: 1302.
36. Papp G, Horvath IF, Barath S, *et al*. Altered T-cell and regulatory cell repertoire in patients with diffuse cutaneous systemic sclerosis. *Scand J Rheumatol* 2011; **40**: 205.
37. Girnita AL, McCurry KR, Zeevi A. Increased lung allograft failure in patients with HLA-specific antibody. *Clin Transpl* 2007: 231.
38. Bharat A, Saini D, Steward N, *et al*. Antibodies to self-antigens predispose to primary lung allograft dysfunction and chronic rejection. *Ann Thorac Surg* 2010; **90**: 1094.
39. Bharat A, Chiu S, Zheng Z, *et al*. Lung-restricted antibodies mediate primary graft dysfunction and prevent allotolerance after murine lung transplantation. *Am J Respir Cell Mol Biol* 2016; **55**: 532.
40. Tiriveedhi V, Gautam B, Sarma N, *et al*. Pre-transplant antibodies to K-alpha-1 tubulin and collagen-V in lung transplantation: clinical correlations. *J Heart Lung Transplant* 2013; **32**: 807.
41. Chiu S, Fernandez R, Subramanian V, *et al*. Lung injury combined with loss of regulatory T cells leads to de novo lung-restricted autoimmunity. *J Immunol* 2016; **197**: 51.
42. Hasegawa S, Ockner DM, Patterson GA, Ritter JH, Cooper JD, Wick MR. Expression of class II major histocompatibility complex antigens and lymphocyte subset immunotyping in chronic pulmonary transplant rejection. *Transplant Proc* 1995; **27**: 1290.
43. Iwata T, Philipovskiy A, Fisher AJ, *et al*. Anti-type V collagen humoral immunity in lung transplant primary graft dysfunction. *J Immunol* 2008; **181**: 5738.
44. Xu Z, Ramachandran S, Gunasekaran M, *et al*. B cell-activating transcription factor plays a critical role in the pathogenesis of anti-major histocompatibility complex-induced obliterative airway disease. *Am J Transpl* 2016; **16**: 1172.
45. Yasufuku K, Heidler KM, Woods K, *et al*. Prevention of bronchiolitis obliterans in rat lung allografts by type V collagen-induced oral tolerance. *Transplantation* 2002; **73**: 500.
46. Bordron A, Dueymes M, Levy Y, *et al*. The binding of some human antiendothelial cell antibodies induces endothelial cell apoptosis. *J Clin Invest* 1998; **101**: 2029.
47. Rizzo M, Sundaresan S, Lynch J, *et al*. Increased concentration of soluble human leukocyte antigen class I levels in the bronchoalveolar lavage of human pulmonary allografts. *J Heart Lung Transplant* 1997; **16**: 1135.
48. Grossman EJ, Shilling RA. Bronchiolitis obliterans in lung transplantation: the good, the bad, and the future. *Transl Res* 2009; **153**: 153.
49. Shilling RA, Wilkes DS. Immunobiology of chronic lung allograft dysfunction: new insights from the bench and beyond. *Am J Transplant* 2009; **9**: 1714.
50. Burke GW 3rd, Vendrame F, Pileggi A, Ciancio G, Reijonen H, Pugliese A. Recurrence of autoimmunity following pancreas transplantation. *Curr Diab Rep* 2011; **11**: 413.
51. Kalache S, Dinavahi R, Pinney S, Mehrotra A, Cunningham MW, Heeger PS. Anticardiac myosin immunity and chronic allograft vasculopathy in heart transplant recipients. *J Immunol* 2011; **187**: 1023.
52. Haque MA, Mizobuchi T, Yasufuku K, *et al*. Evidence for immune responses to a self-antigen in lung transplantation: role of type V collagen-specific T cells in the pathogenesis of lung allograft rejection. *J Immunol* 2002; **169**: 1542.
53. Christie JD, Kotloff RM, Ahya VN, *et al*. The effect of primary graft dysfunction on survival after lung transplantation. *Am J Respir Crit Care Med* 2005; **171**: 1312.
54. Lee JC, Christie JD. Primary graft dysfunction. *Proc Am Thorac Soc* 2009; **6**: 39.
55. Kotloff RM, Thabut G. Lung transplantation. *Am J Respir Crit Care Med* 2011; **184**: 159.
56. Bobadilla JL, Love RB, Jankowska-Gan E, *et al*. Th-17, monokines, collagen type V, and primary graft dysfunction in lung transplantation. *Am J Respir Crit Care Med* 2008; **177**: 660.
57. Feghali-Bostwick CA, Tsai CG, Valentine VG, *et al*. Cellular and humoral autoreactivity in idiopathic pulmonary fibrosis. *J Immunol* 2007; **179**: 2592.
58. Bharat A, Narayanan K, Street T, *et al*. Early posttransplant inflammation promotes the development of alloimmunity and chronic human lung allograft rejection. *Transplantation* 2007; **83**: 150.
59. Bharat A, Fields RC, Trulock EP, Patterson GA, Mohanakumar T. Induction of IL-10 suppressors in lung transplant patients by CD4 + 25 + regulatory T cells through CTLA-4 signaling. *J Immunol* 2006; **177**: 5631.
60. Sakaguchi S, Sakaguchi N. Regulatory T cells in immunologic self-tolerance and autoimmune disease. *Int Rev Immunol* 2005; **24**: 211.
61. Bhorade S, Ahya VN, Baz MA, *et al*. Comparison of sirolimus with azathioprine in a tacrolimus-based immunosuppressive regimen in lung transplantation. *Am J Respir Crit Care Med* 2011; **183**: 379.
62. Cypel M, Rubacha M, Yeung J, *et al*. Normothermic ex vivo perfusion prevents lung injury compared to extended cold preservation for transplantation. *Am J Transplant* 2009; **9**: 2262.
63. van den Berg JW, Hepkema BG, Geertsma A, *et al*. Long-term outcome of lung transplantation is predicted by the number of HLA-DR mismatches. *Transplantation* 2001; **71**: 368.
64. Trello CA, Williams DA, Keller CA, Crim C, Webster RO, Ohar JA. Increased gelatinolytic activity in bronchoalveolar lavage fluid in stable lung transplant recipients. *Am J Resp Crit Care Med* 1997; **156**: 1978.
65. Yano M, Omoto Y, Yamakawa Y, *et al*. Increased matric metallopreteinase-9 activity and mRNA expression in lung ischemia-reperfusion injury. *J Heart Lung Transplant* 2001; **20**: 679.
66. Saini D, Weber J, Ramachandran S, *et al*. Alloimmunity-induced autoimmunity as a potential mechanism in the pathogenesis of chronic rejection of human lung allografts. *J Heart Lung Transplant* 2011; **30**: 624.
67. Morelli AE, Bracamonte-Baran W, Burlingham WJ. Donor-derived exosomes: the trick behind the semidirect pathway of allorecognition. *Curr Opin Organ Transplant* 2017; **22**: 46.
68. Keller S, Sanderson MP, Stoeck A, Altevogt P. Exosomes: from biogenesis and secretion to biological function. *Immunol Lett* 2006; **107**: 102.
69. de la Torre Gomez C, Goreham RV, Bech Serra JJ, Nann T, Kussmann M. "Exosomics" – a review of biophysics, biology and biochemistry of exosomes with a focus on human breast milk. *Front Genet* 2018; **9**: 92.

70. Jeppesen DK, Fenix AM, Franklin JL, *et al*. Reassessment of exosome composition. *Cell* 2019; **177**: 428e18.
71. Gunasekaran M, Sharma M, Hachem R, Bremner R, Smith MA, Mohanakumar T. Circulating exosomes with distinct properties during chronic lung allograft rejection. *J Immunol* 2018; **200**: 2535.
72. Sharma M, Ravichandran R, Bansal S, Bremner RM, Smith MA, Mohanakumar T. Tissue-associated self-antigens containing exosomes: role in allograft rejection. *Hum Immunol* 2018; **79**: 653.
73. Gregson AL, Hoji A, Injean P, *et al*. Altered exosomal RNA profiles in bronchoalveolar lavage from lung transplants with acute rejection. *Am J Resp Crit Care Med* 2015; **192**: 1490.
74. Gunasekaran M, Xu Z, Nayak DK, *et al*. Donor-derived exosomes with lung self-antigens in human lung allograft rejection. *Am J Transpl* 2017; **17**: 474.
75. Liu Q, Rojas-Canales DM, Divito SJ, *et al*. Donor dendritic cell-derived exosomes promote allograft-targeting immune response. *J Clin Invest* 2016; **126**: 2805.
76. Marino J, Babiker-Mohamed MH, Crosby-Bertorini P, *et al*. Donor exosomes rather than passenger leukocytes initiate alloreactive T cell responses after transplantation. *Sci Immunol* 2016; **1**: aaf8759.
77. Braun RK, Molitor-Dart M, Wigfield C, *et al*. Transfer of tolerance to collagen type V suppresses T-helper-cell-17 lymphocyte-mediated acute lung transplant rejection. *Transplantation* 2009; **88**: 1341.
78. Vallabhajosyula P, Korutla L, Habertheuer A, *et al*. Tissue-specific exosome biomarkers for noninvasively monitoring immunologic rejection of transplanted tissue. *J Clin Invest* 2017; **127**: 1375.