FIBROTIC DISEASE AND THE T_H1/T_H2 PARADIGM

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Tissue fibrosis (scarring) is a leading cause of morbidity and mortality. Current treatments for fibrotic disorders, such as idiopathic pulmonary fibrosis, hepatic fibrosis and systemic sclerosis, target the inflammatory cascade, but they have been widely unsuccessful, largely because the mechanisms that are involved in fibrogenesis are now known to be distinct from those involved in inflammation. Several experimental models have recently been developed to dissect the molecular mechanisms of wound healing and fibrosis. It is hoped that by better understanding the immunological mechanisms that initiate, sustain and suppress the fibrotic process, we will achieve the elusive goal of targeted and effective therapeutics for fibroproliferative diseases.

BLEOMYCIN
An antineoplastic antibiotic. It is active against bacteria and fungi, but its cytotoxicity has prevented its use as an anti-infective agent. Treatment with bleomycin is associated with significant pulmonary side effects — including fibrosis — that limit its use. Bleomycin was first noted to cause pulmonary fibrosis in the initial clinical trials in which it was tested. Since that time, it has been used extensively in experimental models to dissect the mechanisms of fibrosis.

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Repair of damaged tissues is a fundamental biological process that allows the ordered replacement of dead or injured cells during an inflammatory response, a mechanism that is crucial for survival. Tissue damage can result from several acute or chronic stimuli, including infections, autoimmune reactions and mechanical injury. The repair process involves two distinct stages: a regenerative phase, in which injured cells are replaced by cells of the same type and there is no lasting evidence of damage; and a phase known as fibroplasia or fibrosis, in which connective tissue replaces normal parenchymal tissue (FIG. 1). In most cases, both stages are required to slow or reverse the damage caused by an injurious agent. However, although initially beneficial, the healing process can become pathogenic if it continues unchecked, leading to considerable tissue remodelling and the formation of permanent scar tissue. In some cases, it might ultimately cause organ failure and death. Fibrotic scarring is often defined as a wound-healing response that has gone awry.

Fibroproliferative diseases are an important cause of morbidity and mortality worldwide. Fibrotic changes can occur in various vascular disorders, including cardiac disease, cerebral disease and peripheral vascular disease, as well as in all the main tissues and organ systems, including the skin, kidney, lung and liver. Fibrosis is a troubling problem for an increasing number of individuals and is a common pathological sequela of many persistent inflammatory diseases, such as idiopathic pulmonary fibrosis, progressive kidney disease and liver cirrhosis (BOX 1). Despite their obvious aetiological and clinical distinctions, most of these fibrotic diseases have in common a persistent inflammatory stimulus and lymphocyte–monocyte interactions that sustain the production of growth factors, proteolytic enzymes and fibrogenic cytokines, which together stimulate the deposition of connective-tissue elements that progressively remodel and destroy normal tissue architecture.

As mechanistic studies of fibrogenesis are difficult to carry out in humans, several animal models have been developed over the past few years (BOX 2). Although combinations of these strategies (such as BLEOMYCIN or schistosomiasis experiments using transgenic mice) have been particularly useful in elucidating the molecular mechanisms of fibrosis, all of these approaches have limitations. The main problem with many of the mouse models has been the difficulty in duplicating the progressive tissue remodelling and fibrosis that is seen in some of the chronic human diseases. Nevertheless, considerable progress has been made over the past few years, particularly in our understanding of the immunological mechanisms that regulate fibrogenesis. Although severe acute (non-repetitive) injuries can also

cause marked tissue remodelling, fibrosis that is associated with chronic (repetitive) injury is unique in that the adaptive immune response is thought to have an important role. So, rather than discussing the basic features of wound healing, tissue remodelling and fibrosis, which have been reviewed elsewhere[1], this review focuses on how the adaptive immune response amplifies, sustains and suppresses the fibrotic process, particularly in chronic progressive disease.

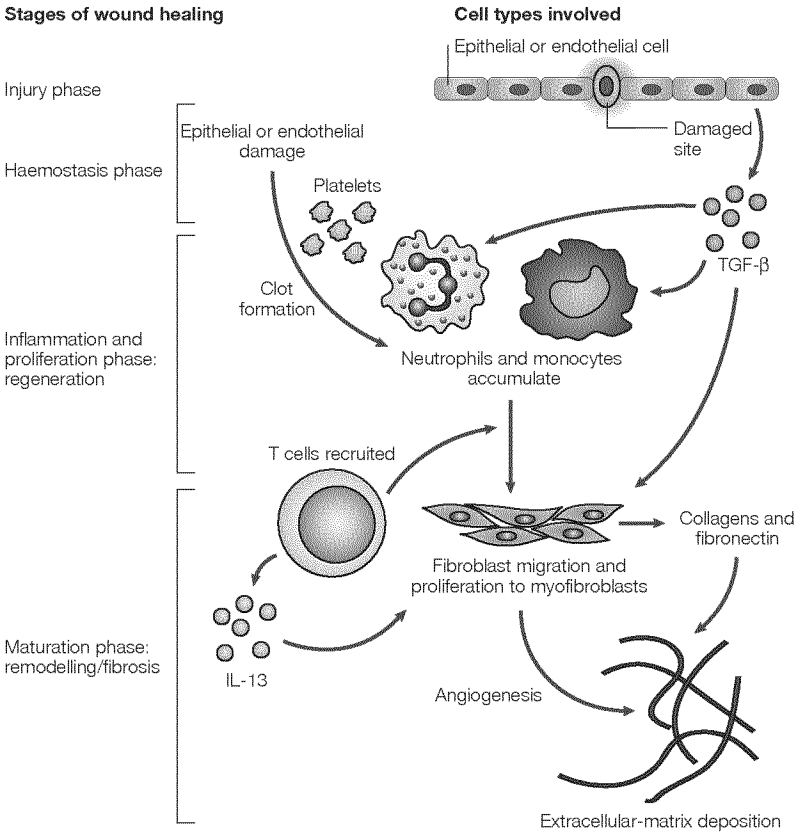

Figure 1 | **The pathogenesis of fibrotic disease.** Healing is the normal reaction of tissues after injury. Damaged epithelial and/or endothelial cells release inflammatory mediators that initiate an antifibrinolytic–coagulation cascade, which triggers blood-clot formation. Next, epithelial and endothelial cells secrete growth factors and chemokines that stimulate the proliferation and recruitment of leukocytes that produce pro-fibrotic cytokines, such as interleukin-13 (IL-13) and transforming growth factor-β (TGF-β). Stimulated myofibroblasts and epithelial/endothelial cells also produce matrix metalloproteinases (MMPs), which disrupt the basement membrane, allowing the efficient recruitment of cells to sites of injury. After this migration, activated macrophages and neutrophils 'clean-up' tissue debris and dead cells. They also produce cytokines and chemokines that recruit and activate T cells, which are important components of granulation tissue as they secrete pro-fibrotic cytokines (such as IL-13). Fibroblasts are subsequently recruited and activated. Fibroblasts can be derived from local mesenchymal cells or recruited from the bone marrow (known as fibrocytes). Epithelial cells can undergo epithelial–mesenchymal transition, providing a rich renewable source of fibroblasts. Revascularization of the wound also occurs at this time. After activation, myofibroblasts cause wound contraction, the process in which the edges of the wound migrate towards the centre. Last, epithelial and/or endothelial cells divide and migrate over the basal layers to regenerate the epithelium or endothelium, respectively, which completes the healing process. However, when repeated injury occurs, chronic inflammation and repair can cause an excessive accumulation of extracellular-matrix components, such as the collagen that is produced by fibroblasts, and lead to the formation of a permanent fibrotic scar. Pro-fibrotic mediators, such as IL-13 and TGF-β, amplify these processes. The net amount of collagen deposited by fibroblasts is regulated by continued collagen synthesis and collagen catabolism. The degradation of collagen is controlled by MMPs and their inhibitors (such as tissue inhibitors of matrix metalloproteinases, TIMPs), and the net increase in collagen within a wound is controlled by the balance of these opposing mechanisms.

Polarized T cells regulate organ fibrosis

In contrast to acute inflammatory reactions, which are characterized by rapidly resolving vascular changes, oedema and neutrophilic infiltration, chronic inflammation is defined as a reaction that persists for several weeks or months and in which inflammation, tissue destruction and repair processes occur simultaneously. When chronic injuries occur, inflammation is characterized by a large infiltrate of mononuclear cells, which include macrophages, lymphocytes, eosinophils and plasma cells. In these cases, lymphocytes are mobilized and stimulated by contact with antigen to produce lymphokines that activate macrophages. Cytokines from activated macrophages, in turn, stimulate lymphocytes, thereby setting the stage for persistence of the inflammatory response. So, there is considerable activation of the adaptive immune response in chronic inflammatory diseases. However, although inflammation typically precedes fibrosis, results from several experimental models show that the amount of fibrosis is not necessarily linked with the severity of inflammation, indicating that the mechanisms that regulate fibrogenesis are distinct from those that regulate inflammation. Findings from our own studies of schistosomiasis-induced liver fibrosis strongly support this hypothesis. In this model, fibrosis develops progressively in response to schistosome eggs that are deposited in the liver, which induce a CHRONIC GRANULOMATOUS RESPONSE. Similar to most experimental models of fibrosis, $CD4^+$ T cells have an important role in the progression of the disease. In particular, the type of $CD4^+$ T-cell response that develops is crucial. Studies using various cytokine-deficient mice showed that fibrogenesis is strongly linked with the development of a T HELPER 2 (T_H2) $CD4^+$ T-CELL RESPONSE, involving interleukin-4 (IL-4), IL-5 and IL-13 (REF. 2). Although an equally potent inflammatory response develops when T_H1 $CD4^+$ T cells, which produce interferon-γ (IFN-γ), dominate[3], under these circumstances, the development of tissue fibrosis is almost completely attenuated. These studies show that chronic inflammation does not always induce the deposition of connective-tissue elements and that the magnitude of fibrosis is tightly regulated by the phenotype of the developing T_H-cell response.

In addition to the system developed in our own laboratory, several other experimental systems have been used to document the potent antifibrotic activity of IFN-γ. In the case of schistosomiasis-induced fibrosis, although treatment with IFN-γ or IL-12 has no effect on the establishment of infection, collagen deposition associated with chronic granuloma formation is substantially reduced[2]. Similar results were obtained in models of pulmonary, liver and kidney fibrosis[4–7]. These findings led to the development of an experimental anti-fibrosis vaccination strategy that involves the use of IL-12 or CpG-CONTAINING OLIGODEOXYNUCLEOTIDES as adjuvants to switch off pro-fibrotic T_H2-cell responses in favour of less damaging T_H1-cell responses[2,8]. The opposing effects of T_H1- and T_H2-cytokine responses in fibrosis have also been substantiated by recent microarray

Box 1 | Important fibroproliferative diseases of humans

The United States government estimates that 45% of deaths in the United States can be attributed to fibrotic disorders. Fibrosis affects nearly all tissues and organ systems. Disorders in which fibrosis is a major cause of morbidity and mortality are listed.

Major-organ fibrosis

- Interstitial lung disease (ILD) — includes a wide range of distinct disorders in which pulmonary inflammation and fibrosis are the final common pathways of pathology. There are more than 150 causes of ILD, including sarcoidosis, silicosis, drug reactions, infections and collagen vascular diseases, such as rheumatoid arthritis and systemic sclerosis (also known as scleroderma). Idiopathic pulmonary fibrosis, which is by far the most common type of ILD, has no known cause.
- Liver cirrhosis — has similar causes to ILD, with viral hepatitis, schistosomiasis and chronic alcoholism being the main causes worldwide.
- Kidney disease — diabetes can damage and scar the kidneys, which leads to a progressive loss of function. Untreated hypertensive diseases can also contribute.
- Heart disease — scar tissue can impair the ability of the heart to pump.
- Diseases of the eye — macular degeneration and retinal and vitreal retinopathy can impair vision.

Fibroproliferative disorders

- Systemic and local scleroderma
- Keloids and hypertrophic scars
- Atherosclerosis and restenosis

Scarring associated with trauma (can be severe when persistent)

- Surgical complications — scar tissue can form between internal organs, causing contracture, pain and, in some cases, infertility
- Chemotherapeutic drug-induced fibrosis
- Radiation-induced fibrosis
- Accidental injury
- Burns

experiments[9,10]: studies investigating the gene-expression profiles (transcriptomes) of diseased tissues found that markedly different programmes of gene expression are induced when chronic inflammatory responses are dominated by T_H1 or T_H2 cytokines[9,10]. Not surprisingly, the transcription of many genes that are associated with IFN-γ activity is upregulated in the tissues of T_H1-polarized mice, with no evidence of significant activation of the fibrotic machinery in this setting[9,10]. Instead, two main groups of genes were identified in T_H1-polarized mice: those that are involved in the acute-phase reaction and those that are involved in apoptosis, which might explain the large amount of cell death and tissue damage that is observed when T_H1-cell responses continue unrestrained[11]. By contrast, the transcription of several genes that are known to be involved in the mechanisms of wound healing and fibrosis is upregulated by T_H2 cytokines[9,10]. The regulation and function of a few of these genes, including those that encode procollagen-I, procollagen-III, arginase[12], lysyl oxidase[13], matrix metalloproteinase 2 (MMP2) (REF. 14), MMP9 (REF. 15) and tissue inhibitor of matrix metalloproteinase 1 (TIMP1) (REFS 16,17), have been investigated in some detail. Moreover, several additional T_H2-linked genes[9,10], including those that encode haem oxygenase, procollagen-III, secreted phosphoprotein 1, procollagen-V, reticulocalbin and fibrillin-1, are also induced in the fibrotic lungs of bleomycin-treated mice[18] and in carbon tetrachloride (CCl_4)-stimulated rat hepatic stellate cells (collagen-producing cells in the liver)[19], providing further proof that fibrogenesis is intimately linked with T_H2-cytokine production (FIG. 2).

CHRONIC GRANULOMATOUS RESPONSE
Granulomas are localized inflammatory reactions that contain T cells and are a form of delayed-type hypersensitivity. They have common features involving persistent antigenic stimulation that is not easily cleared by phagocytic cells. The cellular conglomerate is shielded from the healthy tissue by extracellular matrix. Granuloma formation and the fibrotic scarring that follows can cause progressive organ damage.

IL-13 is the main pro-fibrotic mediator

Each of the main T_H2 cytokines — IL-4, IL-5 and IL-13 — has a distinct role in the regulation of tissue remodelling and fibrosis. IL-4 is found at increased concentrations in the BRONCHOALVEOLAR LAVAGE fluids of patients with idiopathic pulmonary fibrosis[20], in the pulmonary interstitium of individuals with CRYPTOGENIC FIBROSING ALVEOLITIS[21] and in the peripheral blood mononuclear cells of those suffering from periportal fibrosis[22]. Development of post-irradiation fibrosis is also associated with increased concentrations of IL-4 (REF. 23). Although the extent to which IL-4 participates in the progression of fibrosis can vary in each disease, it has long been considered an effective pro-fibrotic mediator. In fact, some studies have indicated that IL-4 is nearly twice as efficient at mediating fibrosis as transforming growth factor-β (TGF-β)[24], another potent pro-fibrotic cytokine that has been widely studied[25] (discussed later). Receptors specific for IL-4 are found on many mouse[26] and human[27] fibroblast subtypes, and *in vitro* studies showed that the extracellular matrix (ECM) proteins, types I and III collagen and fibronectin, are synthesized after stimulation with IL-4 (REFS 24,27,28). Although studies with fibroblasts showed that IL-4 can directly stimulate collagen synthesis *in vitro*, blocking studies were required to confirm its role *in vivo*. One of the first such reports to investigate the contribution of IL-4 was a study of schistosomiasis in mice. In this report, a consistent reduction in hepatic collagen deposition was observed when infected mice were treated with neutralizing antibodies specific for IL-4 (REF. 29). Inhibitors of IL-4 also reduced the development of dermal fibrosis in a chronic skin-graft rejection model and in a putative mouse model of SCLERODERMA[30,31]. However, because IL-13 production decreases in the absence of IL-4 (REF. 29), it was not possible to discern the specific contributions of IL-4 and IL-13 in these early IL-4-blocking studies.

IL-13 shares many functional activities with IL-4 because both cytokines use the same IL-4 receptor α-chain (IL-4Rα)–signal transducer and activator of transcription protein 6 (STAT6) signalling pathway[32]. However, the development of *Il-13*-transgenic and -knockout mice[33,34], as well as IL-13 antagonists[35,36], has revealed unique and non-redundant roles for IL-13 and IL-4 in host immunity. Experiments in which IL-4 and IL-13 were inhibited independently identified IL-13 as the dominant effector cytokine of fibrosis in several models[36–38]. In schistosomiasis, although the egg-induced inflammatory response was unaffected by IL-13 blockade, collagen deposition decreased by more than 85% in chronically infected animals[36,39], despite continued and undiminished production of IL-4 (REFS 36,40). Related studies have also shown a dominant role for IL-13 in the

T HELPER 2 (T_H2) CD4+ T-CELL RESPONSE
CD4+ T cells are classified according to the cytokines that they secrete. T_H2 cells secrete large amounts of interleukin-4 (IL-4), IL-5 and IL-13, which promote antibody production by B cells and collagen synthesis by fibroblasts, whereas T_H1 cells secrete large amounts of interferon-γ and associated pro-inflammatory cytokines. T_H1-type and T_H2-type cytokines can cross-regulate each other's responses. An imbalance of T_H1/T_H2 responses is thought to contribute to the pathogenesis of various infections, allergic responses and autoimmune diseases.

pathogenesis of pulmonary fibrosis. Overexpression of IL-13 in the lung induced considerable subepithelial airway fibrosis in mice in the absence of any additional inflammatory stimulus[34], whereas treatment with IL-13-specific antibodies markedly reduced collagen deposition in the lungs of animals that were challenged with *Aspergillus fumigatus* conidia[37] or bleomycin[41]. By contrast, transgenic mice that overexpressed IL-4 showed little evidence of subepithelial airway fibrosis, despite developing an intense inflammatory response in the lung[42].

Given that IL-4 and IL-13 use similar signalling pathways[32], it was not immediately clear why IL-13 should have greater fibrogenic activity than IL-4. Presumably, both cytokines bind the same signalling receptor (IL-4Rα–IL-13Rα1) that is expressed by fibroblasts[43]. Indeed, studies carried out using several fibroblast subtypes showed potent collagen-inducing activity for both IL-4 and IL-13 (REFS 36,44,45). So, these cytokines are equally capable of functioning as pro-fibrotic mediators *in vitro*. Results from several disease models indicate that differences in ligand density might provide at least one explanation for the differential activities of IL-4 and IL-13 (REFS 36,46–48). When the production of IL-4 and IL-13 are compared, the concentrations of IL-13 often exceed those of IL-4 by a factor of 10–100. Therefore, IL-13 might be the dominant effector cytokine simply because greater concentrations are produced *in vivo*. Nevertheless, this finding alone might not fully explain the differential activities because *Il-4*- and *Il-13*-transgenic mice develop distinct forms of pulmonary pathology, even though both types of animal express high concentrations of cytokine[34,42]. Identical cell-specific promoters were used in each study, yet fibrosis was more marked in the lungs of *Il-13*-transgenic mice. Consequently, a more important role for IL-13 in tissue remodelling could be inferred. Interestingly, two recent studies showed that IL-13-regulated responses[49], including lung fibrosis[46], can develop in the absence of IL-4Rα or STAT6 signalling molecules. So, IL-13 might use a signalling pathway that is in some way distinct from that used by IL-4, which could be an additional mechanism to augment its fibrogenic potential.

In contrast to IL-13, the extent to which IL-5 and eosinophils participate in fibrotic processes varies greatly, with no clear explanation for the widely divergent findings. The differentiation, activation and recruitment of eosinophils is highly dependent on IL-5, and eosinophils could be an important source of fibrogenic cytokines (such as TGF-β and IL-13). IL-5 and tissue eosinophils have been linked with tissue remodelling in several diseases, including skin allograft rejection and pulmonary fibrosis[31,50]. Nevertheless, studies using neutralizing IL-5-specific antibodies and IL-5-deficient mice have yielded conflicting results. Early experiments using IL-5-specific monoclonal antibodies showed no reduction in liver fibrosis after infection with *Schistosoma mansoni*, even though tissue-eosinophil responses were markedly reduced[51]. Although negative findings were reported for some of the skin and lung fibrosis models[51,52], in other studies, significant reductions in tissue fibrosis were observed after IL-5 activity was ablated[31,53–55]. Interestingly, a recent study showed that, although bleomycin-induced fibrosis is exacerbated in transgenic mice that overexpress IL-5, *Il-5*$^{-/-}$ mice remain highly susceptible to fibrosis[56], indicating that IL-5 and/or eosinophils function as amplifiers rather than as indispensable mediators of fibrosis. In mice that are deficient in IL-5 and CC-chemokine ligand 11 (CCL11; also known as eotaxin), tissue eosinophilia is abolished and the ability of $CD4^+$ T_H2 cells to produce the pro-fibrotic cytokine IL-13 is impaired[57]. In addition, IL-5 was recently shown to regulate TGF-β expression in the lungs of mice that were chronically challenged with ovalbumin[55]. So, one of the key functions of IL-5 and eosinophils might be to facilitate the production of pro-fibrotic cytokines, including IL-13 and/or TGF-β, which then function as the main mediators of tissue remodelling.

Box 2 | Experimental models commonly used to study fibrosis

Trauma
- Surgical trauma or organ transplantation (multiple organs and tissues)
- Burns (skin)
- Bile-duct occlusion (liver)
- Irradiation (skin, lungs and other organs)
- Traumatic aorto-caval fistula or rapid ventricular pacing (heart)

Toxins and drugs
- Bleomycin, asbestos, silica or ovalbumin (pulmonary fibrosis)
- Acetaldehyde, carbon tetrachloride or concanavalin A (liver cirrhosis)
- Vinyl chloride (liver and lung fibrosis)
- Trinitrobenzene sulphonic acid or oxazolone (gut)
- Cerulein (pancreas)

Autoimmune disease or malfunctioning immune-mediated processes
- Antibody and immune-complex disease models (kidney)
- Organ-transplant rejection (skin, heart and multiple organs)
- Tight skin (Tsk)-mouse model (progressive systemic sclerosis)
- Ischaemia–reperfusion injury (liver)
- Various models of rheumatoid arthritis (joints)

Chronic infectious diseases
- *Schistosoma* species or chronic viral hepatitis (liver)
- *Aspergillus fumigatus* (lung)
- *Mycobacterium tuberculosis* (lung and liver)
- *Trypanosoma cruzi* (heart or gut)

Genetically engineered mice
- Transforming growth factor-β (TGF-β) or TGF-β-receptor transgenic and knockout mice
- Signalling-molecule-deficient mice: for example, mothers-against-decapentaplegic homologue 3 (SMAD3)-deficient mice
- Mice deficient in molecules that affect TGF-β activation: for example, α_1-integrin or matrix metalloproteinase 9
- Cytokine-gene transgenic and knockout mice: for example, tumour-necrosis factor, interleukin-4 (IL-4), IL-13 or IL-10

Cooperation between TGF-β and IL-13

TGF-β is undoubtedly the most intensively studied regulator of the ECM, and production of TGF-β has been linked with the development of fibrosis in several diseases[58–61]. There are three isotypes of TGF-β found in mammals — TGF-β1, -β2 and -β3 — all of which have similar biological activities[62]. Although various cell types produce and respond to TGF-β[25], tissue fibrosis is mainly attributed to the TGF-β1 isoform, with circulating monocytes and tissue macrophages being the main cellular source. In macrophages, the main level of control is not in the regulation of expression of the mRNA that encodes TGF-β1 but in the regulation of both the secretion and activation of latent TGF-β1. TGF-β1 is stored in the cell in an inactive form, as a disulphide-bonded homodimer that is non-covalently bound to a latency-associated protein (LAP). Binding of the cytokine to its receptors (type I and type II serine/threonine-kinase receptors) requires dissociation of the LAP, a process that is catalysed *in vivo* by several agents, including cathepsins, plasmin, calpain, thrombospondin, $\alpha_v\beta_6$-integrin and MMPs[25,62,63]. After activation, TGF-β signals through transmembrane receptors that stimulate the production of signalling intermediates known as SMAD (mothers-against-decapentaplegic homologue) proteins, which modulate the transcription of target genes, including those that encode the ECM proteins procollagen-I and -III[64]. Dermal fibrosis after irradiation[65] and renal interstitial fibrosis induced by unilateral ureteral obstruction[58] are both reduced in SMAD3-deficient mice, confirming an important role for the TGF-β signalling pathway. So, macrophage-derived TGF-β1 is thought to promote fibrosis by directly activating resident mesenchymal cells, which then differentiate into collagen-producing myofibroblasts. In the bleomycin model of pulmonary fibrosis, alveolar macrophages are thought to produce nearly all of the active TGF-β that is involved in the pathological matrix-remodelling process[66]. Nevertheless, TGF-β1–SMAD3-independent mechanisms of fibrosis have also been proposed[67–69], indicating that additional pro-fibrotic cytokines (for example, IL-4 or IL-13) can function separately or together with the TGF-β–SMAD-signalling pathway to stimulate the collagen-producing machinery.

Interestingly, in addition to inducing the production of latent TGF-β1, IL-13 also indirectly activatates TGF-β by upregulating the expression of MMPs that cleave the LAP–TGF-β1 complex[70,71]. Indeed, IL-13 is a potent stimulator of MMP and cathepsin-based proteolytic pathways in the lung and liver[17,71]. So, the tissue remodelling that is associated with polarized T_H2 responses might involve a pathway in which IL-13-producing $CD4^+$ T_H2 cells stimulate macrophage production of TGF-β1, which then functions as the main stimulus for fibroblast activation and collagen deposition[34,70]. In support of this hypothesis, when TGF-β1 activity was neutralized in the lungs of *Il-13*-transgenic mice, development of subepithelial fibrosis was markedly reduced[70]. However, related studies observed enhanced pulmonary pathology when the TGF-β–SMAD signalling pathway was blocked[72,73], indicating that TGF-β might suppress, rather than induce, tissue remodelling in some settings. The source of TGF-β1 might be crucial to these different effects — macrophage-derived TGF-β1 is often pro-fibrotic[70], whereas T-cell-derived TGF-β1 seems to be suppressive[74]. A recent study investigating the mechanisms of IL-13-dependent fibrosis found no reduction in infection-induced liver fibrosis in MMP9-, SMAD3- or TGF-β1-deficient mice, indicating that IL-13 can function independently of TGF-β[69]; however, the extent to which IL-13 must act through TGF-β1 to induce fibrosis remains unclear. Given that many antifibrotic therapies are focused on inhibiting TGF-β1 (REF. 25), it will be important to determine whether the collagen-inducing activity of IL-13 is mediated solely by the downstream actions of TGF-β and MMPs or whether IL-13 and other pro-fibrotic mediators[44] have direct pro-fibrotic activity, as has been indicated by some studies[36,44,69] (FIG. 3).

The timing, dose and source of IL-13 and TGF-β might also affect their individual contributions to tissue remodelling and fibrosis. Because both mediators might stimulate collagen deposition directly[44], in situations in which IL-13 production exceeds TGF-β production, IL-13 could be the main pro-fibrotic mediator. This might explain the unexpected failure of TGF-β/SMAD inhibitors in some blocking studies[67,68]. We speculate that IL-13 might be the key driver of an 'adaptive' healing programme that is induced during persistent inflammatory responses and is perhaps stimulus specific[69], whereas the TGF-β pathway of fibrosis might be more of an 'innate', and possibly indispensable[75], mechanism of tissue remodelling. IL-13 is produced mainly by cells of the adaptive immune response ($CD4^+$ T_H2 cells)[33], whereas TGF-β is produced by

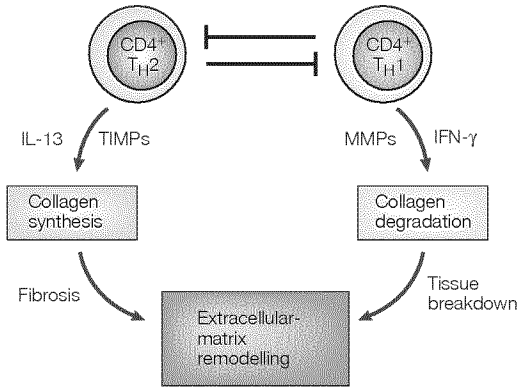

Figure 2 | **Opposing roles for T_H1 and T_H2 cytokines in fibrosis.** The T helper 1 (T_H1)-cell cytokine interferon-γ (IFN-γ) directly suppresses collagen synthesis by fibroblasts. It achieves this through regulating the balance of matrix metalloproteinase (MMP) and tissue inhibitor of matrix metalloproteinase (TIMP) expression, thereby controlling the rates of collagen degradation and synthesis, respectively, in the extracellular matrix. IFN-γ and/or interleukin-12 (IL-12) might also indirectly inhibit fibrosis by reducing pro-fibrotic cytokine expression by T_H2 cells. The main T_H2 cytokines (IL-4, IL-5 and IL-13) enhance collagen deposition by various mechanisms; however, IL-13 seems to be the crucial mediator.

CpG-CONTAINING OLIGODEOXYNUCLEOTIDES
DNA oligodeoxynucleotide sequences that include a cytosine–guanosine sequence and certain flanking nucleotides. They have been found to induce innate immune responses through interaction with Toll-like receptor 9.

BRONCHOALVEOLAR LAVAGE
A diagnostic procedure conducted by placing a fibre-optic scope into the lung of a patient and injecting sterile saline into the lung to flush out free material. The sterile material removed contains secretions, cells and proteins from the lower respiratory tract.

CRYPTOGENIC FIBROSING ALVEOLITIS
Together with various other chronic lung disorders, cryptogenic fibrosing alveolitis is known as interstitial lung disease (ILD). ILD affects the lung in three ways: first, the tissue is damaged in some known or unknown way; second, the walls of the air sacs become inflamed; and third, scarring (or fibrosis) begins in the interstitium (tissue between the air sacs), and the lung becomes stiff.

SCLERODERMA
A chronic autoimmune disease that causes a hardening of the skin. The skin thickens because of increased deposits of collagen. There are two types of scleroderma. Localized scleroderma affects the skin in limited areas and the musculoskeletal system. Systemic sclerosis causes more widespread skin changes and can be associated with internal organ damage to the lungs, heart and kidneys.

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