Editorial

The Immunology of pulmonary fibrosis: The role of Th1/Th2/Th17/Treg cells

Argyris Tzouvelekis[1], Evangelos Bouros[1], Demosthenes Bouros[2]

[1]Medical School, Democritus University of Thrace, Alexandroupolis, Greece
[2]Professor of Pneumonology, Deptartment of Pneumonology, Medical School, Democritus University of Thrace, Alexandroupolis, Greece

PULMONARY FIBROSIS IS DRIVEN BY TH2 IMMUNE RESPONSE

Pulmonary fibrosis is characterized by chronic scar formation and deposition of extracellular matrix, resulting in impaired lung function and respiratory failure. There is growing evidence of immune system involvement in the pathogenesis of pulmonary fibrosis.

Th2 responses characterize a number of pulmonary diseases, many of which culminate in tissue remodelling and fibrosis. A shift towards a Th2 immune response appears to predominate in idiopathic pulmonary fibrosis (IPF) and this promotes fibrosis, primarily via secretion of profibrotic cytokines into the injured epithelium. In murine models of lung disease, animals whose response to tissue injury is predominantly of the Th2 type are more prone to pulmonary fibrosis after lung injury than those with a predominantly Th1 response[1,2].

It has been shown that two CC chemokines that are regulated by Th2 cytokines, specifically CCL17 and CCL22, are associated with pulmonary fibrosis[3,4]. These chemokines and their receptor CCR4 have been found to be elevated in areas of fibrotic lung tissue, compared with normal pulmonary parenchyma. In particular, CCR4 has been found to be expressed mostly by macrophages present in fibrotic areas.

Th2 immune response contributes to a failure of re-endothelialization and reepithelialization, and leads to the release of profibrotic growth factors into the region of injury[1]. These profibrotic cytokines initiate fibroblast migration into the site of injury and promote their proliferation and differentiation into myofibroblasts. It is considered that the inflammation is subsequent to lung injury, and that IPF occurs as a result of a polarization of the immune response of the body to repeated injury (i.e., "multiple hits") to the lung. However, Th2 and Th1 phenotypes are not as well defined in IPF as they are in asthma and animal models[1].

Inflammation and tissue remodelling with pathological fibrosis are common consequences of Th2 responses in the lung and other organs. Interleukin (IL)-13 and transforming growth factor-1 (TGF-1) are frequently co-expressed in these responses and are believed to play an important role in the pathogenesis of Th2-induced pathological conditions.

This Th1/Th2 hypothesis has dominated the understanding of immune

Correspondence to:
Demosthenes Bouros MD, PhD, FCCP
Professor of Pneumonology
Head, Dept of Pneumonology,
and Chairman, Dept of Internal Medicine,
University Hospital of Alexandroupolis
Alexandroupolis, Greece, 68100
Tel./Fax: 25510 75096,
E-mail: bouros@med.duth.gr

regulation, immune pathogenesis and host defenses for decades, despite flaws and its inability to explain certain data regarding T-cell mediated tissue damage.

Recently, two novel CD4 T cell subsets have been described which have revolutionized the understanding of immune function. These are the **Th17**, which develops via different cytokine signals from those of Th1 and Th2 lineages, and the **T regulatory cells (Tregs)**. The Th17 subset is characterized by production of IL-17 and is involved in the pathogenesis of autoimmune tissue injury, including rheumatoid arthritis and allergen-specific responses.

The central players in the generation of the new effector CD4+ Th17 subset are TGF-b, IL-23 and IL-17. Despite intense research, there is still considerable uncertainty regarding the relationship of Th-17 and Th1/Th2 responses in chronic inflammatory and autoimmune disease[9-11]. Although Th17 cells are important agents in mucosal host defence, they can also mediate immunopathology.

The IL-17 receptor has been shown to be upregulated in the lungs of patients with hypersensitivity pneumonitis[5,6]. Tumour growth factor-b (TGF-b) and IL-6 are necessary for the differentiation of naive CD4 T lymphocytes into Th17 cells in mice[7,8]. TGF-b has been shown to be a critical cytokine for the development of pulmonary fibrosis[9], and may promote differentiation of Th17 cells with the adverse consequence of promoting collagen deposition in the lung.

Tregs (i.e., CD4+CD25+foxp3+) represent the first well-defined expansion of the CD4+ T cell functional range[10]. Treg development is specified by the transcription factor forkhead box protein 3 (foxp3), while GATA-3 and STAT1 are the master regulators for Th2 and Th1 differentiation, respectively. Tregs suppress activation of the immune system and help maintain immune homeostasis and tolerance to self-antigens[8].

Tregs suppress both Th1- and Th2-mediated immune responses in such a way that sufficient immunity remains for clearing infectious agents, while unwanted immunopathology is prevented. In the case of a shortage of Tregs, the potential amplitude of Th1 and Th2 responses is increased, resulting in excessive T cell immunity, associated with autoimmune disease, asthma and allergy. An abundance of Tregs, on the other hand, will reduce the potential amplitude of Th1 and Th2 responses and may impair the adequacy of immunity to tumours and infectious diseases[8,10,11].

Tregs appear to represent the resurrection of the old suppressor T cells. Although of a different phenotype, Tregs are able to suppress many T cell mediated immune responses. A distinction is made between naturally occurring regulatory T cells (nTreg), which require cell-cell contact for suppression, and inducible regulatory T cells (iTreg), which mediate suppression predominantly via cytokine dependent pathways. Limited studies on Tregs in human disease are available to date, but the possible clinical applications are under investigation[10,12].

ROLE OF T-REGULATORY CELLS IN THE PATHOGENESIS OF PULMONARY FIBROSIS

Recent findings support a role for Tregs in the pathophysiology and pathogenesis of interstitial lung disease (ILD). Miyara et al.[13] observed a substantial expansion of Tregs in the peripheral blood (PB), bronchoalveolar lavage fluid (BALF) and disease sites of patients with active sarcoidosis. Nevertheless, despite their high numbers and their potent suppression of cell proliferation, which may be responsible for the anergic state seen in sarcoidosis, Tregs were unable to efficiently abrogate the secretion of proinflammatory cytokines such as TNF-α and IFN-γ, thus permitting the development of granulomas.

In addition, another group of investigators found reduced Foxp3 expression in the BALF $CD4^+$ cells of patients with sarcoidosis compared to that of controls, indicative of low numbers, or, alternatively, a qualitative Treg defect[14].

A significant impairment of Treg suppressor function was evident in both the PB and BALF of patients with IPF, most of whom also showed low Treg numbers in PB and BALF compared to both control subjects and patients with NSIP. In contrast, Tregs were overly expanded in the BALF of patients with NSIP, while their suppressor function remained intact. Identical findings were obtained from lung biopsy tissue assessed for the presence of Tregs by dual immunostaining for $Foxp3^+$ and $CD4^+$. Finally, an almost linear correlation of Treg global impairment at both the functional and numerical levels with parameters of disease severity, including pulmonary function tests, was also demonstrated, suggesting that Treg dysfunction may serve as a reliable predictor of disease progressiveness and treatment responsiveness, and may provide clinicians with a novel tool for risk stratification of patients with IPF[15].

Tregs can suppress not only the Th1, but also the Th2 response. In an experimental model of acute lung injury (ALI) it was clearly demonstrated that lymphocyte-, and consequently Treg-deficient ($Rag\text{-}1^{-/-}$) mice showed strikingly impaired recovery of the alveolar injury induced

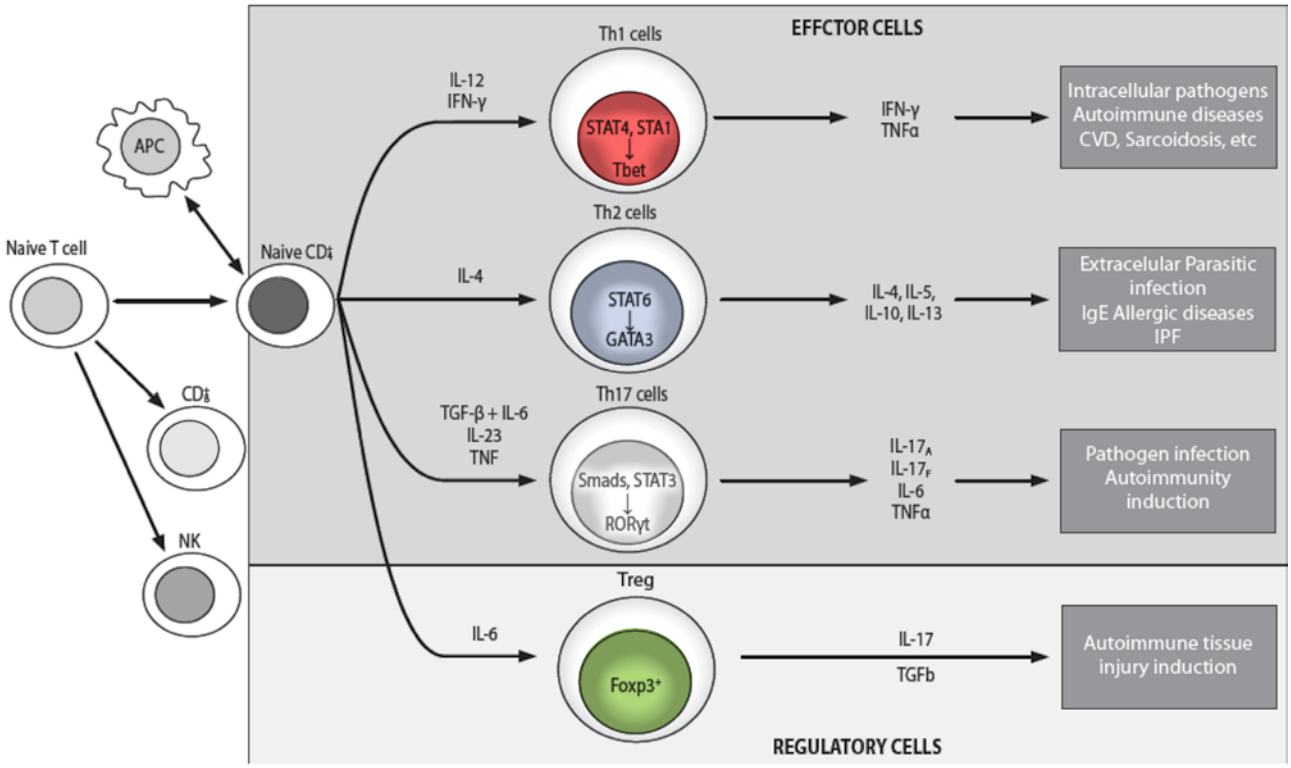

FIGURE 1. T helper cell differentiation and regulation (Bouros D. Pneumon 2007;20:216-218).

by LPS administration, and that exogenous infusion of $CD4^{+}CD25^{high}Foxp3^{+}$ restored the resolution capacity of the Treg deficient mice[16].

It can therefore be surmised that the low numbers of Treg and the systemic and local Treg dysfunction found in IPF patients may either result in inefficient control of the pre-existing overexuberant Th2 response or contribute to a Th2 skew[7]. On the other hand, Tregs were found to be fully functional in NSIP patients, implying the existence of two distinct pathogenetic pathways for the two disorders. In addition, the finding of Treg expansion only in the BALF of patients with NSIP deserves further consideration as it may potentially entail excessive suppression of the local immune response. The local accumulation of Tregs in NSIP may result from either vigorous recruitment of peripheral blood Tregs or local expansion due to the recognition of lung tissue antigens. Alternatively, the high levels of inflammation and the production of proinflammatory cytokines such as IL-2 may release the anergic state of Tregs inducing them to proliferate. It remains to be elucidated whether the observed increase of Tregs in the lung epithelium of patients with NSIP occurs as a primary event inducing aberrant inhibition of local immunity or merely represents a reactive process.

In support of these findings, Mark et al[17] demonstrated that large numbers of highly activated and differentiated CD4+FoxP3+ Tregs localize in the infiltrations of chronically HCV-infected liver, which may result in limitation of the extent of fibrosis. This suggests that CD4+FoxP3+ Tregs play a pivotal role in limiting collateral damage by suppressing excessive HCV-induced immune activation.

In conclusion, although functional CD4+ T cell development has been dominated by the Th1-Th2 paradigm, more recently the discovery of the Th17 pathway and its relationship with Tregs[18] (Figure 1) has opened a new, promising era in the understanding of adaptive immune regulation, resulting in novel and more effective therapeutic approaches in a number of autoimmune and inflammatory diseases.

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