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Idiopathic Pulmonary Fibrosis and a role for autoimmunity.

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Abstract

Idiopathic Pulmonary Fibrosis (IPF) is the most common of the idiopathic interstitial pneumonias. It is typically associated with extensive and progressive fibrosis, is fatal and has limited treatment options. Characteristically IPF patients display large lymphocyte aggregates composed of $CD3^+$ T cells and $CD20^+$ B cells within the lung tissue that are located near sites of active fibrosis. In addition, IPF patients can have autoantibodies to a range of host antigens, suggesting a breakdown in immunological tolerance. In this review we examine the role of T and B cells in IPF pathogenesis and discuss how loss of self-tolerance to lung specific proteins could exacerbate disease progression in IPF. We discuss what these results mean in terms of future prospects for immunotherapy of IPF.

Introduction

Interstitial lung diseases (ILDs) are a heterogeneous group of lung disorders. There are many type of ILDs which recently have been reclassified into three broad categories; 1) Chronic fibrosing interstitial pneumonia (including idiopathic pulmonary fibrosis (IPF)), 2) Smoking related interstitial pneumonia (respiratory bronchiolitis-interstitial lung disease and desquamative interstitial pneumonia) and 3) Acute/Sub-acute interstitial pneumonia (including cryptogenic organizing pneumonia and acute interstitial pneumonia). These groupings are based on clinical, radiological and pathological findings [1].

IPF is a chronic and fatal disease characterized by excessive extracellular matrix (ECM) protein deposition and a subsequent, and progressive decline in lung function. It has no known cause and there are limited treatment options that have minimal impact on survival times for IPF patients [2]. The incidence of IPF is increasing and is currently reported to be about 20 per 100,000 in the USA and Europe [3, 4]. The pathogenesis of IPF remains poorly understood, but it has been proposed that ongoing or repetitive injury to alveolar type II epithelial cells results in increased apoptosis and the induction of chronic inflammation with monocytic and lymphocytic infiltration [5]. The chronic nature of this response leads to fibroblast proliferation and myofibroblast differentiation and the excessive deposition and accumulation of extracellular matrix proteins, predominately type I collagen, within the lung interstitium [6].

There is a growing appreciation for the role of adaptive immune responses in the initiation and/or progression of a range of fibrotic diseases. In IPF patients, the presence of lymphoid aggregates in the lung tissue together with autoantibodies present in the serum, suggest that the immune system is likely to play a role either in initiation or progression of the disease. For example, the loss of self-tolerance in auto-reactive T and B cells specific to antigens derived from damaged or dying lung ECs may drive immune pathology in IPF. In this review we will provide an overview of how dysregulation of the adaptive immune response drives chronic inflammation and fibrosis which are hallmarks of IPF.

Characterization of lymphocyte responses in IPF

IPF has an unknown aetiology but it is characterised by an inflammatory cell infiltrate that includes neutrophils and mononuclear cells [7, 8]. In addition eosinophils and increased numbers of $CD4^+$ T cells together with raised antibody levels have been reported in the bronchoalveolar lavage of patients with pulmonary fibrosis[8-10]. Although these findings suggest a role for innate and adaptive immunity in the disease process until recently there have been no clear links made between antigens that may trigger the immune response and disease pathogenesis. The predominance of T cells in lung tissue and the bronchoalveolar lavage fluid (BALF) of patients with IPF suggested a role for cell mediated immune responses in the disease process. However, at the time biomarkers of T cell subsets did not correlate with disease activity or prognosis [11].

Lymphocyte aggregates in the IPF lung consist of $CD3^+$ T lymphocytes and mature DC cells and are generally proximate to fibroblastic foci[9, 12, 13]. A subset of these aggregates also contain $CD20^+$ B cells which localize in cohesive focal clusters within the centre of the aggregate resulting in an organized appearance [9, 12, 14] (Figure 1). These tertiary lymphoid structures (TLSs) have been classified as ectopic lymphoid structures and contain non-proliferating and non-apoptotic mature $CD45RO^+$ T and B cells evidenced by Ki67 and cleaved caspase 3 staining [9, 12] (Figure 2). This is a unique feature of IPF as pulmonary TLSs present in other diseases such as chronic obstructive pulmonary disease and idiopathic pulmonary arterial hypertension are non-proliferative[9, 12]. Furthermore, TLSs contain mature supporting DCs and large endothelial cells resembling High Endothelial Venules (HEVs) reminiscent of lymph node organization[12]. These inducible TLSs enable B cells to proliferate and differentiate in response to antigen independently of secondary lymphoid organs[12]. However, the fact that these TLSs, evident in IPF lung tissue, contain non-proliferating and non-apoptotic T and B cells suggests another mechanism is occurring. Despite this, the fact that these TLS are a histological feature of IPF and absent in the healthy lung suggests that they are an important feature of the disease pathogenesis.

In an attempt to further characterise the properties of the lymphocyte infiltrate in IPF patients Herazo –Maya et al examined gene expression arrays using DNA prepared from peripheral blood mononuclear cells (PBMCs) and they revealed a unique genetic signature that correlated with disease progression in IPF patients [15]. The genetic signature comprised

four genes that displayed decreased expression levels in IPF patients and these included *Cd28, Icos, Itk* and *Lck* and these markers were confirmed at both the RNA and protein level [15]. Furthermore the signature correlated with a two – four fold increased risk of patients dying of IPF or having a lung transplant [15]. Other genes that were down regulated in IPF patients were associated with the T cell antigen receptor complex such as CD3ε, CD3δ, CD3γ suggesting a general downregulation of TCRs and some of these were confirmed by flow cytometry. This finding was supported independently by Gilani et al. using peripheral blood $CD4^+$ T cells which also showed decreased CD28 levels on $CD4^+$ T cells of IPF patients and confirmed that this phenotype correlated with poor survival [16]. These findings of modulation of CD28 expression and predisposition to IPF disease progression in patients are in stark contrast to results obtained with *Cd28*$^{-/-}$ mice which are protected from bleomycin-induced fibrosis [17]. Currently it is not understood what causes the decreased expression of *Cd28* (and CD3 complex) in IPF patients. Notably all four signature genes are expressed by T cells but expression of Inducible Costimulator (*Icos*) is particularly important as it is constitutively expressed by follicular T helper cells (Tfh) that play a unique role in providing help to B cells to promote antibody synthesis in germinal centres [18]. Other immune biomarkers that also change in IPF patients include surfactant protein A (SP-A), chemokine ligand 18 (CCL18) and metalloproteinase 7, intercellular adhesion molecule (ICAM) and interleukin 8 (IL-8) which have all been found to be highly expressed in PBMCs of IPF patients and correlate with decreased survival [19-21]. Collectively these studies indicate that activation and expansion of T cells in IPF patients can have an important influence on disease progression and some specific biomarkers have been identified that can have prognostic value.

The cytokine response of $CD4^+$ T cells has been examined in a number of studies of IPF patients and consensus indicates an imbalance in the transforming growth factor (TGF)β : IL-10 ratio and the presence of high levels of IL-17 in the serum of IPF patients. Immunostaining of IPF patient lung tissues revealed prominent IL-17 staining in regenerating epithelial cells [14]. The secretion of IL-10 and TGFβ is mediated primarily by $CD4^+Foxp3^+$ T regulatory (Tregs) cells which play a crucial role in immune homeostasis and the maintenance of self-tolerance [22]. In addition Tregs can migrate to non-lymphoid tissues where they appear to play a crucial role in dampening immune responses at mucosal surfaces. In addition there is a growing appreciation that Tregs can play important roles

outside of mucosal tissues like the regulation of metabolic homeostasis in adipose tissue and can promote repair of skeletal muscle following damage [23-25]. Although there is no clear indication of the fate of Tregs in IPF patients, two studies have observed decreased numbers of Treg numbers in BALF, blood and in lung tissue [14, 26]. In addition, Treg cells from IPF patients displayed poor suppressive function *in vitro* [26]. In contrast in mouse models of bleomycin-induced pulmonary fibrosis Tregs can accumulate in large numbers in the lung tissue and can exacerbate disease through their capacity to secrete TGFβ. Reilkoff et al. identified a population of $CD4^+$Sem7a and $CD4^+Sem7a^+$ Tregs in peripheral blood that were expanded in IPF patients, and in mice, the $CD4^+Sem7a^+$ Tregs were responsible for disease progression following bleomycin treatment [27].

A specific lack of Treg cells or a loss in their suppressive activity could have important implications on the maintenance of self-tolerance in humans and rodents and can lead to the development of widespread autoimmunity. Naïve $CD4^+$ T cells if activated in the presence of TGFβ and IL-10 can differentiate to become inducible $CD4^+Foxp3^+$ Tregs (iTregs) [28, 29]. Gagliani et al. demonstrated that $CD4^+$ Th17 cells could transdifferentiate *in vivo* into $CD4^+$ Tregs [30], since Foxp3 expression induced in response to TGFβ signalling could silence RORγt expression which is a nuclear transcription factor required for IL-17 synthesis [31]. Due to plasticity of the $CD4^+$ repertoire the cytokine milieu in which T cells find themselves can strongly influence their path of differentiation. Recently it was shown that a population of $CD4^+IL\text{-}17^+Foxp3^+$ Tregs were expanded in the intestinal lamina propria of Crohn's disease patients [32]. These studies highlight that when $CD4^+$ T cells are exposed for prolonged periods to cytokines this can impact on their differentiation *in vivo*. TGF-β and IL-6 promote the differentiation of $CD4^+$ T cells into the Th17 cell lineage *in vivo* [33], while exposure to IL-10 and TGF-β promotes $CD4^+$ Treg differentiation [22]. Therefore, the environmental cues within inflamed tissues may shape the balance of Th17 and Treg cells that arise, but which cell type dominates during an immune response will be determined by the balance between inflammatory versus tolerogenic signals [32]. A similar scenario may also play out in the lungs of IPF patients where TGF-β, IL-6, IL-17 and IL-10 are detected and it is the manner in which clonal populations of $CD4^+$ T cells respond to the external cytokine signals emanating from

damaged epithelial cells or other innate or lymphocyte cell populations that will determine the outcome of inflammation or immune homeostasis.

Role of B cells in IPF

B cells are the antibody producing cells of the adaptive immune system and follicular B cells are also capable of responding to two different forms of antigens referred to as (i) Thymus (T)-independent (TI) or (ii) T-dependent (TD) antigens. TI antigens are associated with microbial derived products such as cell wall antigens like peptidoglycan, flagellin or lipopolysaccharide (LPS) or alternatively they may recognize DNA or RNA coming from microbes such as bacteria and viruses [34]. TD antigens are usually directed against exogenous proteins and these are processed by professional antigen presenting cells such as dendritic cells to stimulate $CD4^+$ Th cells. The antigen specific Th cell needs to interact with a B cell of the same antigen specificity and the Th cells provide both cognate and soluble signals to direct B cells to differentiate into plasma cells that secrete antibody, and a subset of these cells differentiate into long lasting memory cells [18].TI responses are usually more rapid than TD responses which is thought to help protect the host from blood borne life threatening infections and are limited to low affinity IgM responses. In contrast TD antigens can drive isotype switching and somatic hypermutation of Ig variable genes to increase the affinity of Abs for their antigen. Studies linking IPF to autoimmunity date back to the early-mid 1960s with several reports associating idiopathic pulmonary fibrosis with connective tissue diseases including, rheumatoid arthritis [35, 36]. These studies were the first to demonstrate elevated circulating auto-antibodies in patients with pulmonary fibrosis, albeit with rheumatism. Large foci of B cell aggregates accumulate in the lung of IPF patients but it is not known what draws these cells into the lung tissue during disease pathogenesis [12, 37]. B cell aggregates occur in a range of autoimmune diseases such as Systemic Lupus Erythematosus, Sjogren's disease, Thyroiditis, type 1 diabetes and rheumatoid arthritis [38-40]. Immune complexes formed between antibody and self-antigen can deposit in tissues to promote inflammation. In addition antibodies can mediate antibody dependent cellular cytotoxicity which is mediated via complement activation or via Natural Killer (NK) cell activity. Immune complexes have been identified in the peripheral blood, BALF and lung parenchyma of IPF patients [10, 41-43].

A number of soluble factors that promote B cell growth and differentiation are observed in the blood of IPF patients including B cell activating factor (BAFF) also known as B Lymphocyte stimulator (BLyS), IL-6 and IL-13 [44]. The chemokine CXCL13 is important for directing migration of B cells to inflammatory foci to promote formation of TLSs. In addition high levels of CXCL13 have been detected in serum of patients suffering from a range of autoimmune diseases [45-48]. CXCL13 was also demonstrated to be an important biomarker that was prognostic for IPF disease progression [13]. There was increased staining of CXCL13 in the lung of IPF patients and it was also detected in high levels in the plasma of IPF patients compared to patients with Chronic Obstructive Pulmonary Disease (COPD) [13]. To examine the role of B cells in IPF in a mouse model of pulmonary fibrosis O'Donoghue et al showed that *Rag1*$^{-/-}$ mice which lacked both mature T and B cells were protected from bleomycin induced pulmonary fibrosis [49]. Furthermore they showed that simply eliminating the mature B cell compartment in *gp130757F;uMT*$^{-/-}$ mice protected them from bleomycin-induced gp130/STAT3-mediated lung fibrosis. These studies all support a link between B cells and the pathogenesis of ILDs such as IPF [49].

IPF as an autoimmune disease

In support of a potential autoimmune role for B cell responses in IPF, serum analysis of auto-antibody production in 48 serum samples from patients with IPF [36], reported 32 patients of 48 to have elevated non-organ specific autoantibodies (including rheumatoid factors; DAT and ANF and Complement fixing antibodies). No lung specific autoantibodies were detected in this patient cohort. B cell aggregates and the presence of plasma cells in lung tissue of IPF patients were noted around the same period but their role was largely ignored until more recent times [11, 50, 51]. Numerous studies have highlighted the presence of autoantibodies to self-antigens in IPF patients suggesting a breakdown in central tolerance in T and B cells to T-dependent antigens (See Table 1).

B cells can act as professional antigen presenting cells to memory T cells as they can capture self-antigens and process and present peptide/MHC molecules at the cell surface for recognition by the TCR on an autoreactive T cell. Several studies have identified that T cells from IPF patients can demonstrate proliferative responses to lung derived autoantigens

suggesting a breakdown in T cell tolerance to these tissue specific antigens [16, 26, 43, 52-54]. Feghali-Bostwick et al. demonstrated that $CD4^+$ T cells from IPF patients responded to lung tissue antigens derived from IPF lung material- but did not respond to protein samples prepared from lung material of healthy controls [58]. This indicates that the IPF lung must elaborate self-antigens that have the potential to stimulate self-reactive T cell responses when presented by antigen presenting cells.

Loss of self–tolerance in IPF

The adaptive T cells of the $CD4^+$ and $CD8^+$ lineages express clonally restricted antigen specific receptors. These cells are selected in the thymus to express TCRs with low – moderate affinity for the target antigen [55, 56]. Central tolerance in $CD4^+$ and $CD8^+$ T cells is mediated by antigen presentation via cortical epithelial cells (ECs) in the thymus and is reinforced by antigen presentation in the medulla by medullary thymic epithelial cells (mTECs) and bone marrow derived DCs to ensure self-reactive lymphocytes are deleted within the thymus [57-60].

Studies in mice and humans have revealed the important role for the nuclear transcription factor Autoimmune Regulator gene, *Aire*, which confers to a subset of mTECs the capacity for expression of tissue restricted antigens (TRAs) [61, 62]. *Aire*$^+$ mTECs are able to present a wide range of self-peptides in association with MHC molecules to induce self-tolerance to TRAs [61-64]. Aire-mediated regulation of TRA expression leads to the translation of the self-proteins which are subsequently processed by the mTECs though the MHCI and MHCII pathways leading to formation of peptide/MHC (pMHC) complexes which are presented at the surface of mTECs and these ligands can be scanned by TCRs on thymocytes. If a TCR has high affinity for the pMHC complex this will lead to clonal deletion of the autoreactive T cell. Whereas cells with low/medium affinity will be allowed to complete the differentiation and be exported from the thymus. Autoimmune Polyendocrine Syndrome 1 (APS-1) or Autoimmune Polyendocrinopathy Candidiasis Ectodermal Dystrophy (APCED) is a rare autosomal recessive monogenic autoimmune disorder caused by mutations in the *AIRE* gene [65]. The *Aire* mutation disrupts TRA expression in mTECs leading to loss of central tolerance in the thymus resulting in the export of autoreactive T cells into the peripheral circulation which predisposes to autoimmunity [61-64, 66]. Recently a second gene called *Fezf2* has been

discovered that can direct the expression of a further subset of TRA genes in a subset of mTECs that are different to those controlled by *Aire* [67].

In an effort to provide a molecular basis for the presence of auto-antibodies in patients with IPF, Alimohammadi et al. identified a subset of APS-1 patients that had evidence of pulmonary autoimmunity and identified the potassium channel protein, KCNRG as a putative auto-antigen in these patients [68]. In a separate study Shum et al. identified a human patient with APS-1that developed pulmonary fibrosis and identified vomeromodulin as a putative auto-antigen [69]. Shum also provided evidence that *Aire*$^{-/-}$ mice develop a pulmonary disease similar to that observed in APS-1 patients. In a follow up study Shum et al. identified a strong link between an autoimmune response to the lung-specific protein BPIFB1 (bactericidal/permeability-increasing fold- containing B1) in APS-1 patients [70]. They found 100% of APS-1 patients with ILD had autoantibodies in the serum to BPIFB1, but it was also found to be an autoantigen in non APS-1 patients. Furthermore ~15% of patients with connective tissue disease-associated ILD and 12% of IPF patients displaying auto-antibodies to the antigen [70]. They found transfer of serum containing BPIFB1 autoantibodies did not confer ILD to recipient mice with an intact immune system, but transfer of CD4^{+}BPIFB1^{-}specific T cells to *Rag1*$^{-/-}$ mice developed spontaneous pulmonary fibrosis and inflammation [70]. Furthermore thymic transplant from *Aire*$^{-/-}$ to immunodeficient Balb/c nude mice lead to spontaneous development of BPIFB1 autoantibodies in the blood of recipients. Thymic transplants of *Aire*$^{+/-}$ thymi to nude mice did not yield any autoantibodies [70]. Finally they also showed that immunization of BPIFB1$^{-/-}$ mice with the BPIFB1 protein could induce autoantibodies to BPIFB1 and they went onto develop ILD similar to that observed in *Aire*$^{-/-}$ mice and IPF patients [70].

Tzouvelekis et al., identified patients with combined pulmonary fibrosis and emphysema that displayed increased levels of circulating anti-nuclear antibodies (ANAs) in serum and they had large lymphoid aggregates within lung tissue that consisted of primarily of CD20^{+} B cells [71]. Thus it appears that chronic pulmonary diseases such as IPF can be associated

Table 1 Autoantigens identified in Pulmonary fibrosis

Auto antigen	Cellular expression	Function	References
BP1FB1	Synthesized in mucus production organs e.g. goblet cells in the nasal cavity and the upper airways of the respiratory tract.	Bactericidal & permeability activity against bacteria	[70]
Vomeromodulin	Expressed in cells of nasal/olfactory tissue in lateral nasal glands, posterior septal and vomeronasal glands	Transport function for pheromones of low volatility to gain access to receptors in the vomeronasal organ.	[69]
Periplakin	Epidermal keratinocyte	A component of desmosomes and epidermal cornified envelope in keratinocytes	[72, 73]
HSP70	Ubiquitous in all cells	Chaperone function in protein folding	[43]
KCNRG	Expression in bronchioles	putative potassium channel regulator	[68]
Cytokeratin 18	Glandular Epithelial cells	Connects the cell surface to the nucleus	[42]
IL-1a	Macrophages, monocytes, neutrophils	Proinflammatory cytokine	[74]
Annexin 1	Cells in lung bone marrow and intestine	Ca2+ dependent phospholipid binding proteins	[75]
Vimentin	Mesenchymal cells	Involved in cytoskeletal structure to anchor organelles in the cytosol	[76]
Collagen V	Lung interstitium	Barrier function of basement membrane	[77]
Cytokeratin 8, 18 and 19	Alveolar type II cells, Broncho epithelial cells	Filaments associated with the cytoskeleton of epithelial cells	[42, 78, 79]

with a breakdown in immune tolerance and generation of autoimmunity. Table 1 provides a summary of the known autoantigens that have been described in IPF patients and those antigens highlighted in bold have been shown to be present in APS-1 patients linking it to a defect in central tolerance mediated by AIRE.

Conclusion:

In the last 20 years immunologists have gained greater insight into the links between the innate and adaptive immune response generated not only to pathogens but also to self-antigens in the context of autoimmunity. Susceptibility to IPF is likely to have requirements of genetic and environmental components. Genome wide association studies have identified some key target genes and a number of these have been associated with cellular function of epithelial cells in the airways. Defects in signalling pathways that are crucial to clearing cellular debris may facilitate autoantibody formation and this may tip the scales toward disease pathogenesis. A failure to appropriately regulate the adaptive immune response could be detrimental to the host and predispose to chronic inflammation and damage and tissue remodelling with deposition of collagen leading to fibrotic disease. Th cell differentiation is directed by the presence of cytokines and the balance between proinflammatory or immune regulatory cytokines during the period of damage to lung epithelial cells which could favour development of autoimmunity.

The recent discovery of the role of *Aire* and predisposition to pulmonary fibrosis in both mice and humans provides a new and exciting breakthrough in providing a molecular mechanism to explain the breakdown in immune tolerance to lung derived self-antigens. Although Aire mutations may only be present in a subset of IPF patients it does provide an avenue to understand how a breakdown in T cell tolerance may occur and how the autoreactive T and B cells may be inappropriately activated. Perhaps there are other *Aire*-like genes such as *Fezf2*, that direct TRA expression in the thymus that await to be discovered. Mutations in genes that direct promiscuous expression of TRAs in thymic mTECs could explain the diversity of target autoantigens that have been defined to date in IPF patients. Once the initial priming event has occurred this can lead to formation of memory T and B cells specific for the target autoantigens. Because of the altered costimulatory requirements of memory cells this can prove more challenging in trying to

dampen immune responses to self-antigens. Nevertheless scientists have made some significant advances in the understanding the process of disease pathogenesis in IPF. Although we may still be some distance away from understanding the full pathogenesis of the disease it has revealed some important insights that provide potential targets for immunotherapy in the future.

Future studies may need to focus on how the innate and adaptive immune responses in IPF unfold. There have been significant breakthroughs in our understanding of the diversity of innate cellular repertoire that helps to maintain immune homeostasis in mucosal sites. The innate immune cells play a critical role in directing epithelial cell repair and maintaining the barrier function of the epithelium. The next decade should be able to provide a fertile area of research as the genetic tools for studying disease pathogenesis are being developed and provide a valuable resource to understand how dysregulation of the immune system occurs. Although the mouse models of IPF may not accurately reflect on the inflammatory response in IPF patients, they will still prove valuable to help scientists dissect the cellular and molecular regulation of immune response in what is a complex disease.

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References

1. Travis WD, Costabel U, Hansell DM, King TE, Jr., Lynch DA, Nicholson AG, et al. An official American Thoracic Society/European Respiratory Society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. *Am J Respir Crit Care Med* 2013;**188**:733-748.
2. Maher TM, Evans IC, Bottoms SE, Mercer PF, Thorley AJ, Nicholson AG, et al. Diminished prostaglandin E2 contributes to the apoptosis paradox in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2010;**182**:73-82.
3. Nalysnyk L, Cid-Ruzafa J, Rotella P, Esser D. Incidence and prevalence of idiopathic pulmonary fibrosis: review of the literature. *Eur Respir Rev* 2012;**21**:355-361.
4. Sivakumar P, Das AM. Fibrosis, chronic inflammation and new pathways for drug discovery. *Inflamm Res* 2008;**57**:410-418.
5. Richeldi L, Collard HR, du Bois RM, Jenkins G, Kolb M, Maher TM, et al. Mapping the future for pulmonary fibrosis: report from the 17th International Colloquium on Lung and Airway Fibrosis. *Eur Resp J* 2013;**42**:230-238.
6. Wilson MS, Wynn TA. Pulmonary fibrosis: pathogenesis, etiology and regulation. *Mucosal immunol* 2009;**2**:103-121.
7. Crystal RG, Bitterman PB, Rennard SI, Hance AJ, Keogh BA. Interstitial lung diseases of unknown cause. Disorders characterized by chronic inflammation of the lower respiratory tract. *N Engl J Med* 1984;**310**:154-166.
8. Peterson MW, Monick M, Hunninghake GW. Prognostic role of eosinophils in pulmonary fibrosis. *Chest* 1987;**92**:51-56.
9. Todd NW, Scheraga RG, Galvin JR, Iacono AT, Britt EJ, Luzina IG, et al. Lymphocyte aggregates persist and accumulate in the lungs of patients with idiopathic pulmonary fibrosis. *J Inflamm Res* 2013;**6**:63-70.
10. Wallace WA, Roberts SN, Caldwell H, Thornton E, Greening AP, Lamb D, et al. Circulating antibodies to lung protein(s) in patients with cryptogenic fibrosing alveolitis. *Thorax* 1994;**49**:218-224.
11. Meliconi R, Lalli E, Borzi RM, Sturani C, Galavotti V, Gunella G, et al. Idiopathic pulmonary fibrosis: can cell mediated immunity markers predict clinical outcome? *Thorax* 1990;**45**:536-540.
12. Marchal-Somme J, Uzunhan Y, Marchand-Adam S, Valeyre D, Soumelis V, Crestani B, et al. Cutting edge: nonproliferating mature immune cells form a novel type of organized lymphoid structure in idiopathic pulmonary fibrosis. *J Immunol* 2006;**176**:5735-5739.
13. Vuga LJ, Tedrow JR, Pandit KV, Tan J, Kass DJ, Xue J, et al. C-X-C motif chemokine 13 (CXCL13) is a prognostic biomarker of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2014;**189**:966-974.
14. Nuovo GJ, Hagood JS, Magro CM, Chin N, Kapil R, Davis L, et al. The distribution of immunomodulatory cells in the lungs of patients with idiopathic pulmonary fibrosis. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc* 2012;**25**:416-433.
15. Herazo-Maya JD, Noth I, Duncan SR, Kim S, Ma SF, Tseng GC, et al. Peripheral blood mononuclear cell gene expression profiles predict poor outcome in idiopathic pulmonary fibrosis. *Sci Transl Med* 2013;**5**:205ra136.
16. Gilani SR, Vuga LJ, Lindell KO, Gibson KF, Xue J, Kaminski N, et al. CD28 down-regulation on circulating CD4 T-cells is associated with poor prognoses of patients with idiopathic pulmonary fibrosis. *PLoS One* 2010;**5**:e8959.

17. Okazaki T, Nakao A, Nakano H, Takahashi F, Takahashi K, Shimozato O, et al. Impairment of bleomycin-induced lung fibrosis in CD28-deficient mice. *J Immunol* 2001;**167**:1977-1981.
18. Vinuesa CG, Linterman MA, Yu D, MacLennan IC. Follicular Helper T Cells. *Annu Rev Immunol* 2016;**34**:335-368.
19. Kinder BW, Brown KK, McCormack FX, Ix JH, Kervitsky A, Schwarz MI, et al. Serum surfactant protein-A is a strong predictor of early mortality in idiopathic pulmonary fibrosis. *Chest* 2009;**135**:1557-1563.
20. Prasse A, Probst C, Bargagli E, Zissel G, Toews GB, Flaherty KR, et al. Serum CC-chemokine ligand 18 concentration predicts outcome in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2009;**179**:717-723.
21. Richards TJ, Kaminski N, Baribaud F, Flavin S, Brodmerkel C, Horowitz D, et al. Peripheral blood proteins predict mortality in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2012;**185**:67-76.
22. Sakaguchi S, Vignali DA, Rudensky AY, Niec RE, Waldmann H. The plasticity and stability of regulatory T cells. *Nat Rev Immunol* 2013;**13**:461-467.
23. Burzyn D, Benoist C, Mathis D. Regulatory T cells in nonlymphoid tissues. *Nat Immunol* 2013;**14**:1007-1013.
24. Burzyn D, Kuswanto W, Kolodin D, Shadrach JL, Cerletti M, Jang Y, et al. A special population of regulatory T cells potentiates muscle repair. *Cell* 2013;**155**:1282-1295.
25. Kuswanto W, Burzyn D, Panduro M, Wang KK, Jang YC, Wagers AJ, et al. Poor repair of skeletal muscle in aging mice reflects a defect in local, Interleukin-33-dependent accumulation of regulatory T Cells. *Immunity* 2016;**44**:355-367.
26. Kotsianidis I, Nakou E, Bouchliou I, Tzouvelekis A, Spanoudakis E, Steiropoulos P, et al. Global impairment of CD4+CD25+FOXP3+ regulatory T cells in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2009;**179**:1121-1130.
27. Reilkoff RA, Peng H, Murray LA, Peng X, Russell T, Montgomery R, et al. Semaphorin 7a+ regulatory T cells are associated with progressive idiopathic pulmonary fibrosis and are implicated in transforming growth factor-beta1-induced pulmonary fibrosis. *Am J Respir Crit Care Med* 2013;**187**:180-188.
28. Asseman C, Mauze S, Leach MW, Coffman RL, Powrie F. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. *J Exp Med* 1999;**190**:995-1004.
29. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun CM, Belkaid Y, et al. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *J Exp Med* 2007;**204**:1757-1764.
30. Gagliani N, Amezcua Vesely MC, Iseppon A, Brockmann L, Xu H, Palm NW, et al. Th17 cells transdifferentiate into regulatory T cells during resolution of inflammation. *Nature* 2015;**523**:221-225.
31. Zhou L, Lopes JE, Chong MM, Ivanov, II, Min R, Victora GD, et al. TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgammaτ function. *Nature* 2008;**453**:236-240.
32. Hovhannisyan Z, Treatman J, Littman DR, Mayer L. Characterization of interleukin-17-producing regulatory T cells in inflamed intestinal mucosa from patients with inflammatory bowel diseases. *Gastroenterology* 2011;**140**:957-965.

33. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity* 2006;**24**:179-189.
34. Bekeredjian-Ding I, Jego G. Toll-like receptors--sentries in the B-cell response. *Immunology* 2009;**128**:311-323.
35. Tomasi TB, Jr., Fudenberg HH, Finby N. Possible relationship of rheumatoid factors and pulmonary disease. *Am J Med*1962;**33**:243-248.
36. Turner-Warwick M, Doniach D. Auto-Antibody Studies in Interstitial Pulmonary Fibrosis. *BMJ* 1965;**1**:886-891.
37. Campbell DA, Poulter LW, Janossy G, du Bois RM. Immunohistological analysis of lung tissue from patients with cryptogenic fibrosing alveolitis suggesting local expression of immune hypersensitivity. *Thorax* 1985;**40**:405-411.
38. Cancro MP, D'Cruz DP, Khamashta MA. The role of B lymphocyte stimulator (BLyS) in systemic lupus erythematosus. *J Clin Invest* 2009;**119**:1066-1073.
39. Dillon SR, Harder B, Lewis KB, Moore MD, Liu H, Bukowski TR, et al. B-lymphocyte stimulator/a proliferation-inducing ligand heterotrimers are elevated in the sera of patients with autoimmune disease and are neutralized by atacicept and B-cell maturation antigen-immunoglobulin. *Arthritis research & therapy* 2010;**12**:R48.
40. Glowinska-Olszewska B, Michalak J, Luczynski W, Del Pilar Larosa M, Chen S, Furmaniak J, et al. Organ-specific autoimmunity in relation to clinical characteristics in children with long-lasting type 1 diabetes. *J Ped Endocrinol Metab* 2016;**29**:647-656.
41. Dall'Aglio PP, Pesci A, Bertorelli G, Brianti E, Scarpa S. Study of immune complexes in bronchoalveolar lavage fluids. *Respiration* 1988;**54** Suppl 1:36-41.
42. Dobashi N, Fujita J, Murota M, Ohtsuki Y, Yamadori I, Yoshinouchi T, et al. Elevation of anti-cytokeratin 18 antibody and circulating cytokeratin 18: anti-cytokeratin 18 antibody immune complexes in sera of patients with idiopathic pulmonary fibrosis. *Lung* 2000;**178**:171-179.
43. Kahloon RA, Xue J, Bhargava A, Csizmadia E, Otterbein L, Kass DJ, et al. Patients with idiopathic pulmonary fibrosis with antibodies to heat shock protein 70 have poor prognoses. *Am J Respir Crit Care Med* 2013;**187**:768-775.
44. Xue J, Kass DJ, Bon J, Vuga L, Tan J, Csizmadia E, et al. Plasma B lymphocyte stimulator and B cell differentiation in idiopathic pulmonary fibrosis patients. *J Immunol* 2013;**191**:2089-2095.
45. Brettschneider J, Czerwoniak A, Senel M, Fang L, Kassubek J, Pinkhardt E, et al. The chemokine CXCL13 is a prognostic marker in clinically isolated syndrome (CIS). *PLoS One* 2010;**5**:e11986.
46. Lee HT, Shiao YM, Wu TH, Chen WS, Hsu YH, Tsai SF, et al. Serum BLC/CXCL13 concentrations and renal expression of CXCL13/CXCR5 in patients with systemic lupus erythematosus and lupus nephritis. *J Rheumatol* 2010;**37**:45-52.
47. Meraouna A, Cizeron-Clairac G, Panse RL, Bismuth J, Truffault F, Tallaksen C, et al. The chemokine CXCL13 is a key molecule in autoimmune myasthenia gravis. *Blood* 2006;**108**:432-440.
48. Rioja I, Hughes FJ, Sharp CH, Warnock LC, Montgomery DS, Akil M, et al. Potential novel biomarkers of disease activity in rheumatoid arthritis patients: CXCL13, CCL23, transforming growth factor alpha, tumor necrosis factor receptor superfamily member 9, and macrophage colony-stimulating factor. *Arth Rheumat* 2008;**58**:2257-2267.

49. O'Donoghue RJ, Knight DA, Richards CD, Prele CM, Lau HL, Jarnicki AG, et al. Genetic partitioning of interleukin-6 signalling in mice dissociates Stat3 from Smad3-mediated lung fibrosis. *EMBO Mol Med* 2012;**4**:939-951.
50. Emura M, Nagai S, Takeuchi M, Kitaichi M, Izumi T. In vitro production of B cell growth factor and B cell differentiation factor by peripheral blood mononuclear cells and bronchoalveolar lavage T lymphocytes from patients with idiopathic pulmonary fibrosis. *Clin Exp Immunol* 1990;**82**:133-139.
51. Reynolds HY, Fulmer JD, Kazmierowski JA, Roberts WC, Frank MM, Crystal RG. Analysis of cellular and protein content of broncho-alveolar lavage fluid from patients with idiopathic pulmonary fibrosis and chronic hypersensitivity pneumonitis. *J Clin Invest* 1977;**59**:165-175.
52. Daniil Z, Kitsanta P, Kapotsis G, Mathioudaki M, Kollintza A, Karatza M, et al. CD8+ T lymphocytes in lung tissue from patients with idiopathic pulmonary fibrosis. *Respiratory Res* 2005;**6**:81.
53. Feghali-Bostwick CA, Tsai CG, Valentine VG, Kantrow S, Stoner MW, Pilewski JM, et al. Cellular and humoral autoreactivity in idiopathic pulmonary fibrosis. *J Immunol* 2007;**179**:2592-2599.
54. Parra ER, Kairalla RA, Ribeiro de Carvalho CR, Eher E, Capelozzi VL. Inflammatory cell phenotyping of the pulmonary interstitium in idiopathic interstitial pneumonia. *Respiration* 2007;**74**:159-169.
55. Hogquist KA, Jameson SC, Bevan MJ. Strong agonist ligands for the T cell receptor do not mediate positive selection of functional CD8+ T cells. *Immunity* 1995;**3**:79-86.
56. Jameson SC, Hogquist KA, Bevan MJ. Specificity and flexibility in thymic selection. *Nature* 1994;**369**:750-752.
57. Derbinski J, Schulte A, Kyewski B, Klein L. Promiscuous gene expression in medullary thymic epithelial cells mirrors the peripheral self. *Nat Immunol* 2001;**2**:1032-1039.
58. Gotter J, Brors B, Hergenhahn M, Kyewski B. Medullary epithelial cells of the human thymus express a highly diverse selection of tissue-specific genes colocalized in chromosomal clusters. *J Exp Med* 2004;**199**:155-166.
59. Klein L, Roettinger B, Kyewski B. Sampling of complementing self-antigen pools by thymic stromal cells maximizes the scope of central T cell tolerance. *Eur J Immunol* 2001;**31**:2476-2486.
60. Kyewski BA, Fathman CG, Rouse RV. Intrathymic presentation of circulating non-MHC antigens by medullary dendritic cells. An antigen-dependent microenvironment for T cell differentiation. *J Exp Med* 1986;**163**:231-246.
61. Anderson MS, Venanzi ES, Chen Z, Berzins SP, Benoist C, Mathis D. The cellular mechanism of Aire control of T cell tolerance. *Immunity* 2005;**23**:227-239.
62. Anderson MS, Venanzi ES, Klein L, Chen Z, Berzins SP, Turley SJ, et al. Projection of an immunological self shadow within the thymus by the aire protein. *Science* 2002;**298**:1395-1401.
63. Liston A, Gray DH, Lesage S, Fletcher AL, Wilson J, Webster KE, et al. Gene dosage--limiting role of Aire in thymic expression, clonal deletion, and organ-specific autoimmunity. *J Exp Med* 2004;**200**:1015-1026.
64. Liston A, Lesage S, Wilson J, Peltonen L, Goodnow CC. Aire regulates negative selection of organ-specific T cells. *Nat Immunol* 2003;**4**:350-354.

65. Ramsey C, Bukrinsky A, Peltonen L. Systematic mutagenesis of the functional domains of AIRE reveals their role in intracellular targeting. *Hum Mol Genet* 2002;**11**:3299-3308.
66. Ramsey C, Winqvist O, Puhakka L, Halonen M, Moro A, Kampe O, et al. Aire deficient mice develop multiple features of APECED phenotype and show altered immune response. *Hum Mol Genet* 2002;**11**:397-409.
67. Takaba H, Morishita Y, Tomofuji Y, Danks L, Nitta T, Komatsu N, et al. Fezf2 orchestrates a thymic program of self-antigen expression for immune tolerance. *Cell* 2015;**163**:975-987.
68. Alimohammadi M, Dubois N, Skoldberg F, Hallgren A, Tardivel I, Hedstrand H, et al. Pulmonary autoimmunity as a feature of autoimmune polyendocrine syndrome type 1 and identification of KCNRG as a bronchial autoantigen. *Proc Natl Acad Sci U S A* 2009;**106**:4396-4401.
69. Shum AK, DeVoss J, Tan CL, Hou Y, Johannes K, O'Gorman CS, et al. Identification of an autoantigen demonstrates a link between interstitial lung disease and a defect in central tolerance. *Sci Transl Med* 2009;**1**:9ra20.
70. Shum AK, Alimohammadi M, Tan CL, Cheng MH, Metzger TC, Law CS, et al. BPIFB1 is a lung-specific autoantigen associated with interstitial lung disease. *Sci Transl Med* 2013;**5**:206ra139.
71. Tzouvelekis A, Zacharis G, Oikonomou A, Mikroulis D, Margaritopoulos G, Koutsopoulos A, et al. Increased incidence of autoimmune markers in patients with combined pulmonary fibrosis and emphysema. *BMC Pulm Med* 2013;**13**:31.
72. Mignot S, Taille C, Besnard V, Crestani B, Chollet-Martin S. Detection of anti-periplakin auto-antibodies during idiopathic pulmonary fibrosis. *Clin Chim Acta* 2014;**433**:242.
73. Taille C, Grootenboer-Mignot S, Boursier C, Michel L, Debray MP, Fagart J, et al. Identification of periplakin as a new target for autoreactivity in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2011;**183**:759-766.
74. Ogushi F, Tani K, Endo T, Tada H, Kawano T, Asano T, et al. Autoantibodies to IL-1 alpha in sera from rapidly progressive idiopathic pulmonary fibrosis. *J Med Invest* 2001;**48**:181-189.
75. Kurosu K, Takiguchi Y, Okada O, Yumoto N, Sakao S, Tada Y, et al. Identification of annexin 1 as a novel autoantigen in acute exacerbation of idiopathic pulmonary fibrosis. *J Immunol* 2008;**181**:756-767.
76. Yang Y, Fujita J, Bandoh S, Ohtsuki Y, Yamadori I, Yoshinouchi T, et al. Detection of antivimentin antibody in sera of patients with idiopathic pulmonary fibrosis and non-specific interstitial pneumonia. *Clin Exp Immunol* 2002;**128**:169-174.
77. Vittal R, Mickler EA, Fisher AJ, Zhang C, Rothhaar K, Gu H, et al. Type V collagen induced tolerance suppresses collagen deposition, TGF-beta and associated transcripts in pulmonary fibrosis. *PLoS One* 2013;**8**:e76451.
78. Dobashi N, Fujita J, Ohtsuki Y, Yamadori I, Yoshinouchi T, Kamei T, et al. Circulating cytokeratin 8:anti-cytokeratin 8 antibody immune complexes in sera of patients with pulmonary fibrosis. *Respiration* 2000;**67**:397-401.
79. Fujita J, Dobashi N, Ohtsuki Y, Yamadori I, Yoshinouchi T, Kamei T, et al. Elevation of anti-cytokeratin 19 antibody in sera of the patients with idiopathic pulmonary fibrosis and pulmonary fibrosis associated with collagen vascular disorders. *Lung* 1999;**177**:311-319.

Figure 1. Accumulation of $CD20^+$ B cell aggregates in the lung tissue of IPF patients around areas of pulmonary fibrosis that are normally absent in healthy lungs. A. Masson's trichrome stain of the lung tissue of an IPF patient. B. Immunohistochemical stain of $CD20^+$ B cells in a serial section of the same tissue. The $CD20^+$ aggregates accumulate in areas where there is fibrosis (blue areas in A).

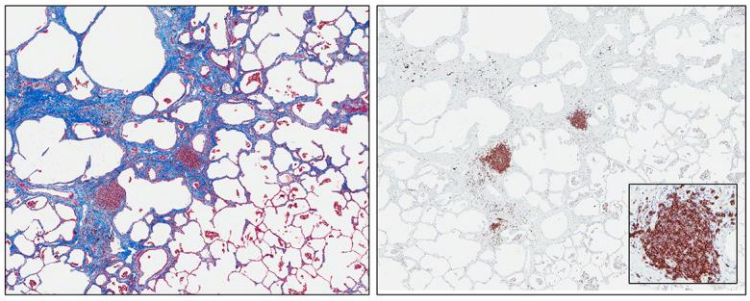

Figure 2. Model of disease pathogenesis of IPF due to breakdown in self-tolerance to lung specific protein antigens. **A.** In the thymus AIRE+ mTECs can present self-antigens to developing thymocytes and self-reactive T cells are eliminated by apoptosis. In patients with APS-1 with mutations in which the Aire gene is faulty- mTECs fail to eliminate lung –specific T cells and they complete maturation in the thymus and migrate to the periphery. **B.** In response to injury in the lung, dendritic cells (DCs) can pick up and process lung specific Ag and migrate to regional lymph nodes or spleen to present Ag to lung-specific Th cells. **C.** The activated Th cells can provide help to Ag-specific B cells and both undergo clonal expansion. Ag-specific B cells can mature as plasma cells and secrete auto-Abs into the blood. **D.** Autoreactive T and B cells migrate to the lung to form tertiary lymphoid structures (TLs) but

they typically lack proliferating B cells and apoptotic cells in these sites which are hallmarks of active germinal centres [9, 12, 44]. Due to chronic tissue damage fibrosis develops and leads to IPF pathogenesis.