

The immunology of fibrosis: innate and adaptive responses

Georg Wick^{1,2}, Aleksandar Backovic^{1,2}, Evelyn Rabensteiner^{1,2}, Nadine Plank^{1,2}, Christian Schwentner³ and Roswitha Sgonc¹

¹Division of Experimental Pathophysiology and Immunology

²Laboratory of Autoimmunity, Biocenter, Innsbruck Medical University, Innsbruck, Austria

³Clinic for Urology, Innsbruck Medical University, Austria

Fibrosis is an important health problem, and its pathogenetic principles are still largely unknown. It can develop either spontaneously, or, more frequently, as a consequence of various underlying diseases. Irrespective of the primary cause, however, fibrotic tissue is always infiltrated by mononuclear immune cells. In most instances the reason for the attraction of these cells to fibrotic tissue and their proliferation remains to be determined; however their cytokine profile shows clear-cut proinflammatory and profibrotic characteristics. In this review, we discuss the innate and adaptive immune reactions associated with the development of fibrosis and the molecular basis of the profibrotic mechanisms taking place in systemic sclerosis (scleroderma), arteriosclerosis and peri-silicone mammary implant fibrosis.

Fibrosis: a disease with an immune-mediated etiology

Fibrosis, i.e. excessive extracellular matrix (ECM) formation, with proliferation and activation of myofibroblasts, is a major global health problem, but its etiology, pathogenesis, diagnosis and therapy have yet to be addressed in detail in either basic or clinical research settings. In principle, fibrosis can occur as a consequence of many different pathologic conditions (Figure 1), the most important of which arise either spontaneously, from tissue damage, inflammatory disease, and in response to foreign implants, or from tumors (see Table 1).

Although the pathologic conditions initiating and perpetuating these processes are rather diverse, from a biochemical and pathohistological view the end stage of the development of fibrosis seems to be very stereotypic. Thus, in all cases studied, the early stages of fibrotic conditions are characterized by immunologic-inflammatory hallmarks, *viz.* a perivascular infiltration by mononuclear cells and the subsequent imbalance of anti- and profibrotic cytokine profiles. In most of these instances, the original antigenic stimuli triggering the lymphoid infiltration have not been identified.

The emphasis of this review is placed on the general role of innate and adaptive immunity, and the respective cytokines involved in the development of fibrosis.

Modulation and amplification of fibrosis by innate immunity

In recent years, an important role of the innate immune system in the development of various fibrotic diseases has become apparent. Early events of fibrosis comprise inflammatory changes, including proliferation of ECM-producing cells and the occurrence of mononuclear inflammatory infiltrates. In this context, macrophages and mast cells have been implicated as important participants in inflammatory processes involving fibrosis. However, the initial events in the activation of host defence mechanisms are still largely unknown. Several mutually non-exclusive hypotheses have been proposed, including infection, reaction to altered self, overproduction of reactive oxygen species (ROS) and nitric oxide (NO), or mechanical stress, e.g. at sites of arterial bifurcation subjected to turbulent flow conditions [1–4] (Box 1, Figure 2). The link between these could be the Nalp3 (also called cryopyrin) inflammasome [5]. Recent studies have shown that various danger signals leading to fibrosis, e.g. the antibiotic bleomycin, silica dust, asbestos, and uric acid (the latter of which is produced upon cellular stress), depend on the activation of the Nalp3 inflammasome [6,7]. Activated macrophages regulate inflammatory ECM turnover through the release of chemokines, cytokines, ROS and growth factors, as well as ECM-degrading enzymes. One of the most prominent activators of mononuclear cells and fibroblasts are hyaluronan fragments that not only induce the expression of various cytokines (IL-1, IL-12, and TNF- α), chemokines (MIP-1A, MCP-1, IL-8) and inducible nitric oxide synthase (iNOS), but also trigger the expression and secretion of macrophage-derived matrix metalloproteinases (MMP) [8], i.e. enzymes essential for ECM cleavage. Macrophages are of essential importance in liver fibrosis, where they use MMP-13 to remodel fibrotic tissue. The CD11b-diphtheria toxin receptor (DTR)-transgenic mouse model that enables selective depletion of scar-associated macrophages, shows a 5-fold reduction in MMP-13 levels in response to chemically induced liver damage [9]. The chemokine MCP-1 and its major receptor, CCR2, are likely to play crucial roles in both renal and pulmonary fibrotic responses. CCR2^{-/-} mice display an impaired profibrotic signalling cascade in response to various stimuli, and seem, at least in part, to be protected from inflammation-induced fibrosis [10].

Another important component of cellular innate immunity are mast cells. These can play a role in fibrosis by their

Corresponding authors: Wick, G. (georg.wick@i-med.ac.at); Sgonc, R. (roswitha.sgonc@i-med.ac.at).

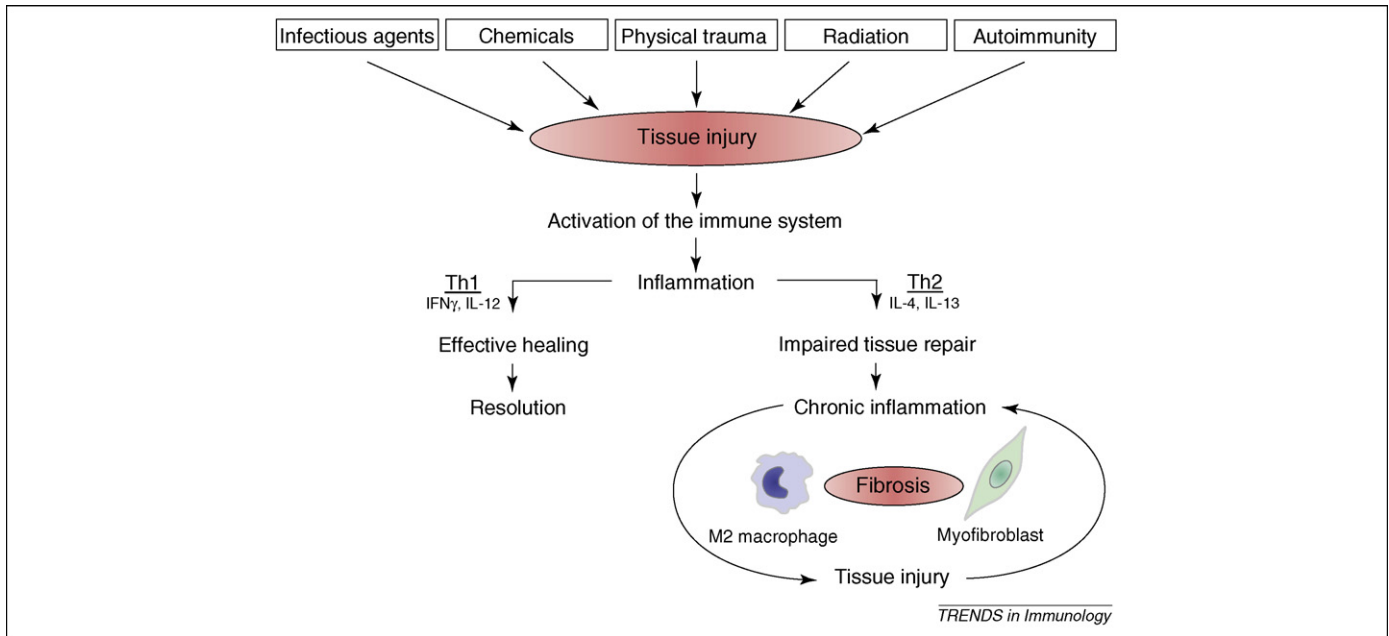


Figure 1. Pathogenesis of fibrosis. Tissue injuries, caused by infection, chemicals, mechanical stress or autoimmune reactions, activate the immune system and repair mechanisms. Effective healing is usually characterized by a dominant Th1 response, whereas a shift of the balance towards Th2 cells, alternatively-activated (M2) macrophages, and myofibroblasts leads to chronic inflammation that can ultimately result in fibrosis.

Table 1. Examples of principal conditions associated with fibromatous lesions.

Tissue damage	Inflammatory diseases	Foreign implants	Spontaneous	Tumors
Post-operative adhesions	Infections	Silicone mammary implants	Keloids	Stroma of parenchymatous tumors
Burns	Arteriosclerosis	Cardiac pacemakers	Dupuytren's contracture	Fibromas
Alcoholic and post-infectious liver fibrosis and cirrhosis	Connective tissue diseases e.g. scleroderma		Peyronie disease	Neurofibromatosis

Box 1. Arteriosclerosis

Arteriosclerosis, the leading cause of human mortality in developed countries, is characterized by thickening and hardening of the arterial wall that, together with other events, finally result in vascular clogging with catastrophic outcomes, such as stroke and myocardial infarction. Arteriosclerosis starts as an inflammatory-immunologic process in the innermost arterial layer, the intima, characterized by an accumulation of mononuclear cells and smooth muscle cells (SMCs) at arterial branching sites where endothelial cells are subjected to turbulent rather than laminar shear stress conditions [77,78]. We have shown that the antigenic trigger for the immune reaction in the intima is the expression of a stress protein (heat shock protein 60 – HSP60) by arterial endothelial cells when they are confronted with classical arteriosclerosis risk factors [4].

Pathophysiologic hallmarks of arteriosclerosis:

- Immigration of T cells into the intima precedes that of monocytes and SMCs [4].
- Classical arteriosclerosis risk factors lead to simultaneous expression of adhesion molecules and HSP60 by endothelial cells [79].
- Mononuclear cells and an elaborate network of dendritic cells are present at arterial branching sites already in healthy children (vascular associated lymphoid tissue – VALT) [80].
- Imbalance of pro-fibrotic vs anti-fibrotic cytokines produced by mononuclear cells lead to fibroblast proliferation and hyperproduction of ECM proteins [81,82].
- Monocytes promote transgression through the endothelium and the basement membrane by increased production of MMPs [83].
- Dysequilibrium between the production and enzymatic cleavage of collagenous ECM components by MMPs, and increased production of tissue inhibitors of metalloproteinases (e.g. TIMP-1) further contribute to arterial thickening and hardening [78].
- SMCs proliferate in the intima and produce ECM proteins upon being subjected to TGF- β 1 and PDGF [84].
- Intralesional T cells produce IFN γ that inhibits collagen production by SMCs but also promotes further T cell and NK-cell activation. Activated T cells secrete CD40L and IL-1, thus triggering macrophages to produce MMP-1, -8 and -13 [85].
- The imbalance between ECM – mainly collagen – production, deposition and cleavage by MMPs results in rupture of arteriosclerotic plaques with deleterious consequences [86,87].
- The distribution pattern of various ECM proteins in different areas of normal and arteriosclerotic arteries can be demonstrated clearly by immunohistology.

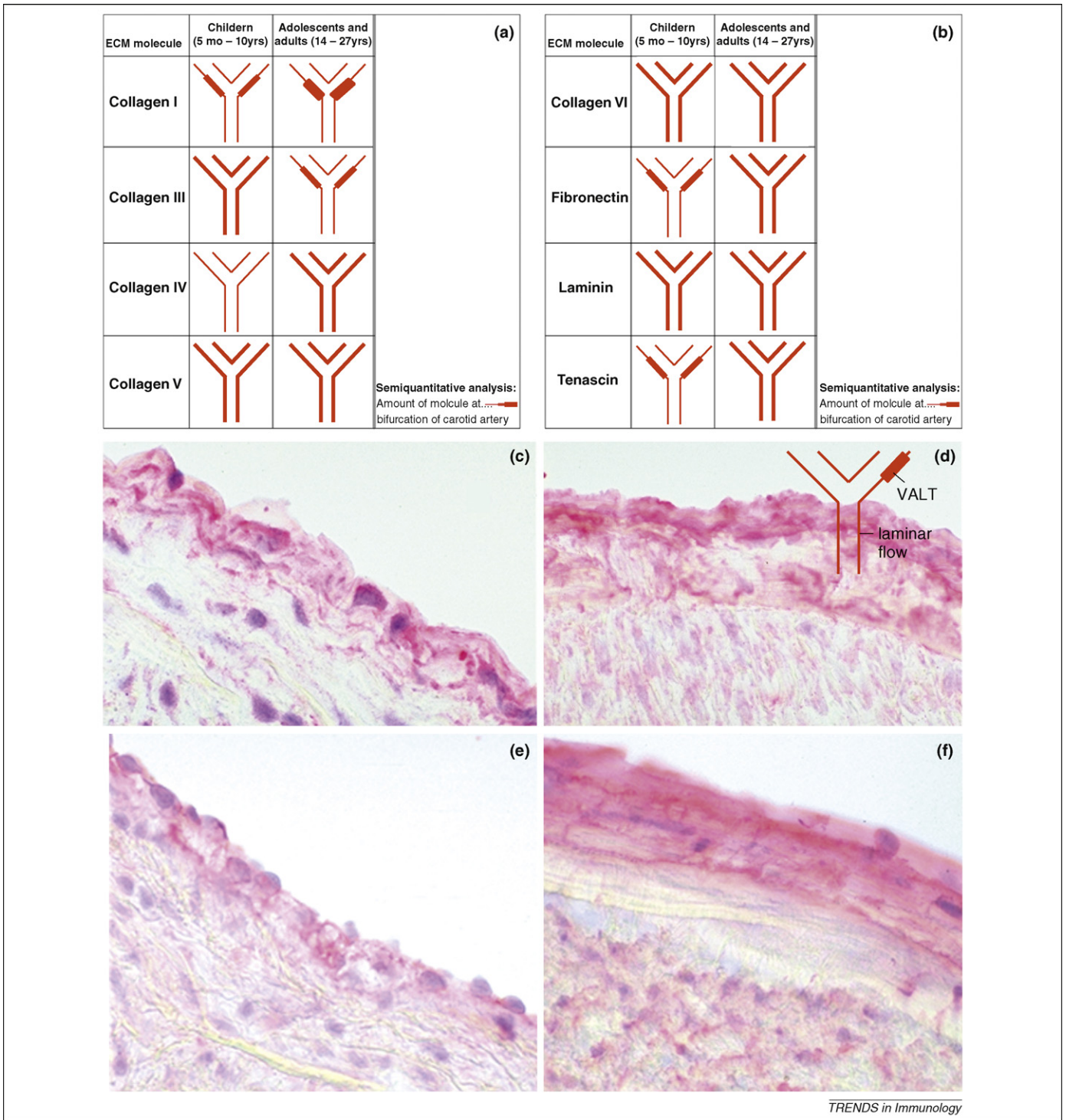


Figure 2. (a) Extracellular matrix (ECM) proteins in the arterial intima. Distribution of collagenous and non-collagenous ECM proteins in the arterial intima of arteries from clinically healthy children and adolescents at sites of laminar and turbulent flow stress, respectively. Thickness of lines schematically depicts the amounts of the respective proteins as assessed by semi-quantitative immunohistochemical analysis, VALT =Vascular Associated Lymphoid Tissue area. (c-f) Representative examples showing quantitative differences of intimal ECM deposition at sites of laminar (c, e) as compared to turbulent (d, f) blood flow conditions. Collagen type I (c, d) and laminin (e, f) were stained by the alkaline phosphatase/anti-alkaline phosphatase (APAAP) method (red). Counterstaining with haemalaun (blue). Original magnification x 400 (C. Schwentner, MD thesis, University of Innsbruck, Medical School, 2000).

secretion of tryptases, thereby contributing to connective tissue breakdown. As a consequence of activation of pro-collagenase and induction of a cascade of MMPs, the connective tissue becomes more penetrable for infiltrating leucocytes during inflammation. Moreover, mast cell-derived tryptase indirectly induces fibroblast proliferation by stimulating the synthesis of cyclooxygenase and pro-

taglandins. These effects are especially prominent in models of cardiovascular diseases [11,12].

Natural killer (NK) cells display predominantly anti-fibrotic properties in several fibrosis model systems. SCID-Beige mice, which lack T and B cells and have defective NK cell function, are more prone to chemically-induced liver and lung fibrosis even when T cell function is reconstituted.

Similarly, natural killer T (NKT) cell-deficient mice challenged with bleomycin show larger fibrotic lesions in the lungs, and have worse clinical outcomes, than wild-type controls [13–15]. Inhibition of liver fibrosis is mediated by NKT-derived interferon (IFN)- γ , which induces cell cycle arrest and apoptosis in hepatic stellate cells in a STAT1 transcription factor-dependant manner [13]. Hepatic stellate cells participate in upregulation of various ECM components, MMPs and tissue inhibitors of metalloproteinases (TIMPs), and their deletion therefore mitigates fibrosis. Furthermore, IFN- γ inhibits the production of the pro-fibrotic cytokine transforming growth factor beta (TGF- β 1) both *in vivo* and *in vitro* [16]. Low numbers of NK cells have been reported in lymphocyte subsets from post-burn hypertrophic scar tissues, lymphocytes isolated from bronchoalveolar lavage fluid from patients with lung fibrosis, and in peripheral lymphocytes of workers occupationally exposed to mineral fibres [17–19]. Patients with chronic and acute liver disease also show impaired NK cell function in the target organ [20].

Initiation and regulation of fibrosis by adaptive immunity

Cells and cytokines of the adaptive immune system play a prominent role in the initiation and progression of fibrosis. Traditionally, Th1 cells are thought to mediate tissue damage, whereas Th2 cells and their corresponding cytokines are linked with fibrogenesis. Th1 and Th2 cytokines play opposing roles in fibrosis: the Th2 cytokines IL-4 and IL-13 are strongly pro-fibrotic, whereas the Th1 cytokines IFN- γ and IL-12 suppress the development of tissue fibrosis [21]. In parasitic infections, a shift from Th1 cytokine (IFN- γ) to Th2 cytokine (IL-4, IL-10, IL-13) production can be observed, with a strong correlation between IL-13 levels and fibrosis in chronic helminth infection such as schistosomiasis [22]. Parasitic antigens exert an immunoregulatory effect by up-regulating IL-10 and down-regulating IL-12 [23]. In addition, the early parasite-induced inflammatory phase is characterized by increased expression of IL-1 β , TNF- α , TGF- β , collagens type I and III, and MMPs (MMP-2, -9), while in later stages, increased expression of TIMP-1, -2 and -3 contributes to fibrosis [24]. Several studies have shown high levels of Th2 cytokines in a variety of fibrotic diseases, including systemic sclerosis (SSc), idiopathic pulmonary fibrosis, and foreign body encapsulation, supporting the notion that fibrosis is mainly a consequence of a Th2 cytokine-dominated inflammatory response. Surprisingly, effector CD8⁺ T cells in patients with SSc produce abnormally high levels of IL-13 associated with increased dermal fibrosis [25,26]. Furthermore, adoptive transfer of Th2 polarized cells induces fibrosis in a murine model of granulomatous lung disease [27].

Since the more recent discovery of the IL-17 producing T cell subset (Th17 cells), IL-17 expression has not only been implicated in the pathogenesis of various autoimmune diseases, but also in some fibrotic disorders. T cells from SSc skin and lung have been shown to express increased IL-17 mRNA levels [28]. Analyzing the influence of IL-17 on fibroblast proliferation and collagen synthesis in monolayer cultures, Kurawawa *et al.* [28] demonstrated the

proliferation of normal and SSc fibroblasts, but no stimulation of collagen synthesis. From these results, it was concluded that IL-17 overproduction could play an important role in the pathogenesis of SSc. However, the demonstration of increased IL-17 expression in affected tissues and of elevated IL-17 serum levels is circumstantial, and does not prove unequivocally a pathogenic role for Th17 cells [28]. IL-17 is not a pure Th17 cell-derived cytokine, but is also made by a variety of other cells, including NK cells, macrophages, neutrophils, and $\gamma\delta$ T cells, the latter often being the major source [29–31]. Interestingly, IL-17-producing $\gamma\delta$ T cells even seem to have a protective function in bleomycin-induced lung injury. $\gamma\delta$ T cell receptor (TCR) knockout mice show increased interstitial pulmonary inflammation and collagen deposition, and delayed epithelial repair after bleomycin administration, whereas wild type (WT) animals display a controlled immune response, characterized by increased IL-17 production by infiltrating $\gamma\delta$ T cells [32]. Therefore, IL-17 seems to have both pathogenic and protective functions during inflammation, and although IL-17 is likely to be an important cytokine in the pathogenesis of inflammatory fibrotic conditions, the exact roles of Th17 cells and IL-17 in the development of fibrosis needs further clarification. Moreover, most of the evidence suggesting that Th17 cells are key mediators of autoimmune disorders have resulted from studies on experimental diseases in mice, while preliminary human studies have revealed significant differences between murine and human Th17 pathways, making it difficult to judge the role of this T cell population in the pathogenesis of human diseases at present [33,34].

An outstanding feature of the Th17 lineage is the low susceptibility to regulation by autologous regulatory T (Treg) cells (CD4⁺CD25^{high}Foxp3⁺) [35]. This sustains the hypothesis that Th17 cells might play an important role in maintaining inflammatory processes, i.e. “auto-immune-like phenomena”, as Treg cells are known to be crucial for the maintenance of peripheral immunologic self-tolerance, as well as for the homeostasis and regulation and immune responses to foreign antigens via suppression of effector cells. Depletion or decreased numbers of Treg cells correlates with allergy and other immunopathological processes, e.g. autoimmune diseases, such as type 1 diabetes and multiple sclerosis [36]. Treg cells also seem to play a role in ameliorating fibrosis, because effective anti-fibrotic therapies are associated with increased numbers of Treg cells [37]. In this context it is important to note that TGF- β 1, also produced by Treg cells, has both anti-inflammatory and pro-fibrotic effects. Which effect predominates seems to depend on the local microenvironment.

Myofibroblasts: the main culprits of fibrosis

Fibroblasts are key effector cells in fibrosis development, and it has recently been recognized that they form a very heterogeneous cell population. Not only do fibroblasts from diseased tissues differ in their cytokine patterns, and chemokine and ECM synthesis from their healthy counterparts [38], but they also seem to have a very different origin as well. They can be derived from local quiescent connective tissue fibroblasts by proliferation, but there is also ample evidence that at least some of them originate from

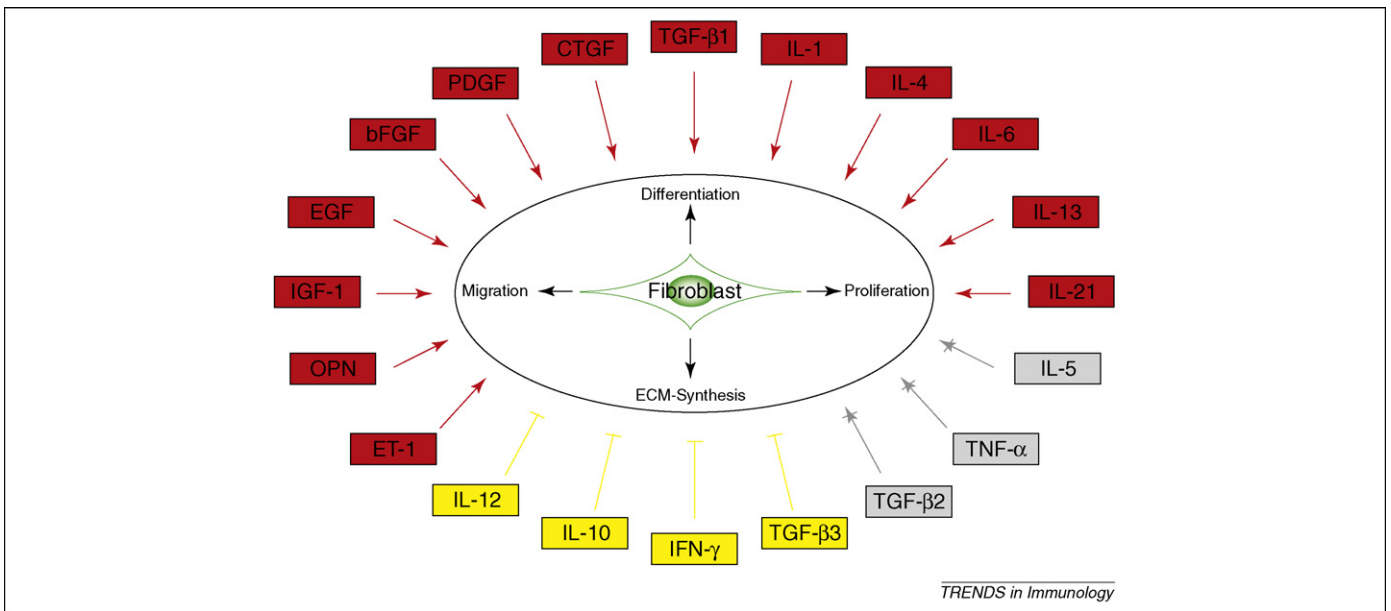


Figure 3. Effect of cytokines on fibroblasts that promote or inhibit fibrosis. Fibroblasts play a key role in the maintenance of extracellular matrix (ECM) homeostasis by continuously synthesizing and degrading ECM molecules. Many different cytokines, chemokines, and growth factors either promote (red arrows) or inhibit (flat yellow arrows) the migration, proliferation, and differentiation of fibroblasts, as well as ECM synthesis and degradation. TGF- β 1 and CTGF are the major pro-fibrotic mediators in various diseases, whereas the role of TGF- β 2, TNF- α and IL-5 on the development of fibrosis is less clear. They can act in either a pro- or anti-fibrotic manner (crossed grey arrows), depending on the experimental setting. Abbreviations: bFGF, basic fibroblast growth factor; CTGF, connective tissue growth factor; EGF, epidermal growth factor; ET-1, endothelin 1; IFN- γ , interferon gamma; IGF-1, insulin-like growth factor 1; OPN, osteopontin; PDGF, platelet-derived growth factor; TGF- β , transforming growth factor beta; TNF- α , tumor necrosis factor alpha.

myeloid precursors in the blood or bone marrow that then migrate to sites of injury [39,40]. Furthermore, reports of fibroblast trans-differentiation from hepatic stellate cells, skeletal muscle cells, cells of the neural crest and other cell types, have shed additional light on their heterogeneity [41,42].

Fibroblasts vary in lineage, origin, expression of cell surface markers (e.g. CD34, CD14, CD11b, CD80, CD86, MHC class II), as well as in their mechanisms of activation (once in an active state, they are designated as myofibroblasts). Fibroblasts also show a surprising diversity of properties. Myofibroblasts express α -smooth muscle cell actin (α -SMA), produce increased amounts of ECM proteins, such as collagen type I and fibronectin, proliferate and show contractile properties. Their usual activators are IL-6, and TGF- β 1, although they can also be activated by a variety of other cytokines, chemokines, growth factors, components of microbial cell walls, and members of the oxidative burst cascade [21].

Fibroblasts also receive stimuli from direct cross-talk with lymphocytes via the CD40-CD40 ligand (CD40L or CD154) pathway. CD40 ligation results in nuclear translocation of the transcription factor NF- κ B, and, subsequently, the synthesis of IL-6 and IL-8, hyaluronan, as well as the adhesion molecules ICAM-1 and VCAM-1. Interestingly, some human lung fibroblasts have been shown to express not only CD40, but also CD40L, and these are found at increased levels in fibroblasts from fibrotic tissue [43]. Thus, fibroblast-derived CD40L might play a role in perpetuating fibroblast activation once inflammatory cells have left the nascent area of fibrosis.

Pro- and anti-fibrotic growth factors and cytokines

As mentioned earlier, the pathophysiology of fibrosis is similar in many fibrotic disorders regardless of the under-

lying primary disease or affected tissue(s). Various stimuli released in the course of the underlying diseases cause the secretion of certain cytokines, chemokines, and growth factors by inflammatory cells and activated resident cells. These cytokines, chemokines, and growth factors perpetuate inflammation, cause further cell injury and induce fibrotic events, e.g. activation, differentiation and proliferation of fibroblasts, as well as increased production of collagen and other ECM proteins (Figure 3).

Among the various pro- and anti-fibrotic cytokines, TGF- β isoforms seem to play a key role in the development of fibrosis (Figure 3). TGF- β represents a large family of closely related proteins that share structure, use similar receptors and signaling pathways, and exert overlapping, but non-identical, biological functions. In mammals, three TGF- β isoforms have been identified, TGF- β 1, - β 2 and - β 3, with cellular actions ranging from anti-inflammatory to fibroblast chemo-attraction and regulation of ECM formation [44]. The effects of the various TGF- β isoforms are target cell-specific and context-dependent. A fibrogenic role of TGF- β 1 has been shown in many experimental models and fibrotic disorders, while TGF- β 3 has anti-fibrotic properties. Studies on the role of TGF- β 2 in the pathogenesis of fibrotic diseases are rare, and the results are contradictory. Findings from our own studies using the University of California at Davis-200 (UCD-200) chicken – the only spontaneous animal model showing all the hallmarks of human SSc, i.e. primary vascular alterations, inflammation, autoimmunity, and fibrosis of skin and internal organs [45] – revealed that TGF- β 2, in contrast to general belief, can act as a potent anti-fibrotic cytokine [46] (Box 2). It was shown *in vitro* by observing that chicken embryonic fibroblasts (CEF) from UCD-200 chickens, which express more of a profibrotic pro α 2(I)mRNA variant compared to the CEF from normal White Leghorn (NWL)

Box 2. Systemic sclerosis

Systemic sclerosis (SSc) also known as scleroderma is a systemic inflammatory-fibrotic autoimmune disease affecting the skin and viscera. The morphological hallmarks of the disease are microvascular lesions, perivascular infiltration by mononuclear cells, and increased deposition of ECM, mainly collagen [88]. Consistent with the notion that the immune system plays an important role in the initiation and perpetuation of the disease, SSc patients show immunologic abnormalities, such as phenotypic and functional alterations of T cell subsets, B cell activation, and autoantibodies directed against endothelial cell antigens (AECA) and various nuclear antigens (ANA) [89]. Although the pathogenesis of SSc is incompletely understood, it seems clear that the complex interplay between the immune system, vascular injury, and fibrotic processes initiates and sustains the characteristic SSc tissue damage.

Pathophysiologic hallmarks of SSc

- A primary pathogenic event in SSc is apoptosis of microvascular endothelial cells caused by AECA dependent cell-mediated cytotoxicity [90,91].
- Endothelial cells undergoing apoptosis release fibrogenic mediators that inhibit apoptosis of fibroblasts, and induce PI3K-dependent myofibroblast differentiation [92].
- Both the resistance of fibroblasts to apoptosis and the presence of myofibroblasts are strongly associated with SSc [93,94].
- A cellular link between the microvascular damage and fibrogenesis could be the pericytes that are α -SMA expressing mural cells of capillaries and venules that can synthesize ECM proteins and fibroblast-activating cytokines, and that have been suggested as precursor cells for myofibroblasts in SSc [95].
- There is also some evidence that microvascular endothelial cells themselves could transform to myofibroblasts after injury [96].
- TGF- β 1 and CTGF are thought to be the profibrotic key mediators in SSc [97,98], whereas TGF- β 2 might have an antifibrotic effect early during disease [99].

chickens, secreted significantly less TGF- β 2 than the CEF of healthy NWL controls. The addition of recombinant TGF- β 2 to the UCD-200-CEF culture reduced the expression of the profibrotic pro α 2(I)mRNA variant to the same levels as found in healthy NWL-CEF [46]. The constitutive overproduction of the profibrotic pro α 2(I) mRNA variant, and diminished TGF- β 2 synthesis found in untreated UCD-200-CEF, suggest that TGF- β 2 might be a key player during the onset of fibrosis. Our hypothesis that the TGF- β 2 level is a critical factor early in fibrogenesis is supported by a microarray study on gene expression patterns in scleroderma skin that showed reduced TGF- β 2 expression in SSc skin biopsies compared to healthy controls [47].

TGF- β 1 is seen as a central mediator of fibrosis in various conditions, but it has also been shown that TGF- β 1 alone is insufficient to cause a persistent fibrotic response. Only the action of TGF- β 1 in synergy with other pro-fibrotic cytokines, such as connective tissue growth factor (CTGF), results in chronic fibrosis [48].

CTGF is a member of the CCN family of matricellular proteins (CCN2) that all function as adaptor molecules connecting the cell surface to the ECM [49]. CTGF is induced by TGF- β 1 and some other pro-fibrotic mediators, and can be expressed by fibroblasts, endothelial cells, smooth muscle cells, chondrocytes, and various cancer cell lines. In fibroblasts, CTGF expression is induced selectively by TGF- β 1, via a unique TGF- β response element in the CTGF promoter. It is abundantly present in the lesions of various fibrotic disorders, e.g. systemic sclerosis, loca-

Box 3. Peri-silicone mammary implant (SMI) fibrosis

Fibrosis is a common consequence of silicone-containing active and passive implants. Excessive peri-SMI connective tissue capsule formation is a paradigmatic example of this.

Pathophysiologic hallmarks of peri-SMI capsule formation

- Ample presence of T cells, macrophages, dendritic cells and scarce B cells in SMI capsules [100–102].
- Serum proteins from many protein families adhere to silicone surfaces and mediate adhesion of fibroblasts, macrophages and ECM proteins [2].
- Macrophages are activated by cryptic or altered protein domains exposed on silicone surfaces or by silicone degradation products that are also ingested [103].
- Activated intracapsular lymphoid cells stimulate transdifferentiation of fibroblasts to myofibroblasts by CTGF, IL-1, and TNF α . Macrophages contribute to this process by the production of TGF β -1 and IL-6 [104].
- Soluble ICAM-1, procollagen III, circulating immune complexes and anti-polymer antibodies are elevated in sera of women with strong fibrotic reactions to silicone [105].
- A special ELISA-based test system (SILISA[®]) demonstrating the “signature” of serum protein adhesion to different silicone types can be used to determine the potential risk of fibrosis development in SMI carriers [106].
- Tight immunoregulatory mechanisms counteract early stages of fibrosis, intracapsular CD4⁺, CD25^{high}, Foxp3⁺ Tregs being the most important candidates (E. Rabensteiner, MD thesis, Innsbruck Medical University, 2009).

lized scleroderma, Dupuytren’s contracture, liver fibrosis, glomerulosclerosis, idiopathic pulmonary fibrosis, and cardiac fibrosis [50]. SSc fibroblasts constitutively express CTGF even in the absence of exogenously added TGF- β 1 [51]. This inappropriate overexpression seems to create a permissive environment for other fibrosis-inducing stimuli. Thus, Balb/c mice that are notoriously resistant to bleomycin-induced pulmonary fibrosis, are transformed into fibrosis-susceptible mice after overexpression of CTGF by adenoviral gene transfer [52]. CTGF mediates many, but not all, pro-fibrotic activities of TGF- β 1, i.e. fibroblast proliferation, ECM production, and the trans-differentiation to myofibroblasts [53,54], a sequence of events that is most impressively manifested during the development of fibrotic reactions to foreign material implants, such as silicone mammary implants (Box 3, Table 2). Whether a cell responds to TGF- β 1 and CTGF with proliferation or with differentiation and collagen synthesis depends on the presence or absence of other cytokines and growth factors. Stimulation of fibroblasts with TGF- β 1 or CTGF in the presence of insulin-like growth factor-2 (IGF-2) alone results in myofibroblast differentiation and increased collagen production, whereas activation in the presence of epidermal growth factor (EGF) or other mitogenic factors leads to proliferation of the cells [55]. Furthermore, it has been demonstrated that the induction of α -SMA by TGF- β 1 is adhesion- and integrin-dependent, supporting the notion that integrins are functional receptors for CTGF [56].

Another cytokine required for TGF- β 1-induced myofibroblast differentiation is osteopontin (OPN). This requirement was demonstrated in the response of cardiac fibroblasts from OPN-null mice to TGF- β 1 and confirmed by selectively downregulating OPN-mRNA in WT fibroblasts using siRNA. In contrast to WT fibroblasts, TGF- β 1-stimulated

Table 2. List of proteins found to be deposited on the surface of silicone mammary implants (SMI) after *in vitro* incubation with human serum^Y.

Mediators of host defence:	Extracellular matrix and associated proteins:	Intracellular proteins:
Immunoglobulin G, A, E	Fibronectin	Actin
Complement C2 precursor	Vitronectin	Heat Shock Protein 60
Complement C1s	Fibrinogen	PR02619
Complement C3 precursor	Collagen I, IV, VII	γ -glutamyltransferase
C reactive protein	Procollagen III	NADH dehydrogenase
Myeloid related protein 8, 14	Laminin	α -spectrin
Alpha1-microglobulin/bikunin precursor	Matrix metalloproteinase 2	Vimentin
HT018	Fibroblast growth factor 11	Myosin binding protein H
AMBP protein precursor		Glyceraldehyde-3 phosphate dehydrogenase TGF- β 1
Integrin beta-4	Transport proteins:	Flavin reductase
Integrin beta-2	Apolipoprotein A1	Creatin Kinase M-type
Integrin beta-2	Transthyretin	Gamma-glutamyl transferase
T cell receptor fragment	Albumin	
Monocyte chemotactic protein-2	Alpha2-macroglobulin precursor	
Eosinophil peroxidase	Haptoglobin 1	
Interleukin 10, 19	Hemoglobin	
Kininogen	Plasma-retinol binding protein	
Coagulation factor V	Vitamin D binding protein fragment	
Von Willebrand Factor	Apolipoprotein A3	
	Apolipoprotein E3	

^YModified from reference [2].

OPN^{-/-} fibroblasts showed no increase in the expression of α -SMA and CTGF, suggesting an early effect of OPN in the fibrotic response [57]. Further studies will be needed to elucidate the role of OPN in CTGF gene expression. OPN has been implicated in the pathogenesis of several fibrotic conditions, e.g. liver fibrosis, cardiac fibrosis, and idiopathic pulmonary fibrosis [58,59]. Studies in OPN-deficient mice also demonstrated the pro-fibrotic effects of OPN, i.e. OPN^{-/-} mice developed altered bleomycin-induced lung fibrosis characterized by a reduced collagen type I expression [60]. A recent study has shown that macrophage- and mast cell-derived platelet-derived growth factor (PDGF) induces the expression of OPN by fibroblasts, and that knockdown of OPN leads to reduced scarring in mouse skin wounds [61]. This suggests that inflammation-triggered OPN expression might contribute to the development of fibrosis. As mentioned above, the Th2 cytokine IL-13, which is considered to be anti-inflammatory, is also strongly pro-fibrotic. Its pro-fibrotic activities involve direct and indirect mechanisms. Thus, IL-13 can, directly and independently of TGF- β , stimulate collagen expression in fibroblasts [62], or induce TGF- β production by signaling through IL-13R α 2 [63].

In addition to the above-mentioned cytokines, IL-4, IL-6, IL-10, IL-21, basic fibroblast growth factor (bFGF), EGF, insulin like growth factor-1 (IGF-1), PDGF, oncostatin M, and endothelin 1 (ET-1) [64] all promote fibrosis, whereas IFN- γ , and IL-12 are anti-fibrotic. IL-5 [21] and TNF α [65] seem to be Janus-like, exerting either pro- or anti-fibrotic activities depending on the disease, animal model, and experimental settings, respectively.

Therapeutic implications

The best approach to treat fibrotic diseases would be the early identification and subsequent elimination or control of the initial triggering factor of a particular fibrotic disorder. However, the ultimate etiology of many fibrotic diseases is still unknown, and the triggers are diverse. A more feasible approach might be a cytokine-directed

therapy. For example, TGF- β 1 has been considered as a promising therapeutic target, but a placebo-controlled phase I/II trial with anti-TGF- β 1 antibody therapy in SSc patients not only showed a lack of efficacy, but also increased morbidity and mortality [66]. This is not surprising, since TGF- β 1 is a pleiotropic cytokine that, in addition to its role in fibrogenesis, has essential functions in normal tissue repair, angiogenesis, and immune regulation [67]. As mentioned above, TGF- β 2, in contrast to TGF- β 1, can act as a potent anti-fibrotic cytokine, at least in an animal model of SSc [46]. While our group has demonstrated a direct anti-fibrotic effect of TGF- β 2 *in vitro*, another group has shown an indirect anti-fibrotic effect via the induction of tolerogenic antigen presenting cell-dependent CD8⁺ Treg cells in a murine model of autoimmune pulmonary interstitial fibrosis [68]. Considering the fact that some studies have shown reduced TGF- β 2 expression in the skin of SSc patients [47], we deem it worthwhile to study the potential therapeutic effect of TGF- β 2 in the UCD-200 model, which shows striking similarities to human SSc. Another approach to avoid clinical problems associated with broadly targeting the TGF- β 1 axis could be to selectively target pro-fibrotic mediators downstream of TGF- β 1. Recently, it has been shown that targeting CTGF expression with siRNA prevents carbon tetrachloride-induced liver fibrosis in rats [69], and anti-CTGF neutralizing antibodies have been shown to ameliorate TGF- β -induced fibrosis in mice [70]. Blocking CTGF can inhibit both TGF- β 1 and CTGF-mediated ECM synthesis. An alternative approach to inhibiting TGF- β 1 signaling is via tyrosine kinase inhibitors. Reduction of ECM protein production *in vitro* reduced the number of myofibroblasts and reduced skin thickness in an experimental dermal fibrosis model [71], but blocking TGF- β 1 activity might lead to overt activation of the immune system and impaired wound healing [72]. A very recent publication described the promising anti-fibrotic effect of Imatinib in experimental animal models of fibrosis. Imatinib is a small molecule tyrosine kinase inhibitor

targeting both the TGF- β and the platelet-derived growth factor (PDGF)-signal transduction pathways. Importantly, this drug not only inhibits the development of fibrosis, but also stops its progression, and even leads to regression, of preexisting fibrosis [73].

Concluding remarks and open questions

The fibrotic consequences of various primary diseases, ranging from tissue damage resulting from inflammatory conditions, reactions against foreign material, to “spontaneous” fibrosis, remains a major unsolved diagnostic and therapeutic medical problem. In our experience, all fibrotic tissues derived from patients and experimental animals with diseases falling into one of these groups display signs of a chronic immune-mediated inflammation during the earliest periods of their development. This fact obviously raises questions about the specificity of lymphocytes occurring in fibrotic tissue, as well as about a possible imbalance of pro- and anti-fibrotic cytokines produced by components of the mononuclear cell infiltrate. One question that remains to be answered is how the Nalp3 inflammasome and the inflammasome-dependent cytokines IL-1 β and IL-33 are involved in the development of fibrotic disorders. Interestingly, IL-1 has been shown to be crucial in regulating the Th2 response in gastrointestinal nematode infection [74], and IL-33 to induce the expression of the Th2-associated cytokines IL-4 and IL-13 [75] – cytokines that, in turn, lead to the development of alternatively activated macrophages [76]. Since optimal biomarkers for the diagnosis and staging of fibrosis are not yet available, more detailed knowledge on the cellular and molecular basis of fibrogenic processes is urgently needed. This is also essential for the development of new evidence-based therapeutic concepts.

Acknowledgements

The authors would like to thank all former collaborators who contributed to our general research topic of “The Immunology of Fibrosis” the work of whom is cited throughout this review. Financial support on this topic over the years was provided by the Austrian Research Fund (FWF)-Grant P198810-B05 FWF-to Georg Wick; P18726-B05 to Roswitha Sgonc; the European Union, FP7 Large Scale Integrated Project Novel approaches to reconstitute normal immune function at old age (TOLERAGE HEALTH-F4-2008-202156) to Georg Wick; the Competence Center Medicine Tyrol (KMT to Georg Wick; and the Lore-and-Udo-Saldow-Donation to Georg Wick. The secretarial help of Claudia Ram, the preparation of artwork by Ilona Lengenfelder, and the editorial assistance of M. Kat Occhipinti-Bender are also gratefully acknowledged.

References

- Ajmal, N. *et al.* (2003) The effectiveness of sodium 2-mercaptoethane sulfonate (mesna) in reducing capsular formation around implants in a rabbit model. *Plast. Reconstr. Surg.* 112, 1455–1461
- Backovic, A. *et al.* (2007) Identification and dynamics of proteins adhering to the surface of medical silicones in vivo and in vitro. *J. Proteome Res.* 6, 376–381
- Demedts, M. *et al.* (2005) High-dose acetylcysteine in idiopathic pulmonary fibrosis. *N. Engl. J. Med.* 353, 2229–2242
- Wick, G. *et al.* (2004) Autoimmune and inflammatory mechanisms in atherosclerosis. *Annu. Rev. Immunol.* 22, 361–403
- Martinon, F. *et al.* (2009) The inflammasomes: Guardians of the body. *Annu. Rev. Immunol.* 27, 229–265
- Cassel, S.L. *et al.* (2008) The Nalp3 inflammasome is essential for the development of silicosis. *Proc. Natl. Acad. Sci. U. S. A.* 105, 9035–9040
- Gasse, P. *et al.* (2009) Uric acid is a danger signal activating NALP3 inflammasome in lung injury inflammation and fibrosis. *Am. J. Respir. Crit. Care Med.* 179, 903–913
- Tedgui, A. and Mallat, Z. (2006) Cytokines in atherosclerosis: Pathogenic and regulatory pathways. *Physiol. Rev.* 86, 515–581
- Fallowfield, J.A. *et al.* (2007) Scar-associated macrophages are a major source of hepatic matrix metalloproteinase-13 and facilitate the resolution of murine hepatic fibrosis. *J. Immunol.* 178, 5288–5295
- Gharaee-Kermani, M. *et al.* (2003) CC-chemokine receptor 2 required for bleomycin-induced pulmonary fibrosis. *Cytokine* 24, 266–276
- Rao, K.N. and Brown, M.A. (2008) Mast cells: multifaceted immune cells with diverse roles in health and disease. *Ann. N. Y. Acad. Sci.* 1143, 83–104
- Levi-Schaffer, F. and Piliponsky, A.M. (2003) Tryptase, a novel link between allergic inflammation and fibrosis. *Trends Immunol.* 24, 158–161
- Gao, B. *et al.* (2007) Activation of natural killer cells inhibits liver fibrosis: a novel strategy to treat liver fibrosis. *Expert. Rev. Gastroenterol. Hepatol.* 1, 173–180
- Kim, J.H. *et al.* (2005) Natural killer T (NKT) cells attenuate bleomycin-induced pulmonary fibrosis by producing interferon-gamma. *Am. J. Pathol.* 167, 1231–1241
- Melhem, A. *et al.* (2006) Anti-fibrotic activity of NK cells in experimental liver injury through killing of activated HSC. *J. Hepatol.* 45, 60–71
- Muhanna, N. *et al.* (2008) Activation of hepatic stellate cells after phagocytosis of lymphocytes: A novel pathway of fibrogenesis. *Hepatology* 48, 963–977
- Castagnoli, C. *et al.* (1997) Characterization of T-cell subsets infiltrating post-burn hypertrophic scar tissues. *Burns* 23, 565–572
- Gruber, R. *et al.* (1996) Determination of $\gamma\delta$ and other T-lymphocyte subsets in bronchoalveolar lavage fluid and peripheral blood from patients with sarcoidosis and idiopathic fibrosis of the lung. *Apmis* 104, 199–205
- Tulinska, J. *et al.* (2004) Immunomodulatory effects of mineral fibres in occupationally exposed workers. *Mutat. Res.* 553, 111–124
- Notas, G. *et al.* (2009) NK and NKT cells in liver injury and fibrosis. *Clin. Immunol.* 130, 16–26
- Wynn, T.A. (2008) Cellular and molecular mechanisms of fibrosis. *J. Pathol.* 214, 199–210
- Caldas, I.R. *et al.* (2008) Human schistosomiasis mansoni: Immune responses during acute and chronic phases of the infection. *Acta Trop.* 108, 109–117
- Zacccone, P. *et al.* (2003) Schistosoma mansoni antigens modulate the activity of the innate immune response and prevent onset of type 1 diabetes. *Eur. J. Immunol.* 33, 1439–1449
- Prakobwong, S. *et al.* (2009) Time profiles of the expression of metalloproteinases, tissue inhibitors of metalloproteinases, cytokines and collagens in hamsters infected with *Opisthorchis viverrini* with special reference to peribiliary fibrosis and liver injury. *Int. J. Parasitol.* 39, 825–835
- Rottoli, P. *et al.* (2005) Cytokine profile and proteome analysis in bronchoalveolar lavage of patients with sarcoidosis, pulmonary fibrosis associated with systemic sclerosis and idiopathic pulmonary fibrosis. *Proteomics* 5, 1423–1430
- Fuschiotti, P. *et al.* (2009) Effector CD8+ T cells in systemic sclerosis patients produce abnormally high levels of interleukin-13 associated with increased skin fibrosis. *Arthritis Rheum.* 60, 1119–1128
- Wangoo, A. *et al.* (2001) Contribution of Th1 and Th2 cells to protection and pathology in experimental models of granulomatous lung disease. *J. Immunol.* 166, 3432–3439
- Kurasawa, K. *et al.* (2000) Increased interleukin-17 production in patients with systemic sclerosis. *Arthritis. Rheum.* 43, 2455–2463
- Ferretti, S. *et al.* (2003) IL-17, produced by lymphocytes and neutrophils, is necessary for lipopolysaccharide-induced airway neutrophilia: IL-15 as a possible trigger. *J. Immunol.* 170, 2106–2112
- Gu, Y. *et al.* (2008) Interleukin 10 suppresses Th17 cytokines secreted by macrophages and T cells. *Eur. J. Immunol.* 38, 1807–1813
- Roark, C.L. *et al.* (2008) gammadelta T cell: an important source of IL-17. *Curr. Opin. Immunol.* 20, 353–357
- Braun, R.K. *et al.* (2008) IL-17 producing $\gamma\delta$ T cells are required for a controlled inflammatory response after bleomycin-induced lung injury. *Inflammation* 31, 167–179
- Wilson, N.J. *et al.* (2007) Development, cytokine profile and function of human interleukin 17-producing helper T cells. *Nat. Immunol.* 8, 950–957

- 34 Yang, L. *et al.* (2008) IL-21 and TGF- β are required for differentiation of human T(H)17 cells. *Nature* 454, 350–355
- 35 Annunziato, F. *et al.* (2007) Phenotypic and functional features of human Th17 cells. *J. Exp. Med.* 204, 1849–1861
- 36 Fehervari, Z. and Sakaguchi, S. (2004) CD4(+) Tregs and immune control. *J. Clin. Invest.* 114, 1209–1217
- 37 Horani, A. *et al.* (2007) Beneficial effect of glatiramer acetate (Copaxone) on immune modulation of experimental hepatic fibrosis. *Am. J. Physiol. Gastrointest. Liver Physiol.* 292, G628–G638
- 38 Brouty-Boye, D. *et al.* (2000) Chemokines and CD40 expression in human fibroblasts. *Eur. J. Immunol.* 30, 914–919
- 39 Isom, C. *et al.* (2007) Breast implant capsules are partially composed of bone marrow-derived cells. *Ann. Plast. Surg.* 58, 377–380
- 40 Pilling, D. *et al.* (2003) Inhibition of fibrocyte differentiation by serum amyloid P. *J. Immunol.* 171, 5537–5546
- 41 Brack, A.S. *et al.* (2007) Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. *Science* 317, 807–810
- 42 Hinz, B. (2007) Formation and function of the myofibroblast during tissue repair. *J. Invest. Dermatol.* 127, 526–537
- 43 Kaufman, J. *et al.* (2004) Expression of CD154 (CD40 ligand) by human lung fibroblasts: Differential regulation by IFN- γ and IL-13, and implications for fibrosis. *J. Immunol.* 172, 1862–1871
- 44 Denton, C.P. and Abraham, D.J. (2001) Transforming growth factor-beta and connective tissue growth factor: key cytokines in scleroderma pathogenesis. *Curr. Opin. Rheumatol.* 13, 505–511
- 45 Wick, G. *et al.* (2006) Avian models with spontaneous autoimmune diseases. *Adv. Immunol.* 92, 71–117
- 46 Prelog, M. *et al.* (2005) Diminished transforming growth factor beta2 production leads to increased expression of a profibrotic procollagen alpha2 type I messenger RNA variant in embryonic fibroblasts of UCD-200 chickens, a model for systemic sclerosis. *Arthritis Rheum.* 52, 1804–1811
- 47 Whitfield, M.L. *et al.* (2003) Systemic and cell type-specific gene expression patterns in scleroderma skin. *Proc. Natl. Acad. Sci. U. S. A.* 100, 12319–12324
- 48 Mori, T. *et al.* (1999) Role and interaction of connective tissue growth factor with transforming growth factor-beta in persistent fibrosis: A mouse fibrosis model. *J. Cell Physiol.* 181, 153–159
- 49 Leask, A. and Abraham, D.J. (2006) All in the CCN family: essential extracellular signaling modulators emerge from the bunker. *J. Cell Sci.* 119, 4803–4810
- 50 Shi-Wen, X. *et al.* (2008) Regulation and function of connective tissue growth factor/CCN2 in tissue repair, scarring and fibrosis. *Cytokine Growth Factor Rev.* 19, 133–144
- 51 Holmes, A. *et al.* (2001) CTGF and SMADs, maintenance of scleroderma phenotype is independent of SMAD signaling. *J. Biol. Chem.* 276, 10594–10601
- 52 Bonniaud, P. *et al.* (2004) Connective tissue growth factor is crucial to inducing a profibrotic environment in “fibrosis-resistant” Balb/c mouse lungs. *Am. J. Respir. Cell Mol. Biol.* 31, 510–516
- 53 Grotendorst, G.R. and Duncan, M.R. (2005) Individual domains of connective tissue growth factor regulate fibroblast proliferation and myofibroblast differentiation. *FASEB J.* 19, 729–738
- 54 Xu, S.W. *et al.* (2006) CCN2 is necessary for adhesive responses to transforming growth factor-beta 1 in embryonic fibroblasts. *J. Biol. Chem.* 281, 10715–10726
- 55 Grotendorst, G.R. *et al.* (2004) Combinatorial signaling pathways determine fibroblast proliferation and myofibroblast differentiation. *FASEB J.* 18, 469–479
- 56 Thannickal, V.J. *et al.* (2003) Myofibroblast differentiation by transforming growth factor-beta 1 is dependent on cell adhesion and integrin signaling via focal adhesion kinase. *J. Biol. Chem.* 278, 12384–12389
- 57 Lenga, Y. *et al.* (2008) Osteopontin expression is required for myofibroblast differentiation. *Circ. Res.* 102, 319–327
- 58 Matsui, Y. *et al.* (2004) Role of osteopontin in cardiac fibrosis and remodeling in angiotensin II - Induced cardiac hypertrophy. *Hypertension* 43, 1195–1201
- 59 Pardo, A. *et al.* (2005) Up-regulation and profibrotic role of osteopontin in human idiopathic pulmonary fibrosis. *PLoS Med.* 2, e251
- 60 Berman, J.S. *et al.* (2004) Altered bleomycin-induced lung fibrosis in osteopontin-deficient mice. *Am. J. Physiol. Lung Cell Mol. Physiol.* 286, L1311–L1318
- 61 Mori, R. *et al.* (2008) Molecular mechanisms linking wound inflammation and fibrosis: knockdown of osteopontin leads to rapid repair and reduced scarring. *J. Exp. Med.* 205, 43–51
- 62 Jinnin, M. *et al.* (2004) Interleukin-13 stimulates the transcription of the human alpha 2(I) collagen gene in human dermal fibroblasts. *J. Biol. Chem.* 279, 41783–41791
- 63 Fichtner-Feigl, S. *et al.* (2006) IL-13 signaling through the IL-13 alpha 2 receptor is involved in induction of TGF-beta 1 production and fibrosis. *Nat. Med.* 12, 99–106
- 64 Sticherling, M. (2006) The role of endothelin in connective tissue diseases. *Rheumatology (Oxford)* 45 (Suppl. 3), iii8–10
- 65 Distler, J.H.W. *et al.* (2008) The controversial role of tumor necrosis factor alpha in fibrotic diseases. *Arthritis. Rheum.* 58, 2228–2235
- 66 Denton, C.P. *et al.* (2007) Recombinant human anti-transforming growth factor beta 1 antibody therapy in systemic sclerosis - A multicenter, randomized, placebo-controlled phase I/II trial of CAT-192. *Arthritis. Rheum.* 56, 323–333
- 67 Blobe, G.C. *et al.* (2000) Mechanisms of disease: Role of transforming growth factor beta in human disease. *N. Engl. J. Med.* 342, 1350–1358
- 68 Zhang-Hoover, J. and Stein-Streilein, J. (2004) Tolerogenic APC generate CD8+ T regulatory cells that modulate pulmonary interstitial fibrosis. *J. Immunol.* 172, 178–185
- 69 Li, G.M. *et al.* (2006) Inhibition of connective tissue growth factor by siRNA prevents liver fibrosis in rats. *J. Gene Med.* 8, 889–900
- 70 Ikawa, Y. *et al.* (2008) Neutralizing monoclonal antibody to human connective tissue growth factor ameliorates transforming growth factor-beta-induced mouse fibrosis. *J. Cell. Physiol.* 216, 680–687
- 71 Akhmetshina, A. *et al.* (2008) Dual inhibition of c-abl and PDGF receptor signaling by dasatinib and nilotinib for the treatment of dermal fibrosis. *FASEB J.* 22, 2214–2222
- 72 Varga, J. and Pasche, B. (2009) Transforming growth factor beta as a therapeutic target in systemic sclerosis. *Nat. Rev. Rheumatol.* 5, 200–206
- 73 Akhmetshina, A. *et al.* (2009) Treatment with Imatinib prevents fibrosis in different preclinical models of systemic sclerosis and induces regression of established fibrosis. *Arthritis Rheumatism* 60, 219–224
- 74 Helmbly, H. and Grecis, R.K. (2004) Interleukin-1 plays a major role in the development of Th2-mediated immunity. *Eur. J. Immunol.* 34, 3674–3681
- 75 Schmitz, J. *et al.* (2005) IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 23, 479–490
- 76 Martinez, F.O. *et al.* (2009) Alternative Activation of Macrophages: An Immunologic Functional Perspective. *Annu. Rev. Immunol.* 27, 451–483
- 77 Hansson, G.K. (2009) Atherosclerosis - An immune disease - The Anitschkov lecture 2007. *Atherosclerosis* 202, 2–10
- 78 Libby, P. (2002) Inflammation in atherosclerosis. *Nature* 420, 868–874
- 79 Amberger, A. *et al.* (1997) Co-expression of ICAM-1, VCAM-1, ELAM-1 and Hsp60 in human arterial and venous endothelial cells in response to cytokines and oxidized low-density lipoproteins. *Cell Stress Chaperones* 2, 94–103
- 80 Millonig, G. *et al.* (2001) The vascular-associated lymphoid tissue: a new site of local immunity. *Curr. Opin. Lipidol.* 12, 547–553
- 81 Grenier, S. *et al.* (2009) Smooth muscle α -actin and calponin expression and, extracellular matrix production of human coronary artery smooth muscle cells in 3D scaffolds. *Tissue Eng. Part A.* 15, 3001–3011
- 82 Speer, M.Y. *et al.* (2009) Smooth muscle cells give rise to osteochondrogenic precursors and chondrocytes in calcifying arteries. *Circ. Res.* 104, 733–765
- 83 Amorino, G.P. and Hoover, R.L. (1998) Interactions of monocytic cells with human endothelial cells stimulate monocytic metalloproteinase production. *Am. J. Pathol.* 152, 199–207
- 84 Mitchell, R.N. and Libby, P. (2007) Vascular remodeling in transplant vasculopathy. *Circ. Res.* 100, 967–978
- 85 Libby, P. (2008) The molecular mechanisms of the thrombotic complications of atherosclerosis. *J. Intern. Med.* 263, 517–527
- 86 Schneider, F. *et al.* (2008) Matrix-metalloproteinase-14 deficiency in bone-marrow-derived cells promotes collagen accumulation in mouse atherosclerotic plaques. *Circulation* 117, 931–939

- 87 Sukhova, G.K. *et al.* (1999) Evidence for increased collagenolysis by interstitial collagenases-1 and-3 in vulnerable human atheromatous plaques. *Circulation* 99, 2503–2509
- 88 Gabrielli, A. *et al.* (2009) Mechanisms of disease: Scleroderma. *N. Engl. J. Med.* 360, 1989–2003
- 89 Chizzolini, C. (2007) Update on pathophysiology of scleroderma with special reference to immunoinflammatory events. *Ann. Med.* 39, 42–53
- 90 Sgonc, R. *et al.* (1996) Endothelial cell apoptosis is a primary pathogenetic event underlying skin lesions in avian and human scleroderma. *J. Clin. Invest.* 98, 785–792
- 91 Sgonc, R. *et al.* (2000) Endothelial cell apoptosis in systemic sclerosis is induced by antibody-dependent cell-mediated cytotoxicity via CD95. *Arthritis Rheum.* 43, 2550–2562
- 92 Laplante, P. *et al.* (2005) Novel fibrogenic pathways are activated in response to endothelial apoptosis: Implications in the pathophysiology of systemic sclerosis. *J. Immunol.* 174, 5740–5749
- 93 Abraham, D.J. *et al.* (2007) New developments in fibroblast and myofibroblast biology: implications for fibrosis and scleroderma. *Curr. Rheumatol. Rep.* 9, 136–143
- 94 Santiago, B. *et al.* (2001) Decreased susceptibility to Fas-induced apoptosis of systemic sclerosis dermal fibroblasts. *Arthritis Rheum.* 44, 1667–1676
- 95 Rajkumar, V.S. *et al.* (2005) Shared expression of phenotypic markers in systemic sclerosis indicates a convergence of pericytes and fibroblasts to a myofibroblast lineage in fibrosis. *Arthritis Res. Ther.* 7, R1113–R1123
- 96 Karasek, M.A. (2007) Does transformation of microvascular endothelial cells into myofibroblasts play a key role in the etiology and pathology of fibrotic disease? *Med. Hypotheses* 68, 650–655
- 97 Ponticos, M. *et al.* (2009) Pivotal role of connective tissue growth factor in lung fibrosis MAPK-dependent transcriptional activation of type I collagen. *Arthritis Rheum.* 60, 2142–2155
- 98 Verrecchia, F. *et al.* (2006) Transforming growth factor-beta signaling through the Smad proteins: Role in systemic sclerosis. *Autoimmun. Rev.* 5, 563–569
- 99 Sgonc, R. and Wick, G. (2008) Pro- and anti-fibrotic effects of TGF- β in scleroderma. *Rheumatology (Oxford)* 47 (Suppl 5), v5–v7
- 100 Kamel, M. *et al.* (2001) The peri-implant breast capsule: An immunophenotypic study of capsules taken at explantation surgery. *J. Biomed. Mater. Res.* 58, 88–96
- 101 Siggelkow, W. *et al.* (2003) Histological analysis of silicone breast implant capsules and correlation with capsular contracture. *Biomaterials* 24, 1101–1109
- 102 Wolfram, D. *et al.* (2004) Cellular and molecular composition of fibrous capsules formed around silicone breast implants with special focus on local immune reactions. *J. Autoimmun.* 23, 81–91
- 103 Shanklin, D.R. and Smalley, D.L. (1999) Dynamics of wound healing after silicone device implantation. *Exp. Mol. Pathol.* 67, 26–39
- 104 Hu, W.J. *et al.* (2001) Molecular basis of biomaterial-mediated foreign body reactions. *Blood* 98, 1231–1238
- 105 Wolfram, D. *et al.* (2008) Altered systemic serologic parameters in patients with silicone mammary implants. *Immunol. Lett.* 118, 96–100
- 106 Backovic, A. *et al.* (2007) Simultaneous analysis of multiple serum proteins adhering to the surface of medical grade polydimethylsiloxane elastomers. *J. Immunol. Methods* 328, 118–127