

## Autoimmunity and lung transplantation

Ankit Bharat<sup>1</sup>, Thalachallour Mohanakumar<sup>2</sup>

<sup>1</sup>Department of Surgery and Cardiothoracic Surgery, Washington University School of Medicine, Campus Box 8109, 660 S. Euclid Avenue, St. Louis, MO 63110, <sup>2</sup>Department of Pathology and Immunology, Washington University School of Medicine, Box 8109-3328 CSRB, 660 S. Euclid Avenue, St. Louis, MO 63110

### TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Clinical risk factors for BOS
  - 3.1. Primary graft rejection
  - 3.2. Acute cellular rejection
  - 3.3. Humoral rejection
  - 3.4. Respiratory viral infection
  - 3.5. Gastroesophageal reflux
  - 3.6. Autoimmunity
4. Pre-existing autoimmunity and its role in lung allograft rejection
  - 4.1. Chronic inflammation in patients with end-stage lung disease can lead to autoimmunity
  - 4.2. Pre-existing autoimmunity induces primary lung allograft dysfunction
5. De novo autoimmunity following lung transplantation
  - 5.1. Lung transplantation provides an optimal environment for lung tissue specific autoimmunity
  - 5.2. Autoimmunity in the pathogenesis of obliterative airway disease in animal models
6. De novo autoimmunity and its role in the pathogenesis of BOS
7. Alloimmunity and induction of de novo autoimmunity
8. Loss of Regulatory T cells and autoimmunity
9. Concluding remarks
10. References

## 1. ABSTRACT

Lung transplantation is a viable treatment option for a variety of end-stage lung diseases. Since the first successful human lung transplant about 20 years ago, tremendous progress has been made in this field. However, lung allografts have the poorest long term survival compared to other solid organs. The predominant reason for this is the development of chronic rejection, also known as bronchiolitis obliterans syndrome (BOS). Although the traditional view supports alloimmunity as the major cause of chronic rejection, emerging evidence reveals a complex interplay of multiple etiologies including perioperative stressors, inflammation, and autoimmunity along with alloimmunity. Identification of autoimmunity in the pathogenesis of BOS is an exciting recent finding in lung transplantation and promises to introduce novel strategies for future therapeutic interventions. In this review, we discuss recent studies and concepts related to the role of autoimmunity in the development of BOS.

## 2. INTRODUCTION

It is remarkable that in just over 20 years since its first success, lung transplantation is providing good palliation for an increasing number of patients world-wide for a variety of end-stage lung diseases. The Registry of the International Society of Heart and Lung Transplantation recorded 24,904 lung transplants between 1985 and 2006 (1). Overall survival after lung transplant has steadily improved with the passage of time. During 1988–1994, median survival was only about 3.9 years. However, between 2000–2006, the median survival increased to 5.5 years, with a 1-year survival of 81.4% and 5-years survival of 53.5%.

Chronic lung allograft rejection, also called Bronchiolitis Obliterans Syndrome (BOS) remains the most common cause of long-term lung allograft failure (1). BOS is characterized by progressive airflow obstruction associated with chronic airway fibrosis, a histopathological

condition also known as Obliterative Bronchiolitis (OB) which predominantly affects distal airways (2). Transbronchial biopsies that are performed for acute rejection are highly insensitive to diagnose OB because of the heterogeneous nature of the disease and limited sampling of distal bronchiolar tissue. Therefore the clinical syndrome of BOS was developed to identify patients with underlying OB. BOS is diagnosed by a persistent decline in the baseline post-transplant forced expired volume after the exclusion of other causes of airway obstruction including anastomotic stricture (3). Unfortunately, BOS gradually progresses toward respiratory failure and death and is unresponsive to current immunosuppressive regimens.

### 3. CLINICAL RISK FACTORS FOR BOS

BOS has a multifactorial etiology. Recent evidence suggests that inflammation leading to augmentation of allo- as well as auto- immunity might be the common link between risk factors and pathogenesis of BOS. The risk factors for BOS that have emerged consistently in multiple studies from different centers are discussed below.

#### 3.1. Primary graft dysfunction

PGD is the most common early post-transplant complication and the incidence can be over 70%. Previous studies by our group demonstrated that higher the grade of PGD (Grade 1-3) 24 hours post-transplant, greater the risk of BOS (4). Further, we demonstrated that PGD induced inflammation promoted the development of alloantibodies and predisposed to BOS (5).

#### 3.2. Acute cellular rejection

Acute rejection has been consistently shown to be a strong clinical risk factor in several series. Histologically, acute rejection could either be perivascular or peribronchiolar. Recent studies have shown that even a single episode of mild acute rejection may predispose to BOS (6). Acute rejection is also postulated to augment the inflammatory milieu in the allograft leading to BOS.

#### 3.3. Humoral rejection

Published evidence supports the role of both pre-formed and de novo HLA class I and II antibodies in pathogenesis of BOS (7-9). Post-transplant inflammation, for instance, as a consequence of PGD, promotes development of de novo HLA antibodies (10). Ligation of target antigens on allograft tissue with specific antibodies can lead to pro-inflammatory and profibrogenic growth factors that contribute to chronic rejection (11).

#### 3.4. Respiratory viral infections

These have emerged as a strong risk factor for BOS (12). We recently demonstrated that respiratory viruses including adenovirus, respiratory syncytial viruses, influenza, and para-influenza viruses lead to apoptosis in regulatory T cells that are known to suppress autoimmunity (13). Loss of regulatory T cells

can promote de novo allo- and auto- immunity that can predispose to BOS.

#### 3.5. Gastroesophageal reflux

Gastric reflux disease can be observed in over 50% of lung transplant recipients (14, 15). Presence of gastric contents can lead to inflammation and has been shown to significantly increase the risk of BOS. In a recent study by Bobadilla *et al*, gastric reflux was shown to correlate with increased immunity to collagen V in transplant recipients (16). As will be discussed later in this review, immune response to the self-antigen collagen V increases the risk of BOS.

#### 3.6. Autoimmunity

The role of autoimmunity in the pathogenesis of chronic rejection remains under-investigated. An inflammatory milieu is conducive for the development of autoimmunity. Due to the exposure of lung allografts to external pathogens or gastric acid in those with post-transplant gastro-esophageal reflux, lung allografts are uniquely predisposed to such an inflammatory milieu and development of autoimmunity. Emerging data is establishing an important role of autoimmunity in lung allograft rejection. Studies from our lab and others have shown that both cellular as well as antibody mediated autoimmunity increases the risk for BOS. Self-antigens that have been identified thus far include collagen type I, collagen type V, and  $\alpha$ -tubulin (17). Patients can develop autoimmunity prior to transplantation or *de novo* post-transplant. In the following text, we will discuss mechanisms that lead to autoimmunity in lung transplant recipients and its role in the pathogenesis of BOS.

### 4. PRE-EXISTING AUTOIMMUNITY AND ITS ROLE IN LUNG ALLOGRAFT REJECTION

This section discusses the role of pre-existing autoimmunity in the development of lung allograft dysfunction.

#### 4.1. Chronic inflammation in patients with end-stage lung disease can lead to autoimmunity

During lymphocyte development, T cells are screened within the thymus and self-antigen reactive T cells are deleted. However, there are sequestered antigens that are not expressed on thymocytes and T-cells specific to these antigens leak out into the periphery. Patients with end-stage lung disease have ongoing inflammation in the native lungs. During inflammation tissue damage can lead to expression of self-antigens that may be presented to such autoreactive T cells. Such an inflammatory milieu is conducive for the development of autoimmunity. Recent findings demonstrating that lung inflammation present in asthmatics and cigarette smokers can promote autoimmunity supports such a hypothesis (18). In the following section we discuss how investigations looking at the pathogenesis of primary lung allograft dysfunction that develops immediately following transplant led to the identification of pre-existing autoimmunity in patients undergoing lung transplantation.

### 4.2. Pre-existing autoimmunity induces primary lung allograft dysfunction

Lung allografts frequently develop a syndrome called primary graft dysfunction (PGD) in the immediate post-transplant period. PGD usually develops within the first 24-48 hours after transplantation and strongly increases the risk of BOS. In one of the previous reports from our center, patients with PGD were found to have over two and a half fold increased risk of developing BOS (4). As discussed in the subsequent sections, development of PGD is associated with inflammation that promotes the both cellular as well as humoral allo-immunity which, in turn, leads to BOS (10). Hence, PGD is an important risk factor for BOS. However, the pathogenesis of PGD is still not well understood. Insults to the lung allograft begin with donor brain death even prior to organ harvest (19). Hypothermic storage associated with organ harvest and subsequent ischemia-reperfusion upon re-implantation then contributes to lung injury that has been postulated to lead to PGD (20).

However, donor or harvest related etiologies do not explain why some recipients develop severe form of PGD despite receiving the best organs in the most optimal circumstances. This led to ongoing search for PGD etiologies and evaluation of pre-transplant variables in the recipient. Westall *et al* evaluated human transbronchial lung biopsy specimens in patients with PGD and found high levels of complement deposition in the perivascular and peribronchial areas (21). This suggested that PGD may be mediated by antibody-complement pathway. We know that HLA mismatch between the donor and recipient does not have any significant correlation with PGD development. Nevertheless, it was possible that pre-existing allo-specific (HLA) antibodies in the recipients could mediate such an immune response. However, when we evaluated human recipients that developed PGD but were negative for HLA antibodies, we found elevated complement C4d in bronchoalveolar lavage specimens (22). Absence of HLA antibodies raised the possibility that pre-existing autoantibodies were likely responsible for the complement activation. This mechanism has been investigated by Iwata *et al* in rat lung transplant model. They transferred autoantibodies against a self-antigen, collagen type V, in rats and subsequently transplanted them with lung isografts. These rat lung isograft recipients that had received anti-collagen V autoantibodies prior to transplant developed a syndrome mimicking human PGD (23). They further showed that lung biopsies obtained from these rat lung isograft recipients that developed the PGD-like syndrome had both autoantibody and complement deposition on the allograft airway epithelium (23).

Direct evidence of pre-existing autoantibodies in human subjects has also been reported. Bobadilla *et al* also found that about 58% of patients with idiopathic pulmonary fibrosis and 16% of patients without idiopathic pulmonary fibrosis have anti-collagen V immunity detected by using the trans-vivo mouse footpad delayed type hypersensitivity model (24). We developed standardized ELISA assays to investigate the presence of autoantibodies against collagen type I, collagen type V, and  $\alpha$ -tubulin. All of these

are non-polymorphic self-proteins present in the lung tissue. We evaluated 142 patients undergoing lung transplantation and found that 41 (28%) had at least one autoantibody against these self-proteins. We were able to correlate a significant increase in the incidence of PGD in patients with circulating autoantibodies (85.4% Vs 65%) with a relative PGD risk of 3.1 compared to allograft recipients without any circulating autoantibody (95% CI 1.2 to 8.1,  $p=0.02$ ). This risk was even higher if patients had all three autoantibodies (RR 7.4, 95% CI 0.93 to 58.9,  $p=0.03$ ) (22). Taken together, these data strongly support the role of autoantibodies in the pathogenesis of primary graft dysfunction which, in turn, predisposes to BOS.

## 5. DE NOVO AUTOIMMUNITY FOLLOWING LUNG TRANSPLANT

### 5.1. Lung transplantation provides an optimal environment for lung tissue specific autoimmunity

Lung allografts undergo continuous injury-repair cycles due to multiple insults sustained during and after transplantation. Such an inflammatory milieu is conducive for the expansion of self-reactive lymphocytes due to: i) release of previously “cryptic” determinants and neo-antigens, ii) lowering of T-cell activation threshold and priming of autoreactive T-cells with “low-affinity” TCRs which were previously below their activation potential (25), and iii) diversification of epitope specificity of alloreactive T-cells to new allo- or auto- antigens through epitope spreading (26, 27). Further, the perioperative stress can trigger inflammation through innate immune cells that include neutrophils, dendritic cells, macrophages and structural epithelial and endothelial cells in the lung allografts. These cells respond to foreign antigens as well as microbial or viral products using pathogen recognition receptors (PRRs). PRRs include toll-like receptors (TLRs), NOD-like receptors and the RIG-like helicases. PRRs may also react with self-antigens, such as hyaluronan or heat shock proteins, that may be released after cellular damage during transplantation and trigger an immune response. For instance, Tesar *et al* reported that hyaluronan degradation products can activate dendritic cells to produce proinflammatory mediators and prime alloimmunity (28). In addition, they showed that presence of hyaluron in human lung allograft samples correlate with BOS, thereby linking autologous TLR ligands to the development of lung allograft rejection (28). The role of the innate immunity in BOS is clearly emerging and may provide insight into developing novel therapeutic strategies to prevent it.

Another important risk factor for the development of both auto- as well as allo- immunity is PGD that can be seen in up to 70% of the lung transplant recipients within the first 24- 48 hours after transplant (22). In a series of 127 patients, we found that 22.8% had no PGD while the rest developed some grade of PGD. Patients with PGD had elevated levels of pro-inflammatory chemokines MCP-1 and IP-10 as well as cytokines IL-1 $\beta$ , IL-2, IFN- $\gamma$ , and IL-12 (29). Hence, PGD can then trigger the immune response and lead to inflammation that can, in turn, augment allo- as well as auto- immunity.

### 5.2. Autoimmunity in the pathogenesis of obliterative airway disease in animal models

Collagen type V (col V) is a non-polymorphic self-antigen present in the peribronchial and perivascular tissues of lung allograft tissue and is normally protected from the immune system since it is incorporated within collagen type I (30). In the rat lung transplant model, col V fragments were detected in the bronchoalveolar lavage specimens during peri-transplant inflammation and acute rejection. Immunohistochemistry revealed col V in the peribronchiolar and perivascular areas as well as interstitium of the transplanted lungs. In contrast, col V expression was not detected in normal control lungs (31). Col V-specific T cells were present in the transplanted lungs of these rats and proliferated in response to col V. Adoptive transfer of col V-specific T cells into normal lungs did not induce obliterative airway disease. However, transfer of col V specific T cells into isograft as well as allograft rat recipients induced rejection (32, 33). Interleukin-17, an autoimmune cytokine, was elevated in fresh isografts after adoptive transfer of col V specific T cells. These data imply a role of autoimmunity against these cryptic collagen epitopes in allograft rejection.

Wilkes and colleagues further tested the hypothesis that inducing tolerance towards col V prior to transplant would protect from rejection (32, 34). They first isolated bronchoalveolar lavage (BAL) cells from wild type C57Bl/6 mice and instilled them into the lungs of MHC-mismatched wild type Balb/c mice. This led to airway epithelium and vascular endothelial damage that was consistent with acute rejection. However, tolerizing these mice towards col V by administration of col V into the lungs prior to instillation of allogeneic BAL cells prevented the development of rejection pathology and down-regulated T-lymphocyte proliferation. They further hypothesized that the allogeneic BAL cells lead to tissue damage and released col V that is normally sequestered in the perivascular and peribronchial tissues. To support this they demonstrated that instillation of col V-pulsed autologous bronchoalveolar cells into lungs of mice that had previously received allogeneic BAL cells perpetuated rejection pathology (34).

In another model, rats were fed col V or diluent, as control, prior to receiving an MHC-mismatched allograft. This was done to induce tolerance against col V by oral feeding. Subsequently, the rats were transplanted with MHC-mismatched lungs in the absence of immunosuppression. At 10 weeks, the authors described that rats that received col V before transplant had much less severe rejection (mild, grade 2). This was in contrast to the diluent fed rats that developed full blown obliterative bronchiolitis. Furthermore, the col V fed rats did not mount immune response to donor antigens at the end of 10 weeks while the diluent fed mice had a robust response to donor antigens suggesting that inducing tolerance towards col V downregulated the alloimmune response. They then showed that adoptive transfer of T cells isolated from transplanted lungs of rats tolerized with col V could confer tolerance into syngeneic rats receiving MHC-mismatched lung transplantation (32, 33). It is debatable that the lesions shown in these studies truly represent chronic lung allograft

rejection as seen in human subjects. Nevertheless, these data suggest that tolerance to col V might abrogate the rejection of MHC mismatch allograft to some degree.

Airway epithelial cells (AEC) are important immunological targets in lung allograft rejection. Using heterotopic and orthotopic tracheal transplants models we demonstrated that tracheal allografts when first transplanted orthotopically get epithelialized with recipient epithelium that protects them from obliterative airway disease in the absence of any immunosuppression. These epithelialized tracheas when re-transplanted heterotopically into same strain recipients are protected from rejection while there was a development of obliterative airway disease (OAD) following transplantation into third-party recipients. These results clearly demonstrated that airway epithelium is a primary target for OAD (35). Anti-AEC antibodies produce pro-inflammatory and fibroblastic growth factors upon ligation with AEC (36). As we will describe later, we have identified a novel self-antigen expressed on AEC that becomes the target of autoimmunity following human lung transplantation (36). We also developed a novel murine model to study the role of allo-antibody induced autoimmunity following lung transplantation which is discussed below. Using this model we showed that administration of anti-MHC class I antibodies result in IL-17 mediated induction of autoantibodies as well as development of OAD-like lesions in the native lungs (37).

### 6. DE NOVO AUTOIMMUNITY AND ITS ROLE IN PATHOGENESIS OF BOS

A self-protein that has been extensively studied in the context of autoimmunity in lung transplant patients is collagen type V (col V). Col V is located within the lung interstitium and expressed by airway epithelial cells. Its expression is enhanced by ischemia reperfusion injury following transplantation and tissue remodeling (38). T cells specific to col V can lead to acute rejection in rat isografts suggesting autoreactive T cells may promote graft failure. Col V specific T cell clones were found to be expanded in patients with BOS (39) and associated with 10-fold increased risk for BOS (40). While dendritic cells are known to be key players in initiating cellular immunity, col V reactivity is reported to be dependent on monocytes (CD14<sup>+</sup>) (40). However, the cellular immune responses to col V were mediated by IL-17A, TNF-alpha, and IL-1beta. Hence coordination between CD4<sup>+</sup> T cells and monocytes may induce IL-17 producing autoreactive Th17 cells and mediate BOS. Lung allografts are infiltrated with many monocytes and macrophages and thus provide a unique environment to promote interactions and expansion of autoreactive T cells.

Th17 cells are involved in mucosal immunity and have been shown to contribute to the development of autoimmunity both in animal models and humans (41). However, the differentiation of T cells into Th17 cells has not been fully characterized. It is speculated that the cytokine milieu and activation status of the antigen presenting cells strongly influence the differentiation of T cell into Th17 cells. Cytokines that have been shown to

promote development of Th17 cells include IL-1 $\beta$ , TGF- $\beta$ , IL-6, IL-23 and IL-21. Vanaudenaerde *et al* found that lung transplant recipients with BOS had increased BAL levels of IL-17 and IL-8 (42). Although, the neutrophilic cytokine, IL-8 has been associated with BOS (43) it is unclear whether neutrophils are a marker of inflammation or mediator of obliterative airway disease. Since IL-17 is also chemotactic for neutrophils, current data suggests that the presence of neutrophils may be secondary to a Th17 mediated alloimmune or autoimmune response.

Since Th-17 cells may be involved in mucosal and anti-epithelial immunity, the association of IL-17 with OAD supports findings from our experiments that airway epithelial cells might be a major target for development of OAD (35). As discussed before, we showed that murine tracheal allografts re-epithelialized by recipient epithelium are protected against obliterative airway disease when re-transplanted heterotopically. Since the initial donor epithelium is not exposed to the recipient immune system in that model, OAD does not develop in this model. Human studies further support the role of anti-epithelial autoimmunity in OAD development. In a recent study from our lab, Goers *et al* found that about a third of human lung transplant recipients develop non-HLA anti-epithelial cell antibodies that lead to the production of pro-fibrogenic growth factors and fibro-proliferation upon ligation with airway epithelium. Further sequencing revealed that these antibodies were specific to a self-antigen, k-alpha 1 tubulin (36). Subsequent studies from our lab have shown that lipid raft mediated ligation of autoantibodies to k-alpha tubulin on epithelial cell membrane results in upregulation of growth factor cascades included pro-fibrogenic growth factors involved in the pathogenesis of BOS (44).

### 7. ALLOIMMUNITY AND INDUCTION OF *DE NOVO* AUTOIMMUNITY

Antibodies against MHC class I antigens pose an increased risk for allograft failure and after lung transplantation. In a prospective analysis, we demonstrated that onset of anti-MHC class I Abs precedes the development of BOS (44, 45). As discussed above, AEC are important immunological targets in lung allograft rejection. When stimulated with anti-MHC class I Abs, AECs undergo proliferation and secretion of pro-fibrogenic growth factors. These include heparin-binding epidermal growth factor (HBEGF), basic fibroblast growth factor (b-FGF), granulocyte monocyte colony-stimulating factor, insulin like growth factor-1, platelet-derived growth factor, and transforming growth factor-beta (TGF- $\beta$ ). Besides augmenting the immune response, they promote fibrogenesis and airway obliteration. Subsequent studies have also demonstrated a correlation between development of *de novo* anti- MHC class II Abs and BOS (10).

We previously used murine models of OAD to better characterize the role of anti-MHC antibodies and chronic lung rejection. Following heterotopic tracheal transplantation, administration of anti-MHC class I Abs into immune-deficient RAG-1-knockout mice resulted in obliterative airway pathology. Up-regulation of growth

factors and pro-apoptotic genes was noted in mice treated with anti-MHC class I Abs but not with control Ab. Alloantibody induced activation of AECs lead to over expression of adhesion, costimulatory, and MHC class II molecules as well as secretion of proinflammatory cytokines and chemokines. Such an inflammatory milieu may be conducive for development of autoimmunity. This further lead us to hypothesize that alloimmunity may induce autoimmunity.

We investigated this hypothesis in our unique murine model in which anti- MHC class I monoclonal antibodies were administered into the trachea and induced autoimmunity and OAD-like lesion in the native lungs (37). In this model, administration of anti-MHC class I antibodies into the native lungs of mice resulted in autoimmunity leading to cellular infiltration, epithelial hyperplasia, endothelitis, fibro-proliferation, collagen deposition and luminal occlusion of the small airways- the central events that are pathognomonic of chronic human lung allograft rejection. Monoclonal antibodies against strain MHC class I antigens were administered intrabronchially into the native lungs of 3 different strains of mice - BALB/C, C57BL/6 and HLA transgenic C57BL/6 mice on days 1, 2, 3, and 6, and then weekly thereafter. Antibodies of the same isotype (C1.18.4) and anti-keratin Abs were administered in control animals. By day 15, histopathological analysis of the anti-MHC class I Ab administered lung tissue revealed the presence of peribronchial and perivascular mononuclear infiltrates, epithelial hyperplasia and fibrosis. By day 30, there was increase in cellular infiltration around vessels and bronchioles. There was also an increase in fibrosis along with luminal occlusion of the small airways. In contrast, there was no evidence of cellular infiltration, epithelial hyperplasia or fibrosis in the isotype or anti-keratin antibody treated animals. Immunohistochemical studies revealed the presence of CD4<sup>+</sup> and CD11b<sup>+</sup> cells around the bronchioles and vessels at both day 15 and day 30 in anti-MHC class I antibody administered mice. The ligation of MHC class I molecules expressed on the lung parenchyma with antibodies significantly increased the expression of pro-inflammatory cytokines, chemokines and their receptors along with increased bone morphogenetic protein (BMP) and fibroblast growth factor (FGF) family of growth factors. Further, in our murine model, IL-17 was the potent proinflammatory cytokine that acted on epithelial cells, airway endothelial cells, and fibrocytes and lead to further cytokine and chemokine secretion (37). There was a significant increase in the frequency of IL-17 producing T cells against K-alpha1T and col V in lung infiltrating T cells of mice administered with anti-MHC Abs. In addition, *de novo* production of autoantibodies against k-alpha 1 and col V was noted. Hence alloantibodies may induce inflammation and lead to the development of autoimmunity.

### 8. LOSS OF REGULATORY T CELLS AND AUTOIMMUNITY

While the mechanisms of tolerance and autoimmunity development are complex, a subset of CD4<sup>+</sup>

T cells characterized by cell surface marker CD25 and transcriptional factor *foxp3* have emerged as one of the key players for maintenance of tolerance in the periphery outside the thymus. Their function seems to be dependent on Interleukin-2 (IL-2). These CD4<sup>+</sup>CD25<sup>+</sup>*Foxp3*<sup>+</sup> cells are known as regulatory T cells or Tregs and their role in tolerance is now well established. For example, CD25 deficient mice develop severe autoimmunity that can be prevented by passive transfer of CD4<sup>+</sup>CD25<sup>+</sup> Tregs from normal syngeneic mice. Furthermore, *in vivo* administration of anti-IL-2 monoclonal antibodies substantially reduced CD4<sup>+</sup>CD25<sup>+</sup> T cells and consequently produced autoimmunity (46-51).

The scurfy strain of mice carries a lethal X-linked recessive mutation in the *foxp3* gene that codes for the scurf protein, a member of the forkhead/winged-helix family of transcription factors. These mice develop hyperactivation of CD4<sup>+</sup> T-cells and consequently severe multi-organ autoimmune disease within a month after birth (52-54). In 1982, X-linked human immunodeficiency syndrome called IPEX (immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance) was described (55). This condition is associated with a severe autoimmune disease similar to that seen in scurfy mice and was subsequently found to have mutations in the human FOXP3 gene (56-58). Recently, *foxp3* has been found to be crucial for the development of CD4<sup>+</sup>CD25<sup>+</sup> Tregs that are capable of preventing autoimmune diseases in the scurfy mice (59-61). Further, females hemizygous for the *foxp3* mutation reveal genetic mosaicism due to random inactivation of the X-chromosome. These “carrier” females do not develop autoimmune diseases due to the presence of residual normal, *foxp3* expressing Tregs (62). Taken together, these findings provide direct evidence that *foxp3*<sup>+</sup> Tregs mediate a dominant form of tolerance and prevent the development of autoimmune diseases both in mice and humans.

In our earlier report, we serially analyzed clones of collagen type V specific T cells in lung transplant recipients. The col-V specific CD4<sup>+</sup> T-cells in LT patients were predominantly IL-10 producing with low IFN-gamma, IL-2, IL-5 and no IL-4 production. The IL-10 producing T cells strongly suppressed the IFN-gamma producing Th-1 type T cells (39). However, in patients that developed BOS, we found that IL-10 producing T cells were lost and there was expansion of the IFN-gamma T cells. The IL-10 producing T cells were distinct from naturally occurring CD4<sup>+</sup>CD25<sup>+</sup>*foxp3*<sup>+</sup> regulatory T cells since they did not express CD25 or *foxp3* constitutively. However, their development was dependent on CTLA signals from natural Tregs (63). Hence, we showed a role for both natural and adaptive regulatory T cells in the pathogenesis of BOS. Also, previous studies both from our lab and others have shown an association between Treg dysfunction and increased incidence of BOS (64).

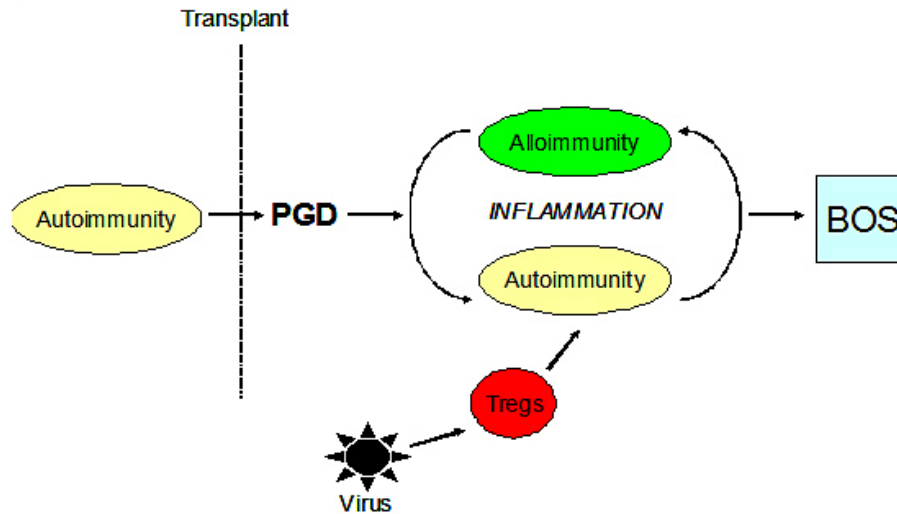
Since regulatory T cells are responsible for peripheral tolerance and prevention of autoimmunity, loss of Tregs would lead to development of autoimmunity. Respiratory viral infections (RVI) represent one of the strongest but preventable risk factors for the development

of BOS (12). In a cohort of 259 patients, Khalifah *et al* demonstrated that respiratory viral infections were distinct risk factors for BOS (12). Furthermore, in our previous report we demonstrated that murine parainfluenza sendai viral (SdV) infection, a correlate of human RVI, increased the severity of obliterative airway disease in murine tracheal transplant model (65). Treg play a major role in peripheral tolerance against self-antigens and loss of Treg leads to autoimmunity (66). Since many viruses have also been implicated in autoimmune disorders (67), we tested the hypothesis that RVI predisposed to BOS by inducing Treg dysfunction and promoting an immune response to self-antigens.

We performed a longitudinal analysis of Tregs in lung transplant patients (13). Over 40% of the study recipients revealed troughs in the frequency of Treg troughs as defined by a decrease greater than 50% of baseline. This was due to apoptosis in the Tregs. Respiratory viral infections were the only risk factor associated with the Treg apoptosis. The viruses tested included influenza, parainfluenza, respiratory syncytial, and adenovirus. Furthermore, patients that showed Treg apoptosis had increased prevalence of antibodies to self antigens collagen type I (23.1% Vs 5.8% pre-trough), collagen V (7.7% Vs 0%), and k-alpha tubulin (30.7% Vs 11.7%, *p*<0.01) at 6-months. Increased number of Treg troughs also correlated with more rapid onset of BOS.

We then tested the mechanisms by which respiratory viruses induce Treg apoptosis using the murine orthotopic tracheal transplant model. Infection of tracheal transplant recipients with murine parainfluenza sendai virus led to increased Treg apoptosis in the allograft draining lymph nodes. Vaccination against sendai virus prior to transplant abrogated apoptosis of Treg. *In vitro*, sendai virus infected, but not naive, tracheal epithelial cells demonstrated upregulation of FasL. Further, upon co-culture of Tregs, the FasL expressing epithelial cells induce apoptosis in the Tregs. We have also recently shown that dendritic cells from virus infected murine orthotopic transplant recipients can also convert Tregs into Th-17 cells in the presence of cognate antigen and IL-6 (Bharat *et al*, Manuscript under preparation). Taken together, these data revealed a new paradigm of regulatory T cells dysfunction, induction of autoimmunity and chronic lung allograft rejection.

Multiple other hypotheses have been postulated for regulatory T cell dysfunction post-transplant. For example, an indirect relationship exists between *Foxp3*, expressed in Tregs, and ROR-gamma-t, a transcription factor expressed in Th17 cells. Both *Foxp3* and ROR-gamma-t are critically dependent on TGF-beta for differentiation. *In vitro*, IL-6 can redirect *foxp3*<sup>+</sup> T cells to express IL-17 and this transition has been described *in vivo* during an inflammatory response in experimental models of autoimmune encephalitis. Since IL-6 and IL-1beta have previously been associated with BOS and are known mediators of Th17 development an intriguing hypothesis is that Tregs with T cell receptors specific for self-antigens get converted to autoimmune T cells in the presence of these cytokines.



**Figure 1.** Autoimmunity, PGD, and BOS: emerging concepts

BOS is believed to affect the majority of lung transplant patients surviving beyond 8-10 years post-transplantation. Contemporary immunosuppression has shown little improvement on its incidence and prognosis. Since the function and development of Treg is dependent on IL-2 (66), calcineurin inhibitors like cyclosporin A and tacrolimus might be detrimental to Tregs. In fact, cyclosporin A has been shown to induce autoimmunity (68-70). Hence, one plausible explanation for the decline of Tregs in transplant recipients could be related to the routine administration of immunosuppressive drugs. Therefore, identification and use of Treg sparing immunosuppressive agents may better promote long-term allograft survival. Such studies are underway and have shown promising preliminary results with mycophenolate mofetil and rapamycin (71, 72).

## 9. CONCLUDING REMARKS

As the literature grows, the pathways linking the pre- and peri- transplant risk factors to the pathogenesis of BOS are becoming more complex. Recognition of autoimmunity in the development of BOS is one of the most exciting recent discoveries in the context of lung transplantation. We now know that patients on lung transplant waiting list may have circulating autoantibodies that not only contribute to immediate PGD but also to BOS. Hence a novel strategy would be to screen these patients and possibly treat them with IVIG or plasmapheresis. Pathways have also been characterized that lead to *de novo* autoimmunity following transplantation. In fact in the post-transplant period, alloimmune responses and the development of *de novo* autoimmunity to self-antigens appear to be key mechanisms in the pathogenesis of BOS (Figure 1). The “injury-response” cycle triggered by tissue inflammation and remodeling provide the substrate for the activation of both cellular and humoral arms of immunity. Further, the new IL-17 pathway seems to substantially contribute to both allo- and auto- immunity and may represent a novel strategy for future therapeutic

interventions. There is ongoing investigation to understand Treg dysfunction and its effect on autoimmunity and BOS. For example, novel pathways have been described by which respiratory viruses induce Treg apoptosis or convert them into Th-17 cells and thereby promote autoimmunity. Future work in the field is needed to characterize the mechanistic role of autoimmunity in the development of BOS and the clinical utility of using autoantibodies as a biomarker for rejection.

## 10. REFERENCES

1. M. I. Hertz, P. Aurora, J. D. Christie, F. Dobbels, L. B. Edwards, R. Kirk, A. Y. Kucheryavaya, A. O. Rahmel, A. W. Rowe, and D. O. Taylor: Registry of the International Society for Heart and Lung Transplantation: a quarter century of thoracic transplantation. *J Heart Lung Transplant* 27(9), 937-42 (2008)
2. J. D. Christie, L. B. Edwards, P. Aurora, F. Dobbels, R. Kirk, A. O. Rahmel, D. O. Taylor, A. Y. Kucheryavaya, and M. I. Hertz: Registry of the International Society for Heart and Lung Transplantation: twenty-fifth official adult lung and heart/lung transplantation report--2008. *J Heart Lung Transplant* 27(9), 957-69 (2008)
3. S. A. Yousem, G. J. Berry, P. T. Cagle, D. Chamberlain, A. N. Husain, R. H. Hruban, A. Marchevsky, N. P. Otori, J. Ritter, S. Stewart, and H. D. Tazelaar: Revision of the 1990 working formulation for the classification of pulmonary allograft rejection: Lung Rejection Study Group. *J Heart Lung Transplant* 15(1 Pt 1), 1-15 (1996)
4. S. A. Daud, R. D. Yusen, B. F. Meyers, M. M. Chakinala, M. J. Walter, A. A. Aloush, G. A. Patterson, E. P. Trulock, and R. R. Hachem: Impact of immediate primary lung allograft dysfunction on bronchiolitis obliterans syndrome. *Am J Respir Crit Care Med* 175(5), 507-13 (2007)
5. A. Bharat, N. Benshoff, T. P. Fleming, J. R. Dietz, W. E. Gillanders, and T. Mohanakumar: Characterization of the

role of CD8+T cells in breast cancer immunity following mammaglobin-A DNA vaccination using HLA-class-I tetramers. *Breast Cancer Res Treat* 110(3), 453-63 (2008)

6.L. D. Sharples, K. McNeil, S. Stewart, and J. Wallwork: Risk factors for bronchiolitis obliterans: a systematic review of recent publications. *J Heart Lung Transplant* 21(2), 271-81 (2002)

7.C. L. Lau, S. M. Palmer, K. E. Posther, D. N. Howell, N. L. Reinsmoen, H. T. Massey, V. F. Tapson, J. J. Jagers, T. A. D'Amico, and R. D. Davis, Jr.: Influence of panel-reactive antibodies on posttransplant outcomes in lung transplant recipients. *Ann Thorac Surg* 69(5), 1520-4 (2000)

8.S. M. Palmer, R. D. Davis, D. Hadjiliadis, M. I. Hertz, D. N. Howell, F. E. Ward, K. Savik, and N. L. Reinsmoen: Development of an antibody specific to major histocompatibility antigens detectable by flow cytometry after lung transplant is associated with bronchiolitis obliterans syndrome. *Transplantation* 74(6), 799-804 (2002)

9.A. L. Girnita, R. Duquesnoy, S. A. Yousem, A. T. Iacono, T. E. Corcoran, M. Buzoianu, B. Johnson, K. J. Spichty, J. H. Dauber, G. Burckart, B. P. Griffith, K. R. McCurry, and A. Zeevi: HLA-specific antibodies are risk factors for lymphocytic bronchiolitis and chronic lung allograft dysfunction. *Am J Transplant* 5(1), 131-8 (2005)

10.A. Bharat, K. Narayanan, T. Street, R. C. Fields, N. Steward, A. Aloush, B. Meyers, R. Schuessler, E. P. Trulock, G. A. Patterson, and T. Mohanakumar: Early posttransplant inflammation promotes the development of alloimmunity and chronic human lung allograft rejection. *Transplantation* 83(2), 150-8 (2007)

11.A. Jaramillo, L. Zhang, and T. Mohanakumar: Binding of anti-HLA class I antibodies to airway epithelial cells induces activation and growth factor production and indirectly upregulates lung fibroblast proliferation. *J Heart Lung Transplant* 20(2), 166 (2001)

12.A. P. Khalifah, R. R. Hachem, M. M. Chakinala, K. B. Schechtman, G. A. Patterson, D. P. Schuster, T. Mohanakumar, E. P. Trulock, and M. J. Walter: Respiratory viral infections are a distinct risk for bronchiolitis obliterans syndrome and death. *Am J Respir Crit Care Med* 170(2), 181-7 (2004)

13.A. Bharat, E. Kuo, D. Saini, N. Steward, R. Hachem, E. P. Trulock, G. A. Patterson, B. F. Meyers, and T. Mohanakumar: Respiratory virus-induced dysregulation of T-regulatory cells leads to chronic rejection. *Ann Thorac Surg* 90(5), 1637-44; discussion 1644 (2010)

14.D. Hadjiliadis, R. Duane Davis, M. P. Steele, R. H. Messier, C. L. Lau, S. S. Eubanks, and S. M. Palmer: Gastroesophageal reflux disease in lung transplant recipients. *Clin Transplant* 17(4), 363-8 (2003)

15.E. Cantu, 3rd, J. Z. Appel, 3rd, M. G. Hartwig, H. Woreta, C. Green, R. Messier, S. M. Palmer, and R. D. Davis, Jr.: J. Maxwell Chamberlain Memorial Paper. Early fundoplication prevents chronic allograft dysfunction in patients with gastroesophageal reflux disease. *Ann Thorac Surg* 78(4), 1142-51; discussion 1142-51 (2004)

16.J. L. Bobadilla, E. Jankowska-Gan, Q. Xu, L. D. Haynes, A. Munoz del Rio, K. Meyer, D. S. Greenspan, N. De Oliveira, W. J. Burlingham, and J. D. Maloney: Reflux-induced collagen type v sensitization: potential mediator of bronchiolitis obliterans syndrome. *Chest* 138(2), 363-70 (2010)

17.R. A. Shilling and D. S. Wilkes: Immunobiology of chronic lung allograft dysfunction: new insights from the bench and beyond. *Am J Transplant* 9(8), 1714-8 (2009)

18.Y. Arnson, Y. Shoenfeld, and H. Amital: Effects of tobacco smoke on immunity, inflammation and autoimmunity. *J Autoimmun* 34(3), J258-65.

19.J. C. Lee and J. D. Christie: Primary graft dysfunction. *Clin Chest Med* 32(2), 279-93 (2011)

20.I. Inci, B. Erne, S. Arni, W. Jungraithmayr, D. Inci, S. Hillinger, P. Vogt, B. Leskosek, and W. Weder: Prevention of primary graft dysfunction in lung transplantation by N-acetylcysteine after prolonged cold ischemia. *J Heart Lung Transplant* 29(11), 1293-301 (2010)

21.G. P. Westall, G. I. Snell, C. McLean, T. Kotsimbos, T. Williams, and C. Magro: C3d and C4d deposition early after lung transplantation. *J Heart Lung Transplant* 27(7), 722-8 (2008)

22.A. Bharat, D. Saini, N. Steward, R. Hachem, E. P. Trulock, G. A. Patterson, B. F. Meyers, and T. Mohanakumar: Antibodies to self-antigens predispose to primary lung allograft dysfunction and chronic rejection. *Ann Thorac Surg* 90(4), 1094-101 (2010)

23.T. Iwata, A. Philipovski, A. J. Fisher, R. G. Presson, Jr., M. Chiyo, J. Lee, E. Mickler, G. N. Smith, I. Petrache, D. B. Brand, W. J. Burlingham, B. Gopalakrishnan, D. S. Greenspan, J. D. Christie, and D. S. Wilkes: Anti-type V collagen humoral immunity in lung transplant primary graft dysfunction. *J Immunol* 181(8), 5738-47 (2008)

24.J. L. Bobadilla, R. B. Love, E. Jankowska-Gan, Q. Xu, L. D. Haynes, R. K. Braun, M. S. Hayney, A. Munoz del Rio, K. Meyer, D. S. Greenspan, J. Torrealba, K. M. Heidler, O. W. Cummings, T. Iwata, D. Brand, R. Presson, W. J. Burlingham, and D. S. Wilkes: Th-17, monokines, collagen type V, and primary graft dysfunction in lung transplantation. *Am J Respir Crit Care Med* 177(6), 660-8 (2008)

25.E. E. Sercarz, P. V. Lehmann, A. Ametani, G. Benichou, A. Miller, and K. Moudgil: Dominance and crypticity of T cell antigenic determinants. *Annu Rev Immunol* 11, 729-66 (1993)



- 26.R. Ciubotariu, A. I. Colovai, G. Pennesi, Z. Liu, D. Smith, P. Berlocco, R. Cortesini, and N. Suciu-Foca: Specific suppression of human CD4<sup>+</sup> Th cell responses to pig MHC antigens by CD8<sup>+</sup>CD28<sup>-</sup> regulatory T cells. *J Immunol* 161(10), 5193-202 (1998)
- 27.Z. Liu, A. I. Colovai, S. Tugulea, E. F. Reed, P. E. Fisher, D. Mancini, E. A. Rose, R. Cortesini, R. E. Michler, and N. Suciu-Foca: Indirect recognition of donor HLA-DR peptides in organ allograft rejection. *J Clin Invest* 98(5), 1150-7 (1996)
- 28.B. M. Tesar, D. Jiang, J. Liang, S. M. Palmer, P. W. Noble, and D. R. Goldstein: The role of hyaluronan degradation products as innate alloimmune agonists. *Am J Transplant* 6(11), 2622-35 (2006)
- 29.A. Bharat, E. Kuo, N. Steward, A. Aloush, R. Hachem, E. P. Trulock, G. A. Patterson, B. F. Meyers, and T. Mohanakumar: Immunological link between primary graft dysfunction and chronic lung allograft rejection. *Ann Thorac Surg* 86(1), 189-95; discussion 196-7 (2008)
- 30.E. Adachi, I. Hopkinson, and T. Hayashi: Basement-membrane stromal relationships: interactions between collagen fibrils and the lamina densa. *Int Rev Cytol* 173, 73-156 (1997)
- 31.M. A. Haque, T. Mizobuchi, K. Yasufuku, T. Fujisawa, R. R. Brutkiewicz, Y. Zheng, K. Woods, G. N. Smith, O. W. Cummings, K. M. Heidler, J. S. Blum, and D. S. Wilkes: 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* 169(3), 1542-9 (2002)
- 32.K. Yasufuku, K. M. Heidler, K. A. Woods, G. N. Smith, Jr., O. W. Cummings, T. Fujisawa, and D. S. Wilkes: Prevention of bronchiolitis obliterans in rat lung allografts by type V collagen-induced oral tolerance. *Transplantation* 73(4), 500-5 (2002)
- 33.Y. Yamada, Y. Sekine, S. Yoshida, K. Yasufuku, I. Petrache, H. L. Benson, D. D. Brand, I. Yoshino, and D. S. Wilkes: Type V collagen-induced oral tolerance plus low-dose cyclosporine prevents rejection of MHC class I and II incompatible lung allografts. *J Immunol* 183(1), 237-45 (2009)
- 34.D. C. Mares, K. M. Heidler, G. N. Smith, O. W. Cummings, E. R. Harris, B. Foresman, and D. S. Wilkes: Type V collagen modulates alloantigen-induced pathology and immunology in the lung. *Am J Respir Cell Mol Biol* 23(1), 62-70 (2000)
- 35.F. G. Fernandez, A. Jaramillo, C. Chen, D. Z. Liu, T. Tung, G. A. Patterson, and T. Mohanakumar: Airway epithelium is the primary target of allograft rejection in murine obliterative airway disease. *Am J Transplant* 4(3), 319-25 (2004)
- 36.T. A. Goers, S. Ramachandran, A. Aloush, E. Trulock, G. A. Patterson, and T. Mohanakumar: De novo production of K-alpha1 tubulin-specific antibodies: role in chronic lung allograft rejection. *J Immunol* 180(7), 4487-94 (2008)
- 37.N. Fukami, S. Ramachandran, D. Saini, M. Walter, W. Chapman, G. A. Patterson, and T. Mohanakumar: Antibodies to MHC class I induce autoimmunity: role in the pathogenesis of chronic rejection. *J Immunol* 182(1), 309-18 (2009)
- 38.S. Yoshida, A. Haque, T. Mizobuchi, T. Iwata, M. Chiyo, T. J. Webb, L. A. Baldrige, K. M. Heidler, O. W. Cummings, T. Fujisawa, J. S. Blum, D. D. Brand, and D. S. Wilkes: Anti-type V collagen lymphocytes that express IL-17 and IL-23 induce rejection pathology in fresh and well-healed lung transplants. *Am J Transplant* 6(4), 724-35 (2006)
- 39.A. Bharat, R. C. Fields, N. Steward, E. P. Trulock, G. A. Patterson, and T. Mohanakumar: CD4<sup>+</sup>25<sup>+</sup> regulatory T cells limit Th1-autoimmunity by inducing IL-10 producing T cells following human lung transplantation. *Am J Transplant* 6(8), 1799-808 (2006)
- 40.W. J. Burlingham, R. B. Love, E. Jankowska-Gan, L. D. Haynes, Q. Xu, J. L. Bobadilla, K. C. Meyer, M. S. Hayney, R. K. Braun, D. S. Greenspan, B. Gopalakrishnan, J. Cai, D. D. Brand, S. Yoshida, O. W. Cummings, and D. S. Wilkes: IL-17-dependent cellular immunity to collagen type V predisposes to obliterative bronchiolitis in human lung transplants. *J Clin Invest* 117(11), 3498-506 (2007)
- 41.T. Korn, E. Bettelli, M. Oukka, and V. K. Kuchroo: IL-17 and Th17 Cells. *Annu Rev Immunol* 27, 485-517 (2009)
- 42.B. M. Vanaudenaerde, S. I. De Vleeschauwer, R. Vos, I. Meyts, D. M. Bullens, V. Reyniers, W. A. Wuyts, D. E. Van Raemdonck, L. J. Dupont, and G. M. Verleden: The role of the IL23/IL17 axis in bronchiolitis obliterans syndrome after lung transplantation. *Am J Transplant* 8(9), 1911-20 (2008)
- 43.D. J. Slebos, D. S. Postma, G. H. Koeter, W. Van Der Bij, M. Boezen, and H. F. Kauffman: Bronchoalveolar lavage fluid characteristics in acute and chronic lung transplant rejection. *J Heart Lung Transplant* 23(5), 532-40 (2004)
- 44.V. Tiriveedhi, N. Angaswamy, J. Weber, and T. Mohanakumar: Lipid raft facilitated ligation of K-alpha1-tubulin by specific antibodies on epithelial cells: Role in pathogenesis of chronic rejection following human lung transplantation. *Biochem Biophys Res Commun* 399(2), 251-5 (2010)
- 45.A. Jaramillo, F. G. Fernandez, E. Y. Kuo, E. P. Trulock, G. A. Patterson, and T. Mohanakumar: Immune mechanisms in the pathogenesis of bronchiolitis obliterans syndrome after lung transplantation. *Pediatr Transplant* 9(1), 84-93 (2005)
- 46.H. Schorle, T. Holtzke, T. Hunig, A. Schimpl, and I. Horak: Development and function of T cells in mice

rendered interleukin-2 deficient by gene targeting. *Nature* 352(6336), 621-4 (1991)

47.A. R. Almeida, N. Legrand, M. Papiernik, and A. A. Freitas: Homeostasis of peripheral CD4<sup>+</sup> T cells: IL-2R alpha and IL-2 shape a population of regulatory cells that controls CD4<sup>+</sup> T cell numbers. *J Immunol* 169(9), 4850-60 (2002)

48.H. Suzuki, Y. W. Zhou, M. Kato, T. W. Mak, and I. Nakashima: Normal regulatory alpha/beta T cells effectively eliminate abnormally activated T cells lacking the interleukin 2 receptor beta *in vivo*. *J Exp Med* 190(11), 1561-72 (1999)

49.D. M. Willerford, J. Chen, J. A. Ferry, L. Davidson, A. Ma, and F. W. Alt: Interleukin-2 receptor alpha chain regulates the size and content of the peripheral lymphoid compartment. *Immunity* 3(4), 521-30 (1995)

50.T. R. Malek, A. Yu, V. Vincek, P. Scibelli, and L. Kong: CD4 regulatory T cells prevent lethal autoimmunity in IL-2Rbeta-deficient mice. Implications for the nonredundant function of IL-2. *Immunity* 17(2), 167-78 (2002)

51.G. C. Furtado, M. A. Curotto de Lafaille, N. Kutchukhidze, and J. J. Lafaille: Interleukin 2 signaling is required for CD4(+) regulatory T cell function. *J Exp Med* 196(6), 851-7 (2002)

52.M. E. Brunkow, E. W. Jeffery, K. A. Hjerrild, B. Paepers, L. B. Clark, S. A. Yasayko, J. E. Wilkinson, D. Galas, S. F. Ziegler, and F. Ramsdell: Disruption of a new forkhead/winged-helix protein, scurf, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat Genet* 27(1), 68-73 (2001)

53.T. A. Chatila, F. Blaeser, N. Ho, H. M. Lederman, C. Voulgaropoulos, C. Helms, and A. M. Bowcock: JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic dysregulation syndrome. *J Clin Invest* 106(12), R75-81 (2000)

54.V. L. Godfrey, J. E. Wilkinson, and L. B. Russell: X-linked lymphoreticular disease in the scurfy (sf) mutant mouse. *Am J Pathol* 138(6), 1379-87 (1991)

55.B. R. Powell, N. R. Buist, and P. Stenzel: An X-linked syndrome of diarrhea, polyendocrinopathy, and fatal infection in infancy. *J Pediatr* 100(5), 731-7 (1982)

56.E. Gambineri, T. R. Torgerson, and H. D. Ochs: Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of FOXP3, a critical regulator of T-cell homeostasis. *Curr Opin Rheumatol* 15(4), 430-5 (2003)

57.C. L. Bennett, J. Christie, F. Ramsdell, M. E. Brunkow, P. J. Ferguson, L. Whitesell, T. E. Kelly, F. T. Saulsbury, P. F. Chance, and H. D. Ochs: The immune dysregulation,

polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat Genet* 27(1), 20-1 (2001)

58.R. S. Wildin, F. Ramsdell, J. Peake, F. Faravelli, J. L. Casanova, N. Buist, E. Levy-Lahad, M. Mazzella, O. Goulet, L. Perroni, F. D. Bricarelli, G. Byrne, M. McEuen, S. Proll, M. Appleby, and M. E. Brunkow: X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat Genet* 27(1), 18-20 (2001)

59.J. D. Fontenot, M. A. Gavin, and A. Y. Rudensky: Foxp3 programs the development and function of CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells. *Nat Immunol* 4(4), 330-6 (2003)

60.S. Hori, T. Nomura, and S. Sakaguchi: Control of regulatory T cell development by the transcription factor Foxp3. *Science* 299(5609), 1057-61 (2003)

61.R. Khattri, T. Cox, S. A. Yasayko, and F. Ramsdell: An essential role for Scurfin in CD4<sup>+</sup>CD25<sup>+</sup> T regulatory cells. *Nat Immunol* 4(4), 337-42 (2003)

62.A. Tommasini, S. Ferrari, D. Moratto, R. Badolato, M. Boniotto, D. Pirulli, L. D. Notarangelo, and M. Andolina: X-chromosome inactivation analysis in a female carrier of FOXP3 mutation. *Clin Exp Immunol* 130(1), 127-30 (2002)

63.A. Bharat, R. C. Fields, E. P. Trulock, G. A. Patterson, and T. Mohanakumar: Induction of IL-10 suppressors in lung transplant patients by CD4<sup>+</sup>25<sup>+</sup> regulatory T cells through CTLA-4 signaling. *J Immunol* 177(8), 5631-8 (2006)

64.S. M. Bhorade, H. Chen, L. Molinero, C. Liao, E. R. Garrity, W. T. Vigneswaran, R. Shilling, A. Sperling, A. Chong, and M. L. Alegre: Decreased percentage of CD4<sup>+</sup>FoxP3<sup>+</sup> cells in bronchoalveolar lavage from lung transplant recipients correlates with development of bronchiolitis obliterans syndrome. *Transplantation* 90(5), 540-6 (2010)

65.E. Kuo, A. Bharat, T. Goers, W. Chapman, L. Yan, T. Street, W. Lu, M. Walter, A. Patterson, and T. Mohanakumar: Respiratory viral infection in obliterative airway disease after orthotopic tracheal transplantation. *Ann Thorac Surg* 82(3), 1043-50 (2006)

66.S. Sakaguchi: Naturally arising Foxp3-expressing CD25<sup>+</sup>CD4<sup>+</sup> regulatory T cells in immunological tolerance to self and non-self. *Nat Immunol* 6(4), 345-52 (2005)

67.A. M. Ercolini and S. D. Miller: The role of infections in autoimmune disease. *Clin Exp Immunol* 155(1), 1-15 (2009)

68.R. Huss: *In vitro* determination of self-reactivity in the early postcyclosporine period. *Transpl Immunol* 1(3), 228-34 (1993)

69.S. Sakaguchi and N. Sakaguchi: Thymus and autoimmunity. Transplantation of the thymus from cyclosporin A-treated mice causes organ-specific autoimmune disease in athymic nude mice. *J Exp Med* 167(4), 1479-85 (1988)

70.S. Sakaguchi and N. Sakaguchi: Organ-specific autoimmune disease induced in mice by elimination of T cell subsets. V. Neonatal administration of cyclosporin A causes autoimmune disease. *J Immunol* 142(2), 471-80 (1989)

71.S. Gregori, M. Casorati, S. Amuchastegui, S. Smioldo, A. M. Davalli, and L. Adorini: Regulatory T cells induced by 1 alpha,25-dihydroxyvitamin D3 and mycophenolate mofetil treatment mediate transplantation tolerance. *J Immunol* 167(4), 1945-53 (2001)

72.M. Battaglia, A. Stabilini, and M. G. Roncarolo: Rapamycin selectively expands CD4+CD25+FoxP3+ regulatory T cells. *Blood* 105(12), 4743-8 (2005)

**Abbreviations:** BOS: Bronchiolitis Obliterans Syndrome, PGD: Primary Graft Dysfunction, HLA: Human Leukocyte Antigen, PRR: Pathogen recognition receptors, TLR: Toll-like receptors, Col V: Collagen type V, BAL: Bronchoalveolar lavage, MHC: Major Histocompatibility Complex, AEC: Airway epithelial cells, OAD: Obliterative airway disease, RVI: Respiratory viral infections

**Key Words:** Autoimmunity, Bronchiolitis Obliterans syndrome, Primary graft dysfunction, Alloimmunity, Rejection, Lung yransplant, Review

**Send Correspondence to:** Ankit Bharat, Department of Surgery and Cardiothoracic Surgery, Box 8234 CSRB, 660 S. Euclid Avenue, St. Louis, MO 63110, Tel: 314-362-6237, Fax: 314-747-4216, E-mail: bharata@wudosis.wustl.edu