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FIBROTIC DISEASE AND THE T_H1/T_H2 PARADIGM

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Abstract

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.

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,

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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.

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 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).

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

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 sub-types, 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.

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 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 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].

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.

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.

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

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 CCchemokine 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.

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.

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 myofibro-blasts. 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-bq (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 nearly all haematopoietic cell populations, which might support such a hypothesis[25]. In addition, IL-13-deficient mice are fertile and show no obvious developmental problems until an invading pathogen or persistent irritant induces damage to tissues and the

development of an immune response[33,36]. By contrast, TGF-β1-deficient mice are considerably impaired during embryonic development and at birth[75], which also supports an intrinsic role for TGF-β but more of a conditional requirement for IL-13 in tissue remodelling and fibrosis.

Chemokines regulate fibrogenesis

Chemokines are potent leukocyte chemoattractants that cooperate with pro-fibrotic cytokines (such as IL-13 and TGF-β) in the development of fibrosis, by recruiting macrophages and other effector cells to sites of tissue damage. Although numerous chemokine signalling pathways are probably involved in fibrogenesis, the CC-chemokine family has been shown to have an important regulatory role. In particular, CCL3 (also known as macrophage inflammatory protein 1α, MIP1α) and related CC-chemokines, such as CCL2 (also known as monocyte chemoattractant protein 1, MCP1), that are chemotactic for mononuclear phagocytes have been identified as essential pro-fibrotic mediators. Macrophages and epithelial cells are thought to be the main cellular sources of CCL3, and early studies using the bleomycin model of pulmonary fibrosis showed that CCL3-specific antibodies could significantly reduce the development of fibrosis[76,77]. Similar results were obtained when CCL2 was neutralized, indicating that several CC-chemokines are involved[78,79]. Subsequent studies with CC-chemokine receptor 1 (CCR1)- and CCR2-deficient mice produced similar results, confirming crucial roles for CCL3- and CCL2-mediated signalling pathways in fibrogenesis[80-84]. Interestingly, in several of these blocking studies, reduced fibrosis was associated with decreased IL-4 and IL-13 expression[81,85], showing a direct link between CC-chemokine activity and the production of pro-fibrotic cytokines. More recently, IL-13 was shown to be a potent inducer of several CC-chemokines — including CCL2, CCL3, CCL4 (also known as MIP1β), CCL6 (also known as C10), CCL11, CCL20 (also known as MIP3α) and CCL22 (also known as macrophage-derived chemokine) — indicating that a positive-feedback mechanism exists between IL-13 and the CC-chemokine family in general[41,86]. As observed using CCL2-specific and CCL3-specific antibody treatment, antibodies specific for CCL6 attenuated lung-remodelling responses in *Il-13*-transgenic mice[86], as well as in mice challenged with bleomycin[41], indicating that various CC-chemokines have non-redundant roles in the pathogenesis of fibrosis. These findings also show how IL-13 has a role in fibrosis that is independent of its direct effects on macrophages and fibroblasts[36,41]: that is, stimulating the production of CC-chemokines by epithelial cells.

Role of macrophages and fibroblasts

Numerous studies have indicated that macrophages and fibroblasts are the main effector cells involved in the pathogenesis of fibrosis. Although $CD4^+$ T cells are clearly important[2,36], their main contribution could be to control the activation and recruitment of macrophages and fibroblasts[8,12]. Macrophage activation was first described as a T_H1 cell–IFN-γ-mediated process; however, it is now clear that macrophages differentiate into at least two functionally distinct populations depending on whether they are exposed to T_H1 or T_H2 cytokines[87].T_H1 cytokines activate nitric-oxide synthase 2 (NOS2) expression in ‘classically activated’ macrophages, whereas the T_H2 cytokines IL-4 and IL-13 preferentially stimulate arginase-1 (ARG1) activity in ‘alternatively activated’ macrophages[87,88]. Interestingly, dendritic cells[89] and fibroblasts[90] show a similar pattern of NOS2 and ARG1 expression when stimulated with T_H1 and T_H2 cytokines, respectively. L-Arginine is the substrate for both enzymes; however, NOS2 generates L-hydroxyarginine, L-citrulline and nitric oxide (NO), whereas ARG1 promotes the production of urea and L-ornithine. L-Ornithine is the substrate for two additional enzymes, ornithine decarboxylase (ODC) and ornithine amino transferase (OAT), which generate polyamines and L-proline, respectively. Because polyamines are crucial for cell growth and proline is a substrate for collagen synthesis, both the ODC and OAT

pathways are thought to be important in repair processes[12]. Collagen synthesis is strictly dependent on the availability of L-proline; therefore, the preferential activation of ARG1 compared with NOS2 in macrophages and/or fibroblasts was recently proposed to be a possible explanation for the potent pro-fibrotic activity of IL-13 (REF. [36]) and the antifibrotic activity of IFN-γ[2,8].

The ARG1 and NOS2 pathways have been investigated in detail in the schistosomiasis model of fibrosis. In these studies, schistosome eggs were compared with *Mycobacterium avium* for their ability to stimulate ARG1 and NOS2 activity[12]. In addition, various cytokine-deficient mice were used to determine whether T_H1 and T_H2 cytokines differentially regulate NOS2 and ARG1 expression *in vivo*. In agreement with previous *in vitro* studies, schistosome eggs preferentially stimulated ARG1 expression, whereas *M. avium* triggered a dominant NOS2 response in the granulomatous tissues; these results are consistent with their ability to promote T_H2- and T_H1-polarized responses, respectively. Studies using both knockout mice[12] and DNA microarrays[10,91] confirmed that ARG1 expression is highly associated with IL-4, IL-13 and STAT6 activities. However, more importantly, these studies were the first to show a strong link between arginase activity and the development of fibrosis, because schistosome granulomas are associated with extensive fibrosis, whereas *Mycobacterium* granulomas are much less fibrogenic. To test the role of ARG1 directly, various approaches were used to inhibit the ARG1 and NOS2 pathways, including using NO and ODC inhibitors, as well as NOS2-deficient mice[8,12,92,93]. The combined results from these studies indicate that NOS2 slows the development of fibrosis, whereas sustained ARG1 activity accelerates the process. T_H2-cytokine-stimulated macrophages produce large quantities of proline through an ARG1-dependent mechanism[12], indicating that there is a paracrine role for alternatively activated macrophages in the stimulation of collagen production by fibroblasts. Because fibroblasts also express high levels of ARG1 when stimulated with T_H2 cytokines[90], direct activation of the ARG1 pathway in fibroblasts could be an additional mechanism to augment their collagen-producing potential. Regardless of the exact mechanisms involved, these findings provide strong support for pharmacologically targeting arginine metabolism in the treatment of fibrosis. They also provide a partial explanation for the anti- and pro-fibrotic activities of T_H1 and T_H2 cytokine responses, respectively (Fig. 3).

$CD4^+CD25^+$ REGULATORY T CELLS

(T_{Reg} cells). A specialized subset of $CD4^+$ T cells that can suppress other T-cell responses. These cells are characterized by expression of the interleukin-2 (IL-2) receptor β-chain (also known as CD25). In some cases, suppression has been associated with the secretion of IL-10, transforming growth factor-β or both.

Endogenous mechanisms that slow fibrosis

Although the ability to repair damaged tissues without scarring would be ideal, in most chronic inflammatory diseases, repair cannot be accomplished solely by the regeneration of parenchymal cells, even in tissues in which considerable regeneration is possible, such as the liver. Repair of damaged tissues must then occur by the replacement of non-regenerated parenchymal cells with connective tissue, which in time produces considerable fibrosis and scarring. Therapeutic strategies that limit fibrosis without adversely affecting the overall repair process would be an important technological advance. Studies of the chronic phase of wound-healing responses have revealed important endogenous regulatory mechanisms that slow the progression of fibrosis.

IL-10 and regulatory T cells

IL-10 was initially recognized for its ability to suppress the activation and function of IFN-γ-producing $CD4^+$ T_H1 cells. However, it is now described as a general immunosuppressive cytokine that downregulates chronic inflammatory responses through many mechanisms[94]. Consistent with its role as a suppressive mediator, IL-10 has shown efficacy in the treatment of fibrosis in numerous models. Mice treated with IL-10 developed less liver, lung and pancreatic fibrosis when challenged with CCl_4, bleomcyin and cerulein, respectively[95-98]. By contrast, IL-10-deficient mice are much more susceptible to the tissue-damaging activities of these compounds. IL-10 suppressed the synthesis of procollagen-I by human scar-tissue-derived fibroblasts[99], indicating that it might function directly to inhibit fibrosis[100]. IL-10 also reduced the severity of liver fibrosis in a subset of patients that were chronically infected with hepatitis C virus[101]. Nevertheless, despite its recent success in the clinic, the mechanism by which IL-10 confers protection in these disorders remains unclear. In the schistosomiasis model, IL-10 deficiency alone had little effect on the progression of hepatic fibrosis[102]. However, when *Il-10*$^{-/-}$ mice were crossed with IFN-γ- or IL-12-deficient animals, liver fibrosis developed at an increased rate after infection, indicating that IL-10 cooperates with T_H1 cytokines to suppress collagen deposition[3,17]. In the IL-10- and T_H1-response-deficient mice, there was a 10-fold increase in the number of IL-13-producing $CD4^+$ T_H2 cells, indicating a possible mechanism for their exaggerated fibrotic response[39]. In support of these findings, a recent study of human *S. mansoni* infection found that most cases of severe periportal fibrosis are associated with low concentrations of IL-10 and IFN-γ[22].

$_{CD}4^+{}_{CD}25^+$ REGULATORY T ($_TReg$) CELLS were identified as the main producers of IL-10 in mice infected with *S. mansoni*, and adoptive-transfer studies showed that the development of chronic liver pathology is largely controlled by this population of T cells[103]. T_{Reg} cells were also implicated in the regulation of bleomycin-induced fibrosis. In these studies, lung fibrosis was ameliorated by administration of a TGF-β1-expressing plasmid, which induced a population of suppressive TGF-β1- and IL-10-producing T cells. The TGF-β1 plasmid had no effect in IL-10-deficient mice, which indicated that its suppressive activity was mediated by the induced IL-10 response[74]. So, induction of tolerance through the production of TGF-β1 and/or IL-10 by T cells might provide an effective mechanism to slow the progression of fibrosis. Nevertheless, it is important to note that another study investigating the mechanisms of pulmonary fibrosis found that IL-10 had considerable pro-fibrotic activity when chronically overexpressed in the lung[104]. Moreover,TGF-β1 that is produced by macrophages can also directly activate the collagen-producing machinery in fibroblasts, which might further decrease the therapeutic utility of this suppressive pathway. Further study is needed before IL-10 or T_{Reg} cells can be advocated as a therapeutic strategy for progressive fibrotic disease (Fig. 4).

IL-13 decoy receptor

The soluble IL-13-Rα2–Fc fusion protein($_{SIL}$-$13_{RA}2$–$_{FC}$) is a highly effective inhibitor of IL-13, which not only blocks the initial production of collagen during an inflammatory response[36], but also ameliorates the progression of established fibrotic disease[39,105]. sIL-13Rα2 inhibits the action of IL-13 by blocking its interaction with IL-13R[35,49,106]. Given the success of this engineered receptor[35], recent studies have focused on elucidating the function of endogenous IL-13Rs. Consistent with its proposed activity as a decoy receptor[107], mice in which IL-13Rα2 was selectively deleted show enhanced responsiveness to IL-13 ($_{REF}$. [108]). When these IL-13Rα2-deficient mice were infected with *S. mansoni*, the development of IL-13-dependent liver fibrosis increased[109]. This increase in fibrosis was attributed to enhanced IL-13 activity, because sIL-13Rα2–Fc treatment reversed the pro-fibrotic phenotype of the knockout animals. Surprisingly, the amount of fibrosis increased despite there being no change in the number, size, or cellular composition of the granulomas at the acute stage post-infection. These findings indicate that IL-13Rα2 does not influence the early development of the

inflammatory response but, instead, targets the ECM-remodelling activity of IL-13. Nevertheless, subsequent studies in chronically infected mice revealed a crucial role for the decoy receptor in the downmodulation of the inflammatory response[110]. In these cases, although granuloma size was similar in acutely infected wild-type and IL-13Rα2-deficient mice, the knockout animals failed to suppress their inflammatory response in the chronic phase of infection, as would normally be expected. In fact, they showed a marked exacerbation in granulomatous inflammation at later time points. The IL-13Rα2-deficient mice also developed severe liver fibrosis, portal hypertension and collateral bypass vessels, and they succumbed to the infection at a highly accelerated rate. However, when the knockout mice were treated with sIL-13Rα2–Fc, granuloma down-modulation was restored[110]. So, the IL-13 decoy receptor was identified as an important life-sustaining inhibitor of T_H2-mediated inflammation and fibrosis.

To understand how IL-13Rα2 is regulated during an immune response, tissue and serum concentrations of the decoy receptor were examined in several cytokine-deficient mice after infection with *S. mansoni*[109]. Surprisingly, the soluble receptor was detected in the serum of uninfected animals, and its concentration was found to increase 5–6-fold shortly after the onset of egg laying[110]. Although expression subsequently decreased, the receptor remained at increased concentrations throughout the infection. Chronically infected humans showed comparable increases in serum IL-13Rα2 (ref. [110]). Interestingly, receptor expression was highly dependent on the presence of IL-10, IL-13, IL-4Rα and STAT6, and it was inhibited by the T_H1-associated cytokines IFN-γ and IL-12, thereby revealing a strong correlation with the T_H2-cytokine response[109]. Similar increases in IL-13Rα2 expression were also observed in transgenic mice that overexpress IL-13 (ref. [111]) and in IL-13-stimulated fibroblasts[112]. So, IL-13Rα2 production is induced by the cytokine that it ultimately inhibits, and it seems to function mainly as a negative-feedback mechanism for the T_H2 response.

Although IFN-γ can induce IL-13Rα2 expression under some circumstances[111,113], *in vivo* studies strongly support a negative regulatory role for T_H1 responses in IL-13Rα2 production[109,114]. In situations in which highly polarized T_H1 responses are generated, there is little need (or stimulus) for IL-13Rα2 because IL-13 expression levels are low[2]. In such a polarized response, IFN-γ is uniformly anti-fibrotic because the pro-fibrotic T_H2 response is completely ablated[2,115] (Fig. 4). However, when IFN-γ is produced concomitantly with T_H2 cytokines, marked exacerbations in T_H2-dependent pathology have been reported[3,116-119]. In this setting, moderate amounts of IFN-γ can markedly augment IL-13 effector function by reducing production of the IL-13 decoy receptor[114]. So, although the relative levels of IL-13 do not change, the concetnration of ‘free’ IL-13 can increase substantially during a mixed T_H1/T_H2 response. Consequently, more IL-13 is able to bind and activate the IL-13Rs linked to signalling pathways, which increases fibrosis[114] and possibly other pathologies, such as mucus and airway hyperreactivity, that are also regulated by IL-13 and IL-13Rα2 (refs [34, 120,121]).

sIL-13Rα2–Fc

A soluble protein that consists of the extracellular domain of the interleukin-13 receptor-α2 (IL-13Rα2) fused to a Gly-SerGly spacer and the sequence encoding the hinge–heavy-chain constant region 2 (CH2)–CH3 regions of human IgG1. The resulting protein is a specific inhibitor of IL-13. The inhibitor prevents IL-13 from binding its signalling receptors and has been used successfully to block progressive fibrotic disease.

Box 3

New experimental antifibrotic therapies

This list is not comprehensive but covers therapies that are relevant to the discussion in the main text.

Therapies at various stages of preclinical or clinical development

- Transforming growth factor-β (TGF-β)-pathway antagonists, such as the soluble type II TGF-β receptor–Fc fusion protein (sTGF-βRII–Fc), pirfenidone (an orally active, small-molecule inhibitor) and the hormone relaxin
- Interleukin-13 (IL-13) inhibitors, such as the soluble IL-13 receptor–Fc fusion protein (sIL-13Rα2–Fc), IL-13-specific antibodies and IL-4R-specific antibodies
- Interferon-γ (IFN-γ), which inhibits IL-13 and TGF-β production or directly inhibits collagen production
- IL-10, which directly suppresses collagen synthesis by fibroblasts
- IL-5 inhibitors, such as IL-5-specific monoclonal antibodies, which reduce the contribution of eosinophils to fibrosis
- Tumour-necrosis factor and platelet-derived growth factor receptor antagonists, which inhibit fibroblasts
- Agents that alter collagen synthesis, such as prolyl hydroxylase inhibitors and halofuginone
- Endothelin-1 receptor antagonists, such as bosentan, which partially blocks bleomycin-induced fibrosis
- Agents that antagonize the activity of fibroblast growth factors, such as suramin
- Eicosanoid and/or leukotriene inhibitors, which inhibit fibroblasts
- CD40 and/or CD40 ligand antagonists, which block collagen synthesis

New approaches indicated by mechanistic studies

- IFN-γ- or IL-12-based immune-deviation strategies for prevention
- Regulatory T cells, which inhibit production of fibrogenic cytokines
- Anti- or pro-apoptotic strategies, which prevent epithelial- and endothelial-cell death or induce death of hepatic stellate cells and myofibroblasts
- Reagents that target arginase (ARG1) activity
- Compounds that modulate the activity of matrix metalloproteinases and/or tissue inhibitors of matrix metalloproteinases
- Antagonists of chemokine receptors
- Compounds that block the adenosine pathway

Key targets of new antifibrotic treatments

Most treatment strategies for fibrotic disorders have been based on eliminating or suppressing the inflammatory agent. Other existing treatment options include general immunosuppressive drugs, such as corticosteroids, immunosuppressive or cytotoxic agents, and antifibrotics, either administered alone or in combination. These drugs reduce the inflammatory cascade by blocking the proliferation of cells and the recruitment of inflammatory leukocytes, or by killing recruited cells. So, these drugs do not necessarily target the underlying fibrotic response, which

typically involves both local cells (stromal cells) and recruited cells (inflammatory leukocytes) (Fig. 1). So far, few pharmacological therapies have been proven unequivocally to alter or reverse the inflammatory process that is associated with fibrosis, thereby emphasizing the need for new and improved treatments that target the fibrotic pathway more specifically (Box 3). As discussed earlier, investigations focusing on the immunological mechanisms that sustain the fibrotic process have identified TGF-β and IL-13 as important therapeutic targets. Several different approaches that block TGF-β function are at various stages of development, including the development of inhibitors that prevent activation of latent TGF-β iso-forms, block TGF-β-receptor engagement or suppress downstream signalling mechanisms[25,62]. Administration of IFN-γ and/or IL-12 might also be effective treatments because they can inhibit the production of TGF-β and IL-13 (REFS [2,122]). T_H1-associated cytokines also directly inhibit collagen synthesis by fibroblasts, further emphasizing their potential as antifibrotic therapies. However, it should be noted that T_H1 cytokines also might exacerbate fibrotic disease by downregulating the IL-13 decoy receptor[109,114]. Therefore, drugs that inhibit IL-13 and TGF-β directly might prove the safest and most effective approach to suppress the fibrotic machinery. Another particularly attractive strategy would be to exploit the natural suppressive mechanisms of the host, which include regulatory T cells[103], IL-10 (REF. [3]) and the IL-13 decoy receptor[110]. Indeed, treatment with IL-10 has already shown efficacy in patients with chronic hepatitis C virus infection for whom IFN-based therapy was not successful[123]. Interestingly, production of IL-13Rα2 is regulated by IL-10, indicating that IL-10 and the IL-13 decoy receptor might cooperate to slow disease progression[109]. Because IL-13 can stimulate TGF-β activity[70], IL-13 inhibitors such as sIL-13Rα2–Fc[35] could have the added benefit of simultaneously suppressing the profibrotic properties of IL-13 and TGF-β. They might also decrease the expression of CD40 ligand by fibroblasts, which was recently implicated in the pathway of IL-13-dependent fibrosis[124,125]. Although the mechanisms of fibrosis are probably similar in numerous fibrotic disorders, the extent to which IL-13, TGF-β and possibly other pro-fibrotic mediators participate and interact should be carefully assessed so that the most effective therapies can be developed for each disease. Fortunately, genomic technologies such as DNA and protein microarrays are rapidly enhancing our knowledge of disease pathways in general[9,126-128], and it is hoped that we will soon have the necessary information to develop targeted therapeutics for these devastating fibrotic disorders.

Conclusion and perspectives

Most research on the T_H1/T_H2 paradigm has focused on the ability of T_H1 and T_H2 cytokines to drive cell-mediated and humoral immune responses, respectively. Another commonly used model depicts T_H1 responses (IFN-γ) as pro-inflammatory and T_H2 responses (IL-13/TGF-β) as anti-inflammatory. Indeed, the T_H2 response is most often described as a mechanism that counter-regulates T_H1 responses[129]. Although these descriptions are accurate, they ignore the underlying role of the T_H2 response as an important regulator of ECM remodelling. T_H2 responses activate collagen deposition, whereas T_H1 responses inhibit this process, indicating opposing roles for T_H1 and T_H2 responses in tissue repair. In fact, this feature of the T_H1/T_H2 paradigm might explain the frequent bimodal nature of immune responses in general. The initial response to invading pathogens or chronic irritants is often a T_H1-dominant response. When the inciting agent is quickly eliminated or controlled, the T_H1 response subsides naturally and leaves little evidence of injury. However, when the stimulus persists, immune suppressive mechanisms need to be activated to prevent the immune response from causing excessive damage to host tissues. In these circumstances, regulatory T cells and T_H2 cytokines often collaborate to suppress the T_H1 response. Perhaps even more importantly, they strongly promote the mechanism of wound healing. So, the ability to preserve and repair host tissues might become the overriding survival mechanism when immune responses enter the chronic phase. Although the end-result of persistent healing is fibrotic tissue remodelling, in most cases, this is a viable compromise for the individual because it might be the only way to ensure long-

term survival. Given this new view, it may be more accurate to describe the T_H2 response as an adaptive tissue-healing mechanism, instead of as a simple counter-regulatory system for the T_H1 response[69]. Hence, the T_H2 response functions as a double-edged sword by facilitating wound healing while simultaneously contributing to fibrotic tissue remodelling. An important goal of future research will be to design therapies that exploit the beneficial aspects of the T_H2 response (wound healing), while preventing or suppressing the detrimental features (fibrosis).

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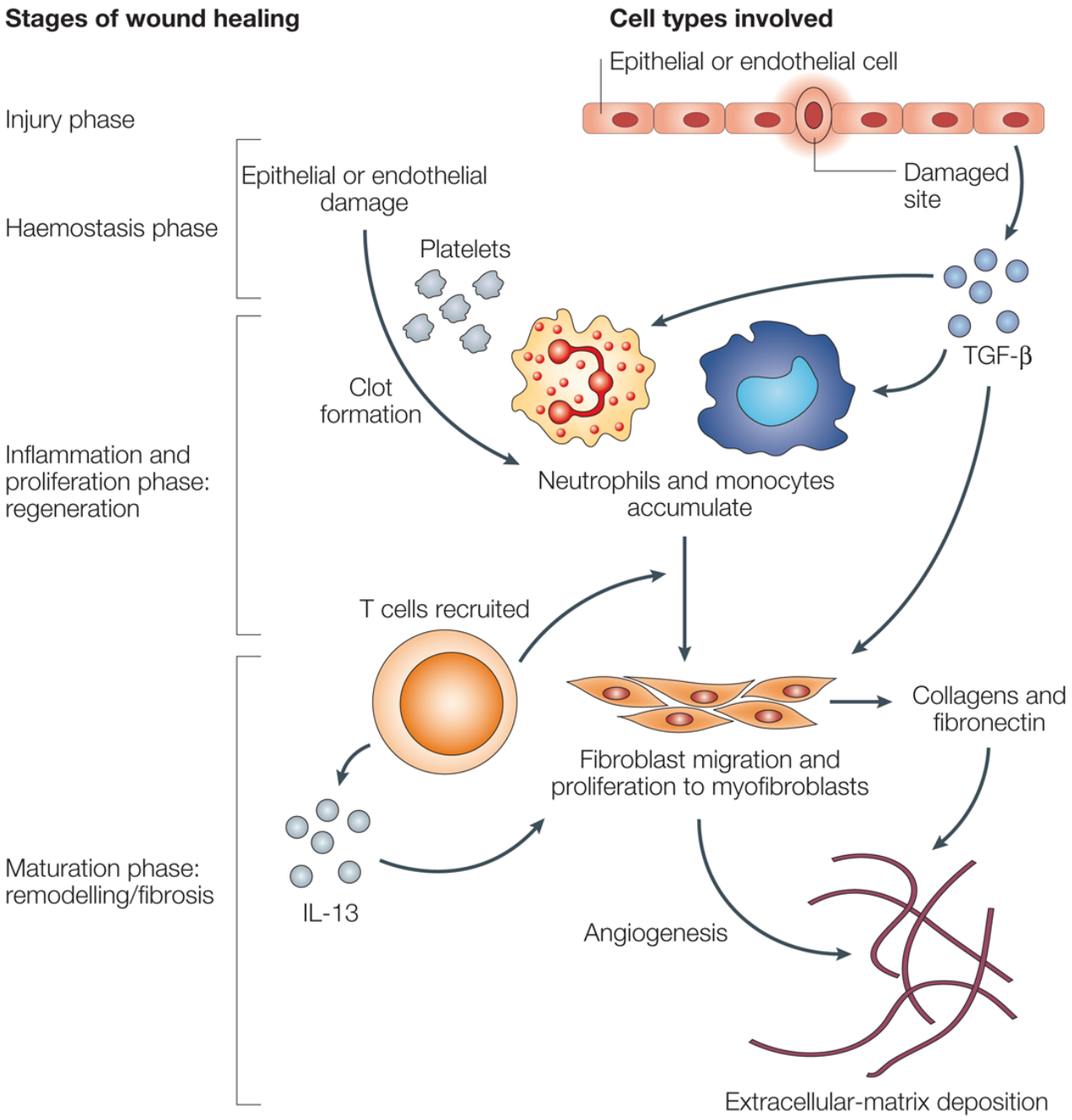

Figure 1. The pathogenesis of fibrotic disease

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.

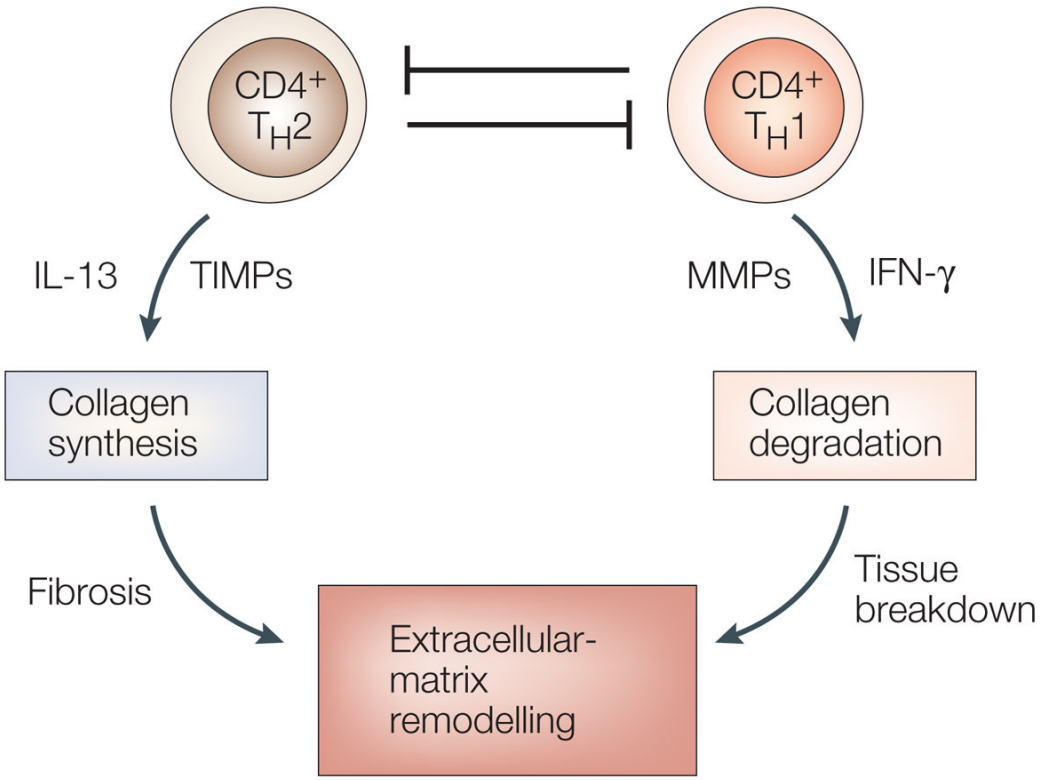

Figure 2. Opposing roles for T_H1 and T_H2 cytokines in fibrosis

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.

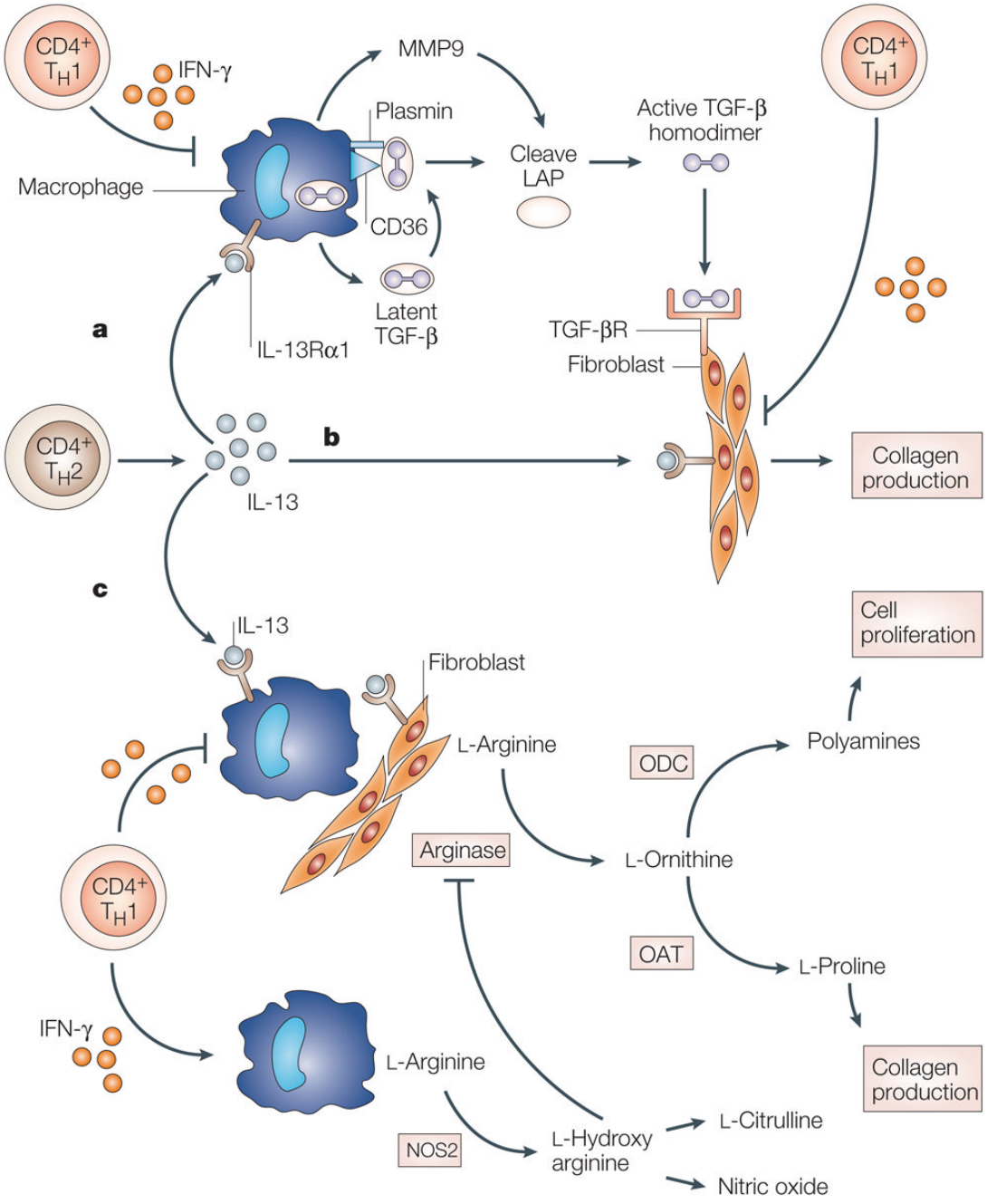

Figure 3. IL-13 and TGF-β might function independently or cooperatively to promote collagen deposition by fibroblasts

Interleukin-13 (IL-13) promotes collagen production by three distinct but possibly overlapping mechanisms. **a** | IL-13 is produced by activated CD4+ T helper 2 (T_H2) cells and stimulates the production of latent transforming growth factor-β (TGF-β) by macrophages. The activation of latent TGF-β is important for both its physiological and pathological actions on target cells, such as fibroblasts. Following the intracellular processing of prepro-TGF-β to its latent complex, the latent TGF-β is secreted and then anchored to the macrophage membrane through thrombospondin-1 bound to its receptor, CD36. Activation of TGF-β might be mediated by plasmin/serine protease- and/or matrix metalloproteinase 9 (MMP9)-dependent

mechanisms[70]. After the latency-associated peptide (LAP) is cleaved, TGF-β is free to bind and activate TGF-β receptors (TGF-βRs) expressed by fibroblasts. In this pathway, the fibrogenic effects of IL-13 are mediated largely by the downstream actions of TGF-β. **b** | Because fibroblasts express IL-13 receptors (IL-13Rs), IL-13 might also directly activate the collagen-producing machinery in fibroblasts[43-45]. **c** | IL-13 can also promote the alternative activation of macrophages and/or fibroblasts. By upregulating arginase activity in these cells, IL-13 increases L-ornithine, L-proline and polyamine concentrations, which promotes fibroblast proliferation, collagen production and ultimately, fibrosis[12]. Interferon-γ (IFN-γ) produced by T_H1 cells seems to antagonize all of these pathways. IFN-γ inhibits alternative macrophage activation, inducing nitric-oxide synthase 2 (NOS2) instead of arginase, and directly decreases collagen synthesis by fibroblasts. NOS2 activity promotes the production of L-hydroxyarginine, L-citrulline and nitric oxide. The intermediate by product L-hydroxyarginine also functions as a potent inhibitor of arginase activity, which can further antagonize the fibrotic pathway. OAT, ornithine amino transferase; ODC, ornithine decarboxylase.

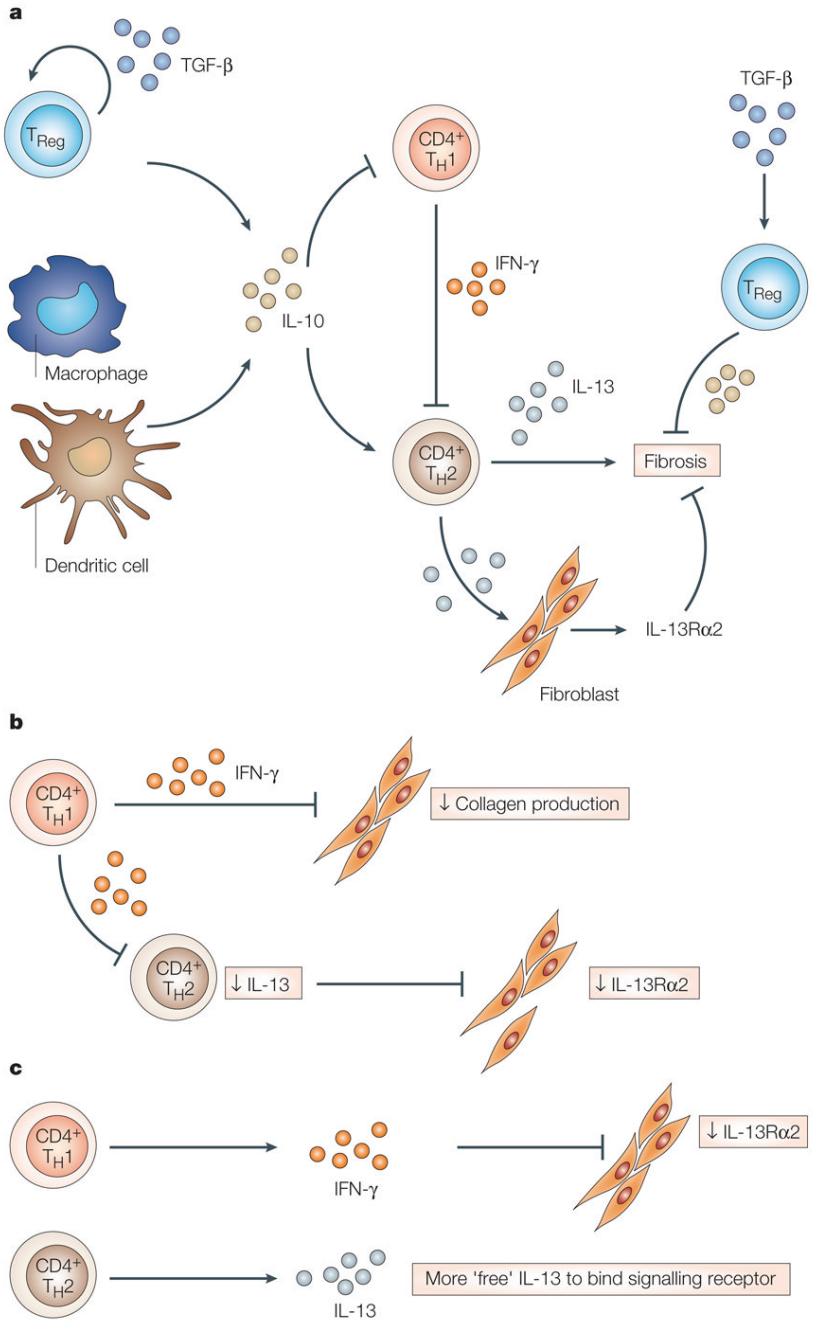

Figure 4. Regulatory T cells, IL-10 and IL-13Rα2 function as endogenous inhibitors of tissue fibrosis

a | Recent studies indicate that $CD4^+CD25^+$ regulatory T (T_{Reg}) cells and non-T cells that express interleukin-10 (IL-10) (possibly macrophages and/or dendritic cells) cooperate to generate polarized T helper 2 (T_H2)-cell responses[103]. Transforming growth factor-β1 (TGF-β1)-producing T_{Reg} cells might also promote the development of IL-10-producing T_{Reg} cells[74]. IL-10 can directly inhibit collagen synthesis by fibroblasts. IL-10 also inhibits interferon-γ (IFN-γ) production by T_H1 cells, while promoting the development of a polarized but controlled T_H2 response[2]. In this setting, IL-13 induces collagen deposition by fibroblasts; however, it also induces expression of its decoy receptor IL-13 receptor-α2 (IL-13Rα2), which

ultimately attenuates the response. Recent evidence indicates that fibroblasts are an important source of IL-13Rα2 (REFS [109-112]). Therefore, both IL-10 produced by T_{Reg} cells and the IL-13Rα2 might cooperate to control fibrosis during polarized T_H2 responses. **b** | When a highly polarized T_H1 response is generated, little IL-13 is produced. Consequently, fibrosis is minimal and decoy-receptor expression remains low[2]. **c** | When a mixed T_H1/T_H2 response develops, IFN-γ might decrease production of the IL-13 decoy receptor and upregulate IL-13 effector function[2,109,114]. In this case, although IL-13 concentrations might slightly decrease or remain unchanged, more IL-13 is free to bind the signalling receptor. This could explain the unusual tendency of mixed responses to trigger severe tissue pathology[116-119].