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REVIEW

# The immunopathology of lung fibrosis: amphiregulin-producing pathogenic memory T helper-2 cells control the airway fibrotic responses by inducing eosinophils to secrete osteopontin

Kiyoshi Hirahara[1,2] · Ami Aoki[1] · Yuki Morimoto[1] · Masahiro Kiuchi[1] · Mikiko Okano[1] · Toshinori Nakayama[1,3]

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**Abstract**
Fibrosis is defined as excessive deposition of the extracellular matrix (ECM) in the parenchyma of various organs, and sometimes leads to irreversible organ malfunction such as idiopathic pulmonary fibrosis (IPF), a fatal disorder of the lung. Chronic inflammatory stimuli induce fibrotic responses in various organs. Various immune cells, including T helper (Th) cells in the lung, protect the host from different harmful particles, including pathogenic microorganisms. However, the dysregulation of the function of these immune cells in the lung sometimes causes inflammatory diseases, such as lung fibrosis. In this review, we will introduce an outline of the cellular and molecular mechanisms underlying the pathogenic fibrotic responses in the lung. We will also introduce the concept of the "Pathogenic Th population disease induction model," in which unique subpopulations of certain Th cell subsets control the pathology of immune-mediated inflammatory diseases. Finally, we introduce our recent findings, which demonstrate that amphiregulin-producing pathogenic memory Th2 cells control airway fibrosis through the osteopontin produced by inflammatory eosinophils. The identification of this new pathogenic Th cell population supports the concept of "Pathogenic Th population disease induction model", and will provide novel strategies for treating intractable diseases, including lung fibrosis.

**Keywords** Fibrosis · Extracellular matrix (ECM) · Asthma · "Pathogenic Th population disease induction model" · Amphiregulin-producing pathogenic memory Th2 cells · Osteopontin · Inflammatory eosinophils

## The molecular and cellular mechanism of lung fibrosis

The lungs and bronchus are continuously exposed to exogenous stimuli from sources such as smoking, infection from microorganisms, foreign materials, and drugs. Thus, the bronchi and alveolar epithelial cells are at risk of being damaged and need to be repaired repeatedly. Excessive acute inflammation leads to massive damage in lung tissue, inducing pulmonary alveolar and airway endothelial cells, which causes advanced fibrosis. Moreover, the chronic inflammation induced various sources of stimulation, including tissue injury, infection, autoimmune responses, exogenous foreign agents, tumors, aging, and a genetic predisposition cause fibrotic responses in vivo, in other words: "no inflammation, no fibrosis" [1, 2].

The tissue regeneration occurs after inflammation and tissue damage through two mechanisms: (1) the proliferation of common differentiated cells and (2) the differentiation of stem cells or progenitor cells. In alveolar epithelial injury, common differentiated cells proliferate; however, rare airway stem cells have also been shown to be induced by injury and to play a role in tissue regeneration [3]. The airway stem cells, which are lineage specific marker-negative, cytokeratin 5-positive, can repair the influenza infection-damaged tissue via Notch signaling in mice [4]. Excess Notch signaling is also known to promote fibrotic responses such as cystic honeycombing [4].

Kiyoshi Hirahara and Ami Aoki contributed equally to this work.

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✉ Kiyoshi Hirahara
hiraharak@chiba-u.jp

[1] Department of Immunology, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan

[2] AMED-PRIME, AMED, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan

[3] AMED-CREST, AMED, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan

Acute inflammation damages vascular endothelial cells, which is followed by the secretion of various types of growth factors that recruit fibroblasts and immune-related cells such as T cells, macrophages, neutrophils, and eosinophils into the inflamed lung. At the same time, activated inflammatory cells produce various types of pro-inflammatory cytokines, including Interleukin-1β (IL-1β), IL-6, IL-25, IL-33, thymic stromal lymphopoietin (TSLP), tumor necrosis factor-α (TNF-α), and granulocyte-macrophage colony stimulating factor (GM-CSF) [5]. The damage of vascular endothelial cells also induces innate wound-healing responses, such as coagulation responses by activated platelets. These tissue repair responses in the vascular endothelium are proceeded by activated platelets and immune cells that show the enhanced production of several growth factors (e.g., platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF)). Tissue-resident fibroblasts proliferate in the inflammatory tissue and differentiate into myofibroblasts, which are a key cell population in the fibrotic response. Activated myofibroblasts show the enhanced expression of α-smooth muscle actin (SMA) accompanied by the massive production of the extracellular matrix (ECM). The intratracheal transfer of resident fibroblasts clearly showed that the transferred resident fibroblasts were activated and produced a significant amount of collagen in bleomycin-induced pulmonary fibrosis [6].

Both myofibroblasts and fibroblasts play important roles in pathogenic fibrotic responses and wound repair [7]; however, the origin of myofibroblasts remains controversial. Originally, it was reported that myofibroblasts were derived from tissue resident fibroblasts [8]. Subsequently, fibrocytes, which are circulating monocytic progenitor cells with characteristic features of both monocytes and fibroblasts, were reported to transform into myofibroblasts [9, 10]. Fibrocytes are known to be derived from the bone marrow and circulate in the peripheral blood with the expression of CD34 (a stem cell marker), CD45 (a pan-hematopoietic marker), CD14, and CD11 (both monocyte markers) and produce collagen l, collagen lll, and vimentin [10]. In healthy subjects, fibrocytes make up among approximately 0.5% of the peripheral blood. Pro-inflammatory and fibrogenic chemokines, such as CXCL12, CCL12, CCR3, and CCR5, are involved in the recruitment and infiltration of fibrocytes to the injured lung [11, 12]. Interestingly, fibrocytes from patients with idiopathic pulmonary fibrosis (IPF) show higher expression levels of CXCR4 and CXCL12 (a ligand for CXCR4), in the peripheral blood and the lung [13]. Thus, hyperactivation of fibrocytes may be involved in shaping the pathology of the fibrotic responses in IPF patients. Lung epithelial cells or endothelial cells are another source for myofibroblasts, as these cell populations transform into myofibroblasts via epithelial/endothelial mesenchymal transition (EMT) [14, 15]. Recent technological developments have allowed us to investigate the RNA expression landscape at the single-cell level using single-cell RNA sequencing (scRNA-Seq) [16]. An scRNA-Seq analysis identified a rare cell population, Foxi1+ ionocytes, in the lung [17]. Ionocytes express high levels of cystic fibrosis transmembrane conductance (Cftr), in which more than 1000 mutations have been identified in patients with cystic fibrosis [17]. Thus, this rare cell population is involved in the pathology of lung fibrotic disease (Fig. 1).

Oxidative stress is one of the crucial chemical stresses that are involved in the induction of pathogenic fibrotic responses. Glutathione (GSH) is decreased in the lungs of patients with IPF, which causes an imbalance between oxidants and antioxidants [18]. N-Acetyl-L-cysteine (NAC), a precursor of GSH, was developed as a treatment for patients with IPF; however, the clinical trial failed due to insufficient efficacy [19]. Interestingly however, glutaredoxin-1 (GLRX), which reverses a post-translational modification of GSH, S-glutathionylation (PSSG), was reported as a potential therapeutic agent in recent years. The intratracheal administration of exogenous GLRX to mice with bleomycin-lung fibrosis reversed increases in collagen in the lungs [20].

## Lessons from recent mouse models of fibrosis

Bleomycin, an anti-cancer medication, acts by inducing DNA strand breaks. The intratracheal administration of bleomycin into the lung can induce massive inflammation followed by the severe fibrotic responses [21]. Thus, mice with a bleomycin-induced lung fibrosis are among the animal models most commonly used to investigate the pathology of lung fibrosis. In addition to this model, in recent years, other mouse models have been used to investigate the fibrotic responses in the lung. The lungs of genetically engineered mice with the overexpression of AP-1 (activator protein-1) family members show a spontaneous fibrotic response. The AP-1 family is formed by a large number of proteins, including Fos proteins (c-Fos, FosB, Fra-1, Fra-2) and Jun (JunB, c-Jun, JunD) proteins. Fos and Jun proteins, which are activated by various types of stress signals, control a variety of cellular responses, including proliferation, apoptosis, inflammation, and wound healing [22]. Fra2-transgenic mice show skin and lung fibrosis with vasculopathy that are similar to systemic sclerosis in humans [23, 24]. Interestingly, the expression of osteopontin, an AP-1 targeting gene, is increased in Fra2-transgenic mice. Osteopontin is a phosphoglycoprotein and is induced in various types of myeloid cells and epithelial cells by inflammation, infection, and irradiation. Osteopontin exists as two isoforms, intracellular osteopontin and secreted osteopontin, which have distinct functions. Intracellular osteopontin regulates myeloid cells and works as a scaffold protein in signal transduction pathways [25]. Secreted

osteopontin controls lymphocytes to maintain the balance of the leukocyte population in hematopoiesis and is associated with fibrosis [25]. Systematic fibrosis is also induced in c-Jun transgenic mice [26]. These data suggest that AP-1 transcription factor is one of the central mediators of fibrosis. A cell surface molecule, tetraspanin is another essential protein for the pathology of pulmonary fibrosis. Tetraspanins consist of four transmembrane domains that bind adhesion proteins, such as integrins, and thereby generate a hierarchical network of interactions. CD151, a member of the Tetraspanin family, is predominantly expressed in alveolar epithelial cells and CD151-deficient mice spontaneously exhibit pulmonary fibrosis with age [27]. The deletion of CD151 causes the loss of epithelial integrity that is associated with an exacerbation of pulmonary fibrosis. Telomere dysfunction is also associated with fibrosis. Genetically engineered mice with short telomeres (telomerase-deficient mice) show spontaneous pulmonary fibrosis due to severe telomerase dysfunction in alveolar type II cells, which suggests that cellular senescence is involved in the pathology of pulmonary fibrosis [28].

## The involvement of immune cells in the pathology of fibrosis

Various types of immune cells are involved in the pathology of fibrosis. It is well known that type 2 immunity contributes to the development of fibrotic responses [29]. Th2 responses result in tissue injury and fibrotic responses, while the Th1 response ameliorates fibrotic responses [2]. Pulmonary alveolar macrophages produce profibrotic cytokines, including PDGF, transforming growth factor-β (TGF-β), and IL-13 in the fibrogenic phase. TGF-β promotes the differentiation of fibroblasts to myofibroblasts and enhances the production of ECM from differentiated myofibroblasts [7]. IL-13 is also involved in shaping the pathology of the fibrotic responses both directly and indirectly [1]. Group 2 innate lymphoid cells (ILC2) are also involved in the development of fibrosis in influenza virus and *S. mansoni* infection [30, 31].

M2 macrophages, which are a macrophage subpopulation, are activated by Th2 cytokines such as IL-4 and IL-13, which are involved in shaping the pathology of fibrosis [32]. M2 macrophages express high levels of

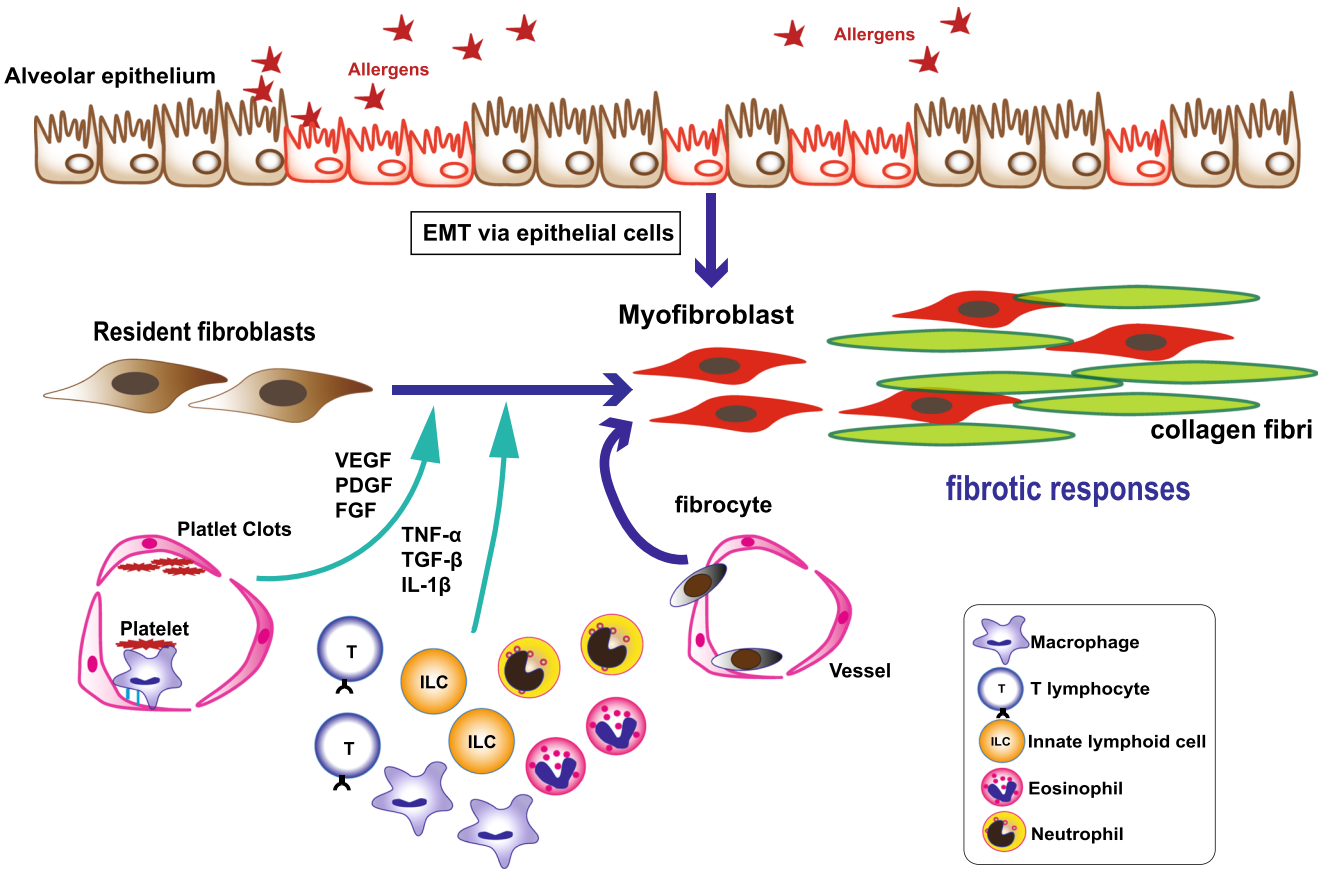


**Fig. 1** A schematic illustration of the cellular and molecular mechanisms of the induction of lung fibrosis. Three major pathways for the induction of myofibroblasts are depicted. (1) Lung epithelial cells or endothelial cells transform into myofibroblasts via epithelial/endothelial mesenchymal transition (EMT). (2) Tissue resident fibroblasts differentiate into myofibroblasts. (3) Fibrocytes transform into myofibroblasts. At the same time, various types of immune cells contribute to shaping the pathology of fibrosis via the production of pro-inflammatory cytokines and growth factors

arginase-1(Arg1) that control the production of L-proline, which is required for collagen synthesis [33]. An scRNA-Seq analysis of macrophages from the lungs of mice with bleomycin-induced fibrosis revealed a population of disease-associated $CX3CR1^{+}SiglecF^{+}$ macrophages with a unique gene expression profile [34]. A specific subpopulation of $Ceacam1^{+}Msr1^{+}Ly6C^{-}F4/80^{-}Mac1^{+}$ monocytes called segregated-nucleus-containing atypical monocytes (SatM) has also been shown to be crucial for the induction of fibrosis [35].

## The pathogenic Th population disease induction model

Leading-edge techniques, including scRNA-Seq, have increased our knowledge regarding the complexity and heterogeneity of immune cells and we now need to revisit issues regarding the pathogenicity of immune-related inflammatory diseases. In the case of T cells, a functional imbalance of helper T cell subsets has been suggested to cause the pathology of immune-related inflammatory diseases ('Th1/Th2 balance disease induction model). However, the diversity of CD4 T cells suggests us that a unique subpopulation of Th cells controls the pathology of specific inflammatory diseases. Recently, we proposed a "pathogenic Th population disease induction model," in which—despite the balance between Th1 and Th2 cells—the pathogenesis of so-called Th1- and Th2-mediated diseases was mostly dependent on the "pathogenic subpopulations" of each helper T cell subset generated in vivo and possessed an effector function, which was a distinct feature [36]. In the model, a pathogenic subpopulation of T helper cells that was induced under certain conditions was found to be critical for the pathogenesis of immune-mediated diseases rather than the balance among T helper cell subsets [36]. In particular, type 2 immunity-mediated pathologies, including atopic dermatitis, pollen allergy, allergic airway inflammatory disease, and IL-5-producing Th2 cell subpopulations, are considered to be pathogenic populations. Indeed, several groups—including our own—have revealed that identified distinct pathogenic helper T cell subpopulations that play key roles in shaping the pathology of various types of immune-mediated inflammatory diseases in both mice and humans. For example, IL-5-producing Th2 cells, so-called pathogenic Th2 (Tpath2) cells, are identified in eosinophilic gastrointestinal diseases, allergic airway inflammation, pollen allergy, and eosinophilic chronic rhinosinusitis in both mice and humans [36–38]. Th1 cells, which express high levels of CXCR3, are crucial for the pathogenicity of type 1 diabetes [39]. In the case of Th17 cells, pathogenic Th17 cells are induced without TGF-β1 or with TGF-β3 [40, 41]. AIM (CD5L) also controls Th17 cell pathogenicity through the regulation of lipid biosynthesis [42]. In the case of human Th17 cells, the transcription factor c-MAF controls the immunopathology of Th17 cells [43]. Thus, a comprehensive understanding of the "pathogenic Th population disease induction model" is a key to elucidating the precise pathogenicity of immune-mediated inflammatory diseases.

## Pathogenic Th2 cells are induced by the epithelial cytokine IL-33

Various environmental stimuli are known to induce pathogenic populations of helper T cells [36]. IL-33, a member of the epithelial cytokines, was identified as a ligand of ST2 (an IL-1 receptor) [44]. IL-33 works as a transcriptional repressor in the nuclei of many types of cells under a steady state because it is constitutively localized in the nuclei and is associated with chromatin by a chromatin-binding motif [45]. The finding that IL-33 has no signal sequence suggests that IL-33 differs from conventional secreted cytokines [46]. In contrast to being secreted, mechanical damage, cellular activation through ATP signaling, or necrotic cell death induces the release of IL-33 into the extracellular space [47, 48]. The expression pattern and the mode of IL-33 release differ between mice and humans (i.e., vascular endothelial cells do not express IL-33 constitutively in mice, while human endothelial cells preserve IL-33 universally) [49–51].

In humans, IL-33 is involved in different types of diseases, such as bacterial and viral infections, cardiovascular disease, allergies, and metabolic disorders [51]. At the same time, the ST2 and IL-33 gene loci are often associated with asthma in different genome-wide association studies (GWAS) [52]. Moreover, polymorphism in the IL-33 signal pathway is associated with the clinical phenotype of childhood asthma [53]. IL-33 induces potent type 2 immune responses accompanied by massive eosinophil infiltration through the activation of both innate and adaptive immune cells, including mast cells, eosinophils, and ILC2s [54–57]. Moreover, we recently found that IL-5-producing pathogenic Th2 (Tpath2) cells selectively express a high level of ST2, and the exposure of memory Th2 cells to IL-33 resulted in a significant increase in the production of IL-5 in both mice and humans [58]. Thus, the IL-33-ST2 axis is crucial for the induction and enhancement of the pathogenicity of memory Th2 cells in allergic airway inflammation.

## Maintenance of memory Tpath2 cells within the iBALT

After the induction of airway inflammation, a type of ectopic lymphoid tissue called inducible bronchus-associated lymphoid tissue (iBALT), which consists of T cells, B cells,

dendritic cells (DCs), and follicular DCs (FDCs), is induced in the lung parenchyma [59, 60]. A recent study revealed that type I interferon (IFN) after influenza A infection causes the activation of a subpopulation of lung fibroblasts, which can convert non-lymphoid tissue into functional tertiary lymphoid structure formations [61]. FDCs represent a unique cell population that is required for the development of proper B cells [62]. Furthermore, $CD11c^+$ DCs are involved in maintaining the iBALT structure [63]. Antigen-specific memory CD4 T cells, including Tpath2 cells, are maintained in the iBALT [59, 64]. During chronic allergic airway inflammation, Thy1-positive IL-7-producing lymphatic endothelial cells (LECs), which are localized within iBALT structures, are a key population for the maintenance of antigen-specific memory Tpath2 cells [59]. Lung-infiltrating memory Tpath2 cells preferentially localize within the iBALT and are attached to IL-7-producing LECs, which increase in number under inflammatory conditions. Thus, the survival of antigen-specific memory Tpath2 cells is induced by the modification of the lung microenvironment. Interestingly, $Thy1^+$IL-7-producing LECs also produce IL-33, which is critical for maintaining the pathogenic ability (e.g., type 2 cytokine production) memory of Tpath2 cells [59]. Taken together, Thy1-positive IL-7-producing LECs show dual effects on memory Tpath2 cells (i.e., IL-7 from LECs supports the survival of memory Tpath2 cells, while IL-33 is responsible for maintaining the pathogenic functions of Tpath2 cells in the airway).

## IL-33 induces the enhanced production of IL-15 by tissue-resident memory Th2 cells within iBALT

Three major subpopulations are known to exist in memory Th2 cells: central memory T cells ($T_{CM}$), effector memory T ($T_{EM}$) cells, and tissue resident memory T ($T_{RM}$) cells, which have recently been identified [65, 66]. $T_{CM}$ cells with the high expression of CCR7 and CD62L can respond rapidly to pathogens in the lymphoid organs [67]. $T_{EM}$ cells show low expression levels of CD62L and CCR7 and circulate among non-lymphoid tissue, secondary lymphoid organs and the blood [67]. In contrast, $T_{RM}$ cells permanently reside in the peripheral tissue with the expression of CD69 and CD103 [68]. $CD4^+$ $T_{RM}$ cells reside in various mucosal organs, including the reproductive organs, skins, and lungs [69–71]. Interestingly, lung $T_{RM}$ cells are retained in the lungs, and migrate back to the lung in the adaptive transfer mouse model [72]. Lung $CD4^+$ $T_{RM}$ cells play crucial roles in shaping the pathology of allergic airway inflammation [73]. Visceral adipose tissue has been reported to be a reservoir of CD4 $T_{RM}$ cells [74]. The involvement of CD4 $T_{RM}$ cells in shaping the pathology of lung fibrosis has been uncertain. Further investigations are needed to clarify the precise roles of CD4 $T_{RM}$ cells in the lung.

## Amphiregulin-producing pathogenic memory T helper-2 cells drive airway fibrosis

Asthma is a common disease worldwide, and is characterized by chronic airway inflammation with persistent cough and airflow limitation. Long-standing asthma causes airway remodeling and exacerbates bronchial hyperresponsiveness with various symptoms and airway narrowing. Airway remodeling involves a loss of normal bronchial epithelial cells, mucous-gland hyperplasia, and deposition of the collagen subepithelial layer [75]. Adult patients with severe asthma show a decreased lung function (FEV1 or FEV1 /VC < 75% predicted) accompanied by fibrotic changes [76].

Various stimuli, such as allergens, viruses, bacteria, and fungi, which cause exacerbations of asthma, injure the bronchus and induce the release of epithelial cytokines (IL-25, IL-33, and TSLP). These epithelial cytokines activate both innate and adaptive immune cells, including ILC2s and memory-type Tpath2 cells that produce large amounts of Th2 cytokines and induce massive infiltration of eosinophils in the airway. It was reported that the fibrotic responses in allergic airway inflammation were associated with IL-25, IL-33, and TSLP [77]. Fibrotic responses involve the massive deposition of collagenous and non-collagenous extracellular matrix components in the lung parenchyma as a result of the activation and proliferation of fibroblasts, myofibroblasts, and various types of immune cells, as discussed previously. Th2 cells, especially Tpath2 cells, which have the ability to produce large amounts of IL-5, are central players in shaping the pathology of both allergic airway inflammation and fibrosis [36]. Eosinophils, a key population for allergic inflammation, can develop, infiltrate inflammatory sites, and are activated through IL-5 stimulation [78]. Eosinophils are involved in the pathogenesis of airway remodeling characterized by collagen fibril deposition [79]. Activated eosinophils produce inflammatory mediators, including cytokines, lipid mediators, and reactive oxygen species (ROS), which damage airway tissue and nerves. This is followed by inflammation accompanied by enhanced airway hyper responsiveness [80]. However, the precise cellular and molecular mechanisms underlying the pathology of fibrotic responses have not been clarified.

We used an experimental mouse model in which repetitive exposure of house dust mite (HDM) induced massive fibrotic responses together with eosinophilic airway inflammation, to investigate this point. The repeated exposure of the HDM mice increased the expression of *Il33*, *Il25*, and *Tslp* and molecules relevant to the fibrotic response, such as *Spp1, Tenascin C (Tnc), collagen type 1 alpha 1 (Col1a1),* and *Actin alpha 2 smooth muscle aorta (Acta 2)* in the inflamed lung. The genetic deletion of *Il33* or *Il1rl1* encoding ST2 (a component of the IL-33 receptor) resulted in a decreased fibrotic response. At the same time, the lungs of *Il33*-deficient mice that received OVA-

specific memory Th2 cells or wild-type mice that received *Il1rl1*-deficient OVA-specific memory Th2 cells showed significantly decreased in collagen deposition. Thus, the IL-33-ST2 axis in memory Th2 cells plays an important role in the induction of the fibrotic responses in allergic inflammation.

**Fig. 2** Amphiregulin-producing Tpath2 cells induce pathogenic fibrotic responses by instructing inflammatory eosinophils to produce osteopontin. Amphiregulin produced by pathogenic memory Th2 cells reprogrammed eosinophils to produce osteopontin and trigger airway fibrosis. The image illustrates amphiregulin-producing pathogenic memory Th2 cells as a "witch" sharing "poisoned apples" (amphiregulin) to "dwarves" (eosinophils). The dwarves who ate a poisoned apple are confused and are fixing the lung of a robot with improper items such as "tin cans" (osteopontin)

To identify the key molecule(s) of the fibrotic responses, we performed an RNA sequencing (RNA-Seq) analysis using OVA-specific $ST2^{hi}$ memory Th2 cells stimulated with IL-33 in vitro. IL-33 stimulation induced the high expression of *Areg,* encoding amphiregulin, in $ST2^{hi}$ memory Th2 cells. In vivo, memory Th2 cells producing amphiregulin increased in the lung after exposure to HDM. The production of amphiregulin by memory Th2 cells stimulated by IL-33 decreases with the application anti-ST2 antibodies, which blocks the IL-33-ST2 signaling pathway. At the same time, lung fibrotic responses in *Areg*-deficient mice were ameliorated with HDM inhalation, although the expression levels of *Il33*, *Il5*, and *Il13* were comparable between wild-type and *Areg*-deficient mice. Furthermore, the fibrotic responses decreased in recipients of *Areg*-deficient memory Th2 cells. These data suggest that IL-33 induces the production of amphiregulin by $ST2^{hi}$ memory Th2 cells and that amphiregulin is a key molecule in the induction of fibrotic responses in vivo.

Amphiregulin is a member of the epithelial growth factor proteins and is a key molecule for tissue repair [81]. Various immune cells, including mast cells, ILC2s, and Treg cells, also produce amphiregulin [82–84]. In the brain, amphiregulin from Treg cells contributes to the suppression of neurotoxic astrogliosis [84]. In the lung, the stimulation of epithelial cells with amphiregulin results in proliferation and enhanced mucin production [82, 85]. Amphiregulin also induces the proliferation of fibroblasts in the lung [86]. In addition to these well-known target cells, we found that eosinophils expressed EGF receptor and that amphiregulin stimulation resulted in global transcriptional reprogramming in eosinophils, marked by the upregulation of a number of inflammatory genes. *Spp1*, which encodes osteopontin (a major component of non-collagenous ECM) was one of the genes upregulated by amphiregulin stimulation in eosinophils. Osteopontin is known to contribute to shaping the pathogenesis of fibrosis [87, 88]. Thus, eosinophils directly produce non-collagenous ECM and contribute to tissue fibrosis in allergic airway inflammation

Finally, we wanted to determine whether the observed IL-33-ST2-amphiregulin pathway was important for shaping the fibrotic pathology in inflammatory lesions of human chronic allergic diseases. Eosinophilic chronic rhinosinusitis (ECRS) is a chronic upper airway inflammatory condition that is accompanied by the formation of nasal polyps with the massive infiltration of eosinophils [89]. Patients with ECRS often suffer from allergic airway inflammation such as asthma, and their polyps exhibit eosinophilic infiltration, suggesting that excessive type 2 immunity is involved in shaping the pathology of ECRS [90]. Thus, the pathogenesis of asthma is closely related to that of ECRS [91]. Interestingly, we found that polyps from patients with eosinophilic chronic rhinosinusitis (ECRS) showed enhanced fibronectin disposition. We also found that $CD45RO^{+}CRTH2^{hi}CD161^{hi}CD4^{+}$ T cells in polyps specifically produced amphiregulin. Furthermore, the eosinophils infiltrating the polyps showed higher osteopontin expression levels in comparison to the eosinophils in peripheral circulation. Taken together, the IL-33-amphiregulin-osteopontin axis controls the fibrotic responses in chronic allergic inflammation in both mouse and human systems, and these molecules may be potential therapeutic targets for intractable chronic allergic diseases (Fig. 2).

## Conclusion

The regeneration of fibrotic organs has not yet been achieved. Because of the poor understanding of the pathology underlying the development of fibrosis, the diagnosis and therapy are under development; however, the prognosis of pulmonary fibrosis remains poor, with a median survival period of 3–5 years. The small molecule receptor tyrosine kinase inhibitor nintedanib is one of the few Food and Drug Administration-approved treatments for lung fibrosis is. Unfortunately, the current goal of anti-fibrotic treatment is to delay the progression of fibrosis rather than cure the disease [92–94]. Thus, we must explore not only novel biomarkers of the progression of fibrosis but also effective therapeutic strategies for lung fibrosis. To this end, intensive studies are needed to understand the mechanisms underlying pathogenic fibrotic changes.

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### Compliance with ethical standards

**Competing interests** The authors declare that they have no conflict of interest.

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