



Regulation of immune cells in lung fibrosis: the reign of regnase-1?

Antoine Froidure ¹ and Bruno Crestani^{2,3}

Affiliations: ¹Service de pneumologie, Cliniques universitaires Saint-Luc et Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain, Brussels, Belgium. ²Université de Paris, Inserm U1152, Labex Inflamex, Paris, France. ³APHP, Service de Pneumologie A, Reference center for rare pulmonary diseases, DHU APOLLO, Hôpital Bichat, Paris, France.

Correspondence: Antoine Froidure, Service de pneumologie, Cliniques universitaires Saint-Luc, Avenue Hippocrate, 10, 1200 Bruxelles, Belgium. E-mail: antoine.froidure@duclouvain.be



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Regnase-1, a natural regulator of ILC2 function, regulates the production of pro-Th2 cytokines (IL-25, IL-33, TSLP) within the fibrotic lung <https://bit.ly/32eVRiJ>

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Since the failure of the PANTHER trial in 2012 [1] and the demonstration that immunosuppressants could be harmful for idiopathic pulmonary fibrosis (IPF) patients, the role of immunity in lung fibrosis has been somehow neglected. Later, the negative results of clinical trials that evaluated the effect of a targeted inhibition of tumour necrosis factor- α [2], CCL2 [3], or interleukin (IL)-13 and/or IL-4 [4, 5], have reinforced the opinion that immune-related cytokines were not a target of choice in IPF. However, recent data have emerged suggesting that immune cells present within fibrotic areas of the lung might either accelerate or slow down the fibrotic process, through their interplay with alveolar epithelial cells and fibroblasts [6]. Ever since, the potential role of lymphoid cells in IPF is under serious consideration, supported by the identification of lymphoid populations essential for the reparative process after organ injury [7].

Innate lymphoid cells (ILC) are the innate counterparts of T lymphocytes. ILC are able to induce immune response in an antigen-independent manner [8] and react to epithelial-derived cytokines. Group 2 ILC (ILC2) trigger Th2 immune responses following stimulation by IL-25, IL-33 and thymic stromal lymphopoietin (TSLP). In the absence of allergen-driven immune response, ILC2 are the main source of IL-5, IL-4 and IL-13 in non-allergic hypereosinophilic asthma and nasal polyposis [9]. Beyond their role in inflammatory Th2 diseases, ILC2 are implicated in tumour immunity [10], psoriasis [11] and liver fibrosis [12].

The role of ILC2-related cytokines in lung fibrosis has already been explored to some extent. In 2014, HAMS *et al.* [13] demonstrated that IL-13 release by ILC2 was able to drive collagen deposition in the lung. The authors also showed an increased expression of IL-25 in the lungs of IPF patients, along with the presence of ILC2 within the fibrotic tissue. The same year, it was shown that IL-33, a pro-Th2 alarmin produced by epithelial cells, could induce the expression of IL-13 and transforming growth factor (TGF)- β 1 by alveolar macrophages [14]. This action was mediated by ST2, an IL-33 receptor, which was expressed on macrophages. Both ST2 deficiency and IL-33 depletion resulted in attenuated fibrosis in mice exposed to bleomycin, suggesting that the IL-33/ST2 axis was profibrotic in this model [14]. Finally, WANG *et al.* [15] demonstrated that TGF- β 1 induced ST2 expression on ILC2 progenitors, further demonstrating how fibrotic and immune pathways intertwine.

In this issue of the *European Respiratory Journal*, NAKATSUKA *et al.* [16] explore the role of regnase-1, an enzyme that degrades immune-related RNA [17], and its expression in ILC2 in lung fibrosis. Regnase-1 is

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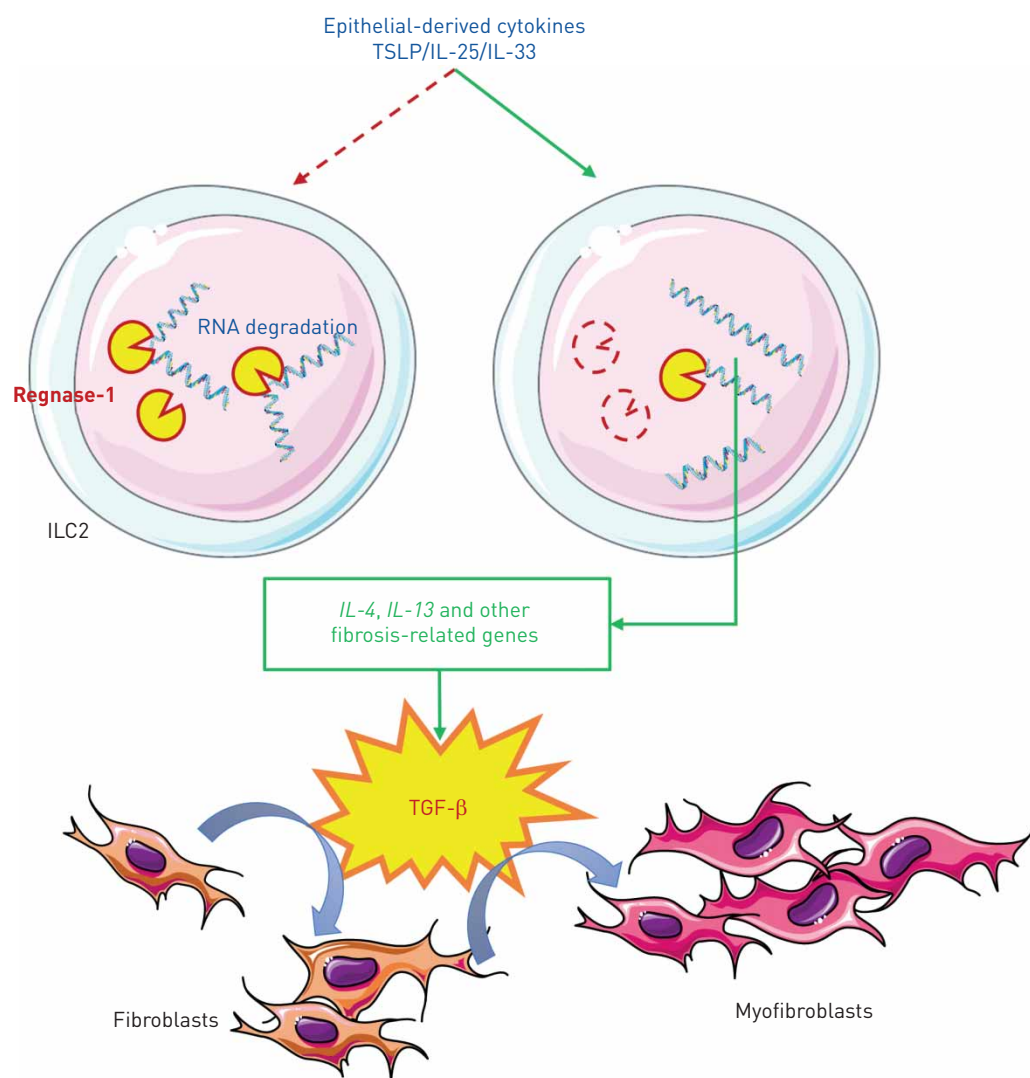


FIGURE 1 Regnase-1 degrades RNA and regulates the expression of fibrosis-related genes. Lower expression of regnase-1 allows the effects of the epithelial-derived cytokines thymic stromal lymphopoietin [TSLP], interleukin (IL)-25 and IL-33. This results in the upregulation of fibrosis-related genes, including IL4 and IL13, ultimately leading to transforming growth factor (TGF)- β -dependent fibroblasts migration, proliferation, and differentiation.

a member of the CCCH zinc finger family, that removes polyA tail from messenger RNA, resulting in higher mRNA turnover. In brief, regnase-1 is an enzyme that selectively degrades RNA to regulate gene expression and polarise target cell function [18]. A recent study showed that regnase-1 degradation was required for the activation of ILC2 by IL-33 and IL-25 [19]. Using cutting-edge techniques, NAKATSUKA *et al.* [16] investigated the expression of regnase-1 in ILC2 and the effect of regnase-1 deficiency in ILC2 on fibrotic pathways. They showed that the absence of regnase-1 in ILC2 increased the expression of fibrosis-related genes and that the transfer of regnase-1-deficient ILC2 in mice submitted to bleomycin instillation resulted in a greater extent of fibrosis, compared to mice receiving bleomycin alone. Finally, switching from bench to bedside, they showed that regnase-1 levels negatively correlate with the number of ILC2 in bronchoalveolar lavage, and that there is a link between the number of circulating ILC2 and prognosis in IPF patients: the higher the ILC2 count, the worse the outcome. Interestingly, this last finding was not linked to Th2 inflammation itself (*i.e.* different levels of IL-4, IL-5 or IL-13 in serum), which rather suggests a local interaction between Th2 cytokines and fibrosis.

This study points to the previously unidentified role of ILC2 as modulators of the fibrotic process in the lung, suggesting that these cells may exert profibrotic functions, under the control of regnase-1 activity.

There is mounting evidence that Th2 cytokines may trigger or enhance tissue fibrosis. For instance, IL-25 activates lung fibroblasts and is able to modulate the phenotype of type 2 alveolar epithelial cells (AEC2)

[6]. Similarly, both IL-33 and TSLP are upregulated in the lungs of IPF patients, and both cytokines display profibrotic effects *in vitro* [20, 21], while IL-4 and IL-13 are overexpressed in IPF lungs, and targeting those cytokines attenuates lung fibrosis in experimental models *in vivo* [22]. Recently, it was also shown that IL-13 prevents AEC2 renewal and promotes the expression of bronchial and airway-associated markers [23]. Despite this evidence, all trials looking for the targeted inhibition of the IL-4/IL-13 pathway in IPF patients failed, with some data suggesting that the outcome was worse in treated patients as compared with placebo [4, 5, 24]. Failure may have multiple reasons, but targeting pro-Th2 cytokines (IL-25, IL-33, TSLP), or modulating ILC2 activity may prove more efficient to tune down the fibrotic process.

Whether circulating ILC2 might constitute a clinically relevant biomarker requires confirmation in a validation cohort. Interestingly, dermal and circulating ILC2 were proposed as biomarkers for systemic sclerosis, as their number is correlated with the extent of dermal and lung fibrosis [25]. This further supports the role of ILC2 and ILC2-derived cytokines in pulmonary fibrosis.

In conclusion, recent evidence suggests that an aberrant production of pro-Th2 cytokines (IL-25, IL-33, TSLP) occurs within the fibrotic lungs. Those epithelial-derived cytokines act on their target cells, including ILC2. Consequently, Th2 cytokines (IL-5, IL-4 and IL-13) exert profibrotic effects on alveolar epithelial cells and fibroblasts. The findings of NAKATSUKA *et al.* [16] suggest that a lower expression of regnase-1, a natural regulator of ILC2 function, could be involved in this complex loop (figure 1). However, why, and how the function of regnase-1 is altered in lung fibrosis remains unclear at this point. Whether acting on regnase-1 expression can counteract this profibrotic Th2 activation also remains uninvestigated. So, altogether, these new results highlight the potential influence of Th2-related cells and cytokines on fibrosis and pave the way for future research in the field.

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