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MOLECULAR MECHANISMS OF PULMONARY FIBROSIS

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1. ABSTRACT

Pulmonary fibrosis is the end-point of a numerous and heterogeneous group of disorders known as interstitial lung diseases (ILD). Lung fibrotic remodeling is characterized by fibroblast/myofibroblast activation, and excessive extracellular matrix accumulation leading to progressive organ dysfunction and usually terminal outcome. Treatment is largely ineffective primarily because few of the molecular mechanisms have been well defined to design appropriate targets for therapy. While the pathogenesis is incompletely understood, a growing body of evidence suggests two different pathogenic routes for developing pulmonary fibrosis. The inflammatory pathway, where a shift to the so-called T-helper 2 type cytokine networks is critical, and the epithelial pathway represented by idiopathic pulmonary fibrosis, by far the most aggressive ILD. In this pathway the inflammatory process is irrelevant, and the physiopathology seems to be dominated by epithelial cell injury and activation. Both routes may trigger a number of cytokines/growth factors inducing fibroblast migration/proliferation and phenotype change to myofibroblasts, with a consequent accumulation of extracellular matrix. An imbalance in matrix metalloproteinase/tissue inhibitors of metalloproteinases may contribute to alteration in extracellular matrix turnover and remodeling. This review will focus in some of the mechanisms involved in both prefibrotic pathways, as well as those involved in fibroblast activation and abnormal matrix deposition.

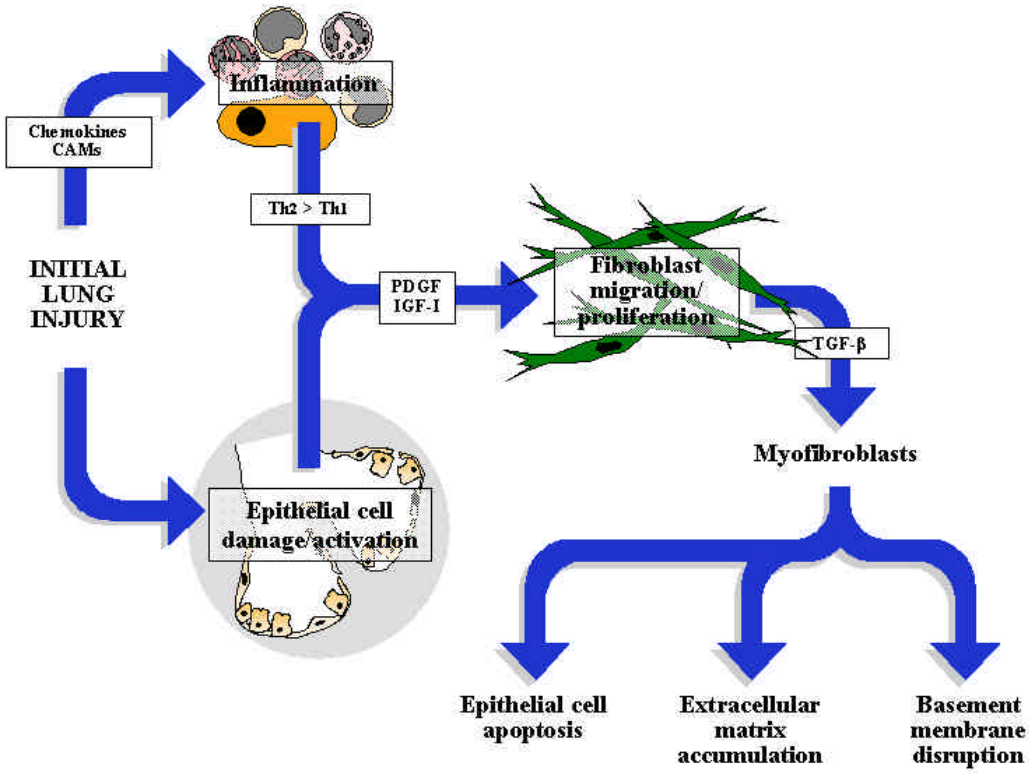

Figure 1. Hypothetical scheme of the two routes leading to pulmonary fibrosis. An initial injury of known or unknown etiology provokes an unresolved inflammation or alveolar epithelial cell activation. Inflammatory and epithelial cells release factors inducing fibroblast migration and proliferation and changes in cell phenotype (myofibroblasts). In the microenvironment of the injured lung, myofibroblasts induce epithelial cell apoptosis, and irreversible changes in extracellular matrix architecture resulting in an erratic remodeling of the lung parenchyma.

2. INTRODUCTION

Pulmonary fibrosis is the final common pathway of a diverse group of lung disorders known as interstitial lung diseases [ILD (1)]. Although the fibrotic changes are typically conceptualized as the fibrous thickening of the interstitium, --- the anatomic space interposed between the alveolar epithelial and endothelial basement membranes, --- an important part of the lung remodeling occurs in the alveolar spaces (2). Therefore, it would be more appropriate to refer them as diffuse parenchymal lung diseases. Under all circumstances, development of fibrosis provokes dramatic and irreversible changes in the lung architecture resulting in progressive respiratory insufficiency and a terminal outcome in a relatively short period of time.

Despite that ILD are different in a variety of features, they are grouped together because they share many clinical, radiographic and physiological characteristics (1). A number of them are of known etiology, i.e. exposure to organic (hypersensitivity pneumonitis) or inorganic particles (e.g. asbestosis), induced by drugs, or associated to collagen-vascular disease, such as systemic sclerosis and rheumatoid arthritis. However, around 40-50% of the ILD are of unknown etiology and these are now classified as idiopathic interstitial pneumonias (IIP) (3). The most common IIP and by far the most aggressive ILD is idiopathic pulmonary fibrosis (IPF), which represents a chronic, progressive and usually lethal lung disorder. In this sense, it is important to emphasize that while most of ILD patients present a heterogeneous clinical behavior, that is, they may heal, improve, or evolve to fibrosis, IPF always progress until the destruction of the lung parenchyma (4).

Regarding the pathogenic sequence of events, it is generally agreed that ILD are initiated by an injury that results in the development of an inflammatory response which, if unresolved, is followed by fibroblast proliferation/activation and finally by the exaggerated accumulation of extracellular matrix (ECM) proteins. According to this view, inflammation is a crucial event that precedes the development of pulmonary fibrosis. However, it has been recently hypothesized that this pathway operates for most ILD but not for IPF in which fibrosis appears to be an inflammatory-independent pathological process (4).

In this context, we propose that there are two different routes for developing diffuse pulmonary fibrosis: a) **the inflammatory pathway** represented by almost all the non-IPF interstitial lung diseases, where there is an early, clearly distinguishable phase of alveolitis, and a late fibrotic phase, and b) **the epithelial pathway**, represented by IPF [Figure 1 (4, 5)].

3. LESSONS FROM THE INFLAMMATORY PATHWAY

The pathogenesis of fibrosis in most ILD is likely associated with a post-injury immune and inflammatory response leading to tissue remodeling. Naturally, the mechanisms of alveolitis and the inflammatory infiltrate may vary according to the damaging agent, but several essential common cytokine networks are involved. In this pathway, interplay between immune and inflammatory cells and mediators control initiation, progression and eventually resolution of inflammation.

3.1. The paradigm of type 1 or type 2 cytokine network commitment

A critical aspect of the immune response is the control of helper T cell differentiation into subsets which predominantly produce cytokines as: interferon-gamma (IFN-gamma), interleukin (IL)-2, IL-12, IL-18, and TNF-beta (T helper-1) or IL-4, IL-5, IL-10, and IL-13 (T helper-2). T helper 1 and 2 cells have not precommitted phenotypes but rather are endpoints of a multistep process where common precursor cell populations achieve different cytokine-secretion profiles (6). It is important to emphasize that although these networks have been classically referred to the subsets of CD4+ T helper lymphocytes, it is now recognized that a number of other cell types, namely mast cells and macrophages, can produce similar cytokine patterns (6-8). Therefore, the criteria defining these responses should be widened and it is now more appropriate to refer to type 1 and type 2 cytokine responses rather than Th1 and Th2.

One emerging hypothesis is that the polarized type 1 or type 2 cytokine profiles displayed during the immune/inflammatory response likely determine the phenotype responsible for either resolution of the pathological process or progression to fibrosis. Indeed, a growing body of evidence supports the notion that the type 1 phenotype has a profound antifibrotic effect, primarily mediated by IFN-gamma. This cytokine substantially decreases fibroblast proliferation and ECM synthesis *in vitro* and reduces the lung fibrotic response *in vivo* (9, 10). Likewise, it has been shown that IFN-γ strongly decreases expression of alpha-smooth muscle actin in TGF-beta pretreated fibroblasts, suggesting that this cytokine is able of reverting the myofibroblast phenotype at least *in vitro* (11). Moreover, presently IFN-gamma represents one of the most encouraging drugs for IPF treatment and preliminary data have shown that in combination with low dose of corticosteroids, it leads to improvement or stabilization of this disorder (12). In contrast, IL-4 and IL-13, which typically represent the type 2 cytokine response, activate fibroblasts, and induce production of ECM macromolecules (13, 14). Moreover, in human skin keloid fibroblasts, IL-13 inhibits the synthesis of matrix metalloproteinase (MMP)-1 and MMP-3, and enhances production of tissue inhibitor of metalloproteinase (TIMP)-1, suggesting that it might generate a non degradative matrix microenvironment (14). In addition, it has been demonstrated that both, IL-4 and IL-13 are capable of modulating the phenotype of human lung fibroblasts to myofibroblasts through a c-Jun NH2-terminal kinase-dependent pathway (15).

In vivo experimental models have strongly supported the assumption that the type 2 reaction is related to fibrosis. Indeed, a predominant type 2 response has been shown in radiation- and bleomycin-induced pulmonary fibrosis (16-18). In the radiation model, a selective increase of lung IL-4 loaded CD4+ T-cells was observed preceding the thickening of alveolar walls, peaking 4 weeks after irradiation. When experimental animals were depleted of these specific lymphocytes, the post-radiation fibrotic reaction was significantly reduced (16). In the bleomycin model the fibrotic lesion has been related with increased expression of both IL-4 and IL-5 by mononuclear cells (17, 18). Indeed, the use of anti-IL-5 antibody caused significant reduction in lung eosinophilia, cytokine expression, and fibrosis (17).

Studies in human fibrotic pulmonary diseases are scanty, but a type-2 like dominant pattern has been found by *in situ* hybridization and immunohistochemistry in the lung parenchyma of patients with fibrosing alveolitis (19). Similarly, it has been shown that CD^{8+} T cells obtained through bronchoalveolar lavage (BAL) from the majority of patients with systemic sclerosis --- an autoimmune disease highly associated with pulmonary fibrosis --- express IL-4 and/or IL-5 mRNA, and that this type 2 dominant response is associated with a greater decline in pulmonary function (20). With regards to the type 1 response, it has been found that ILD patients with higher serum IFN-γ levels respond better to corticosteroids. This observation included several diseases such as sarcoidosis, systemic sclerosis and fibrosing alveolitis (21). Furthermore, the *in vitro* production of IFN-γ by lymphocytes was impaired in most patients that subsequently showed spontaneous lung functional deterioration, suggesting that impaired IFN-γ release might enhance the fibrotic response in these interstitial lung diseases (21).

Altogether these studies suggest that this complex interplay between type 1/type 2 mediators, may shift the balance to either resolution of the process or progression to fibrosis.

A hypothetical scenario exemplifying this process could be drawn with hypersensitivity pneumonitis (HP). HP is a diffuse granulomatous lymphocytic alveolitis provoked by the exposure to a variety of organic particles, which is believed to be a predominantly T helper type 1 lung disorder (22). Likewise, it is known that while a number of patients improve or heal after therapy, around 30% of them progressively evolve to fibrosis even if they avoid further exposure (23). The fibrotic response is accompanied by a change in inflammatory infiltrate with an increase of neutrophils loaded with collagenase-2 and gelatinase B and the disease usually progresses (23, 24). Therefore, it can be speculated that in this ILD, the host reaction to the organic particle or the chronicity of the disorder or some unknown event may turn on switch to a response dominated by the type-2 cytokine phenotype, whose consequence is the development of progressive fibrosis.

3.2. Chemokines

Chemokines are a group of small, mostly basic, structurally related molecules that regulate cell trafficking

of various types of leukocytes (25). Chemokines have been divided in two major subfamilies based on the arrangement of two N-terminal cysteine residues, CXC and CC, depending on whether the first two cysteine residues have an aminoacid between them (CXC) or are adjacent (CC) (25).

3.2.1. Chemokines in inflammatory/fibrotic lung disorders.

A number of members of the CC chemokine family have been identified as important in development of inflammation in both experimental models and in human interstitial lung diseases. Macrophage inflammatory protein-1 alpha (MIP-1 alpha) and monocyte chemoattractant protein-1 (MCP-1), which are chemotactic for macrophages, basophils, eosinophils, and subsets of T-lymphocytes, are upregulated in bleomycin-challenged rodents, and neutralization of both chemokines significantly reduce inflammatory cell accumulation (26). Levels of these chemokines have also been found significantly elevated in patients with systemic sclerosis, and correlate with the presence of pulmonary fibrosis (27). MCP-1 is strongly expressed in the epidermis, inflammatory mononuclear cells, and endothelial cells from sclerotic skin but is not expressed in normal skin.

Likewise, MIP-1 alpha and IL-8, the latter a potent chemoattractant for neutrophils, are increased in BAL fluid of patients with fibrosing alveolitis and sarcoidosis (28). Interestingly, a strong mRNA signal for both chemokines is detected in patients showing progressive disease. RANTES, a potent eosinophil and lymphocyte chemoattractant, has been found elevated mainly in sarcoidosis (29), with the alveolar macrophage being its main cellular source. Recently, we have found that CCL18/DC-CK-1 a chemokine involved in naive T cell recruitment, is strongly upregulated in the lung of patients with hypersensitivity pneumonitis (30). Macrophages, dendritic and alveolar epithelial cells are the source of this chemokine, and a direct relationship between the number of lymphocytes and mRNA levels of CCL18/ DC-CK-1 is observed.

Overall, all these studies support an important role for a number of chemokines in the maintenance of inflammation and in the development of pulmonary fibrosis under certain circumstances.

3.2.2. Chemokines and Th1/Th2 cytokines circuit

Recent data suggest that the expression of some chemokine receptors might play a role in the development of Th1 or Th2 immune responses which, as mentioned above are related to resolution or progression to fibrosis. Thus, for example, the chemokine receptors CCR4 and CCR8 are strongly upregulated in T lymphocytes polarized to the type 2 phenotype (37, 38). Moreover, a defective T helper type 2 immune response has been reported in several experimental models developed in CCR8 knock-out mice. In contrast, the Th1 type immune response is unaffected by CCR8 deficiency, indicating that CCR8 has an important role in Th2 functional responses *in vivo* (39). In another study, it was found that CCR5 exhibited the same pathway of Th1 association, while CCR4 expression although not limited to Th2 cells *in vivo*, was markedly upregulated by IL-4 and downregulated by IFN-gamma *in vitro* (40).

Overall these studies suggest the existence of different programs of chemokine receptors expression during the development of Th1 and Th2 cells. However, whether the presence of these receptors and their chemokine ligands participate in recruitment of committed Th1 or Th2 cells, or whether they directly influence differentiation of naive T cells into Th1 or Th2 phenotypes, need further elucidation.

3.2.3. Chemokines and angiogenesis

Neovascularization, a fundamental process in tissue repair after injury, is mediated by a variety of molecules, including CXC chemokines, which regulate the process in opposite directions (31). Thus for example, IL-8 and the growth-related genes alpha, beta, and gamma are angiogenic chemokines, while platelet factor-4 and two interferon-γ inducible proteins (IP-10 and MIG) are angiostatic (31).

With the existing knowledge, it is unclear whether angiogenesis plays a role in the resolution of the inflammatory process or in the progression of lung fibrotic lesions. Although an old report showed extended angiogenesis in patients with diffuse interstitial fibrosis (32), some recent evidence in humans suggests that the development of fibrosis, at least in part, may be related to reduced angiogenesis. Thus, neovascularization is a prominent feature in bronchiolitis obliterans organizing pneumonia (BOOP), a usually reversible fibrogenic process, while fibrosis in IPF involves dense collagen fiber deposition without vascularization (33). Similar results have been obtained in fibrotic skin lesions where a significant reduction in the vascular density has been found in keloids when compared with surgical and hypertrophic scars (34). Thus, formation of keloid scars may be at least partially related to their reduced level of angiogenesis mediated through tissue hypoxia (34).

In contrast, different results have been found in experimental models of pulmonary fibrosis, where angiogenesis and associated chemokines appear to be increased. For example, in bleomycin-induced lung fibrosis, MIP-2 an angiogenic chemokine and IP-10 an angiostatic one, were found to be directly and inversely correlated, respectively, with the lung fibrotic response (35, 36). Furthermore, depletion of MIP-2 or exogenous increase of IP-10 resulted in marked attenuation of lung fibrosis. Thus, further work is necessary to better understand the role of angiogenesis in human fibrotic lung disorders.

In summary, chemokines play a pivotal role in the regulation of inflammation/fibrosis processes. Upregulation/downregulation of certain chemokines and receptors may participate in the control of the responses at least at three levels, i.e., inducing and perpetuating inflammation, altering angiogenesis, and polarizing the immune response to a type-2 profibrotic reaction.

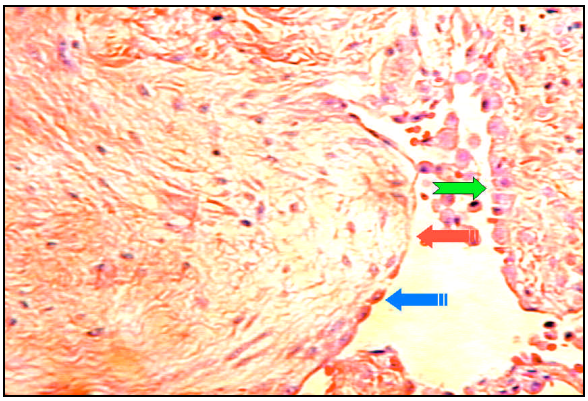

Figure 2. Subepithelial fibroblastic foci in an IPF lung. Several abnormalities can be noticed in the alveolar epithelium including, hyperplasia of type 2 pneumocytes (green arrow), reactive alveolar epithelial cells (blue arrow), and areas of loss of epithelium (red arrow).

It is important to emphasize that recruitment of leukocytes to the injured tissues requires, in addition to chemokine mediated chemotaxis, a finely regulated multistep process involving leukocyte rolling along the endothelial cell surface, firm adhesion and activation, and transendothelial migration toward interstitial spaces (41, 42). Control of these events depends on a variety of specific cell-surface proteins, named cell adhesion molecules, some of which are upregulated in a number of human and experimental fibrotic lung disorders (43, 44).

4. THE EPITHELIAL PATHWAY. TYING LOOSE ENDS

The alveolar surface of the lung is lined by two distinct epithelial cells. Nearly 95% is lined by flattened, greatly expanded type 1 epithelial cells. The geometry of these cells is ideally suited for gas exchange. Importantly, type 1 pneumocytes are very vulnerable to injury by inhaled and bloodborne agents and they represent an end-stage of cell differentiation, with little if any proliferative potential. The remaining 5% of alveolar surface is covered by cuboidal type 2 pneumocytes which project into the lumen often occupying a niche in the corner of the alveoli. They are metabolically very active secretory cells, and synthesize among other molecules, surfactant, a complex surface-active compound which covers the alveolar lining lowering the surface tension at the alveolar air-liquid interface. Type 2 epithelial cells are also capable of proliferating and differentiating into type 1 pneumocytes.

4.1. Idiopathic pulmonary fibrosis and the alveolar epithelium

Several lines of evidence strongly suggest that inflammation is not an important pathogenic event in IPF. Evidence includes the presence of similar mild/moderate alveolitis either in early or late disease, and the lack of response to potent and long-term anti-inflammatory therapy (4, 5). Additionally, experimental models and some human diseases have made evident that it is possible to have inflammation-independent fibrosis. In this context, an evolving hypothesis proposes that IPF may result from epithelial microinjuries with alveolar epithelial cell activation and abnormal wound healing (4).

A marked disruption in the integrity of the alveolar epithelium with presence of several altered phenotypes is a distinctive feature in IPF lungs. Usual morphologic phenotypes include a) cuboidal cells (hyperplasia of type 2 pneumocytes), b) reactive large and elongated epithelial cells (putative transitional cells among type 2 and type 1 pneumocytes), and c) bronchiolar-like epithelial cells lining areas of honeycomb lesions (Figure 2). These morphological changes are also accompanied by modifications in specific expressed cytokeratins, --- that reflect differentiation, functional specialization, and pathological alterations --- suggesting that epithelial cells are not only altered in shape, but also in their state of differentiation and function (45).

In addition, epithelial dysfunction may enhance extravasation of blood constituents that in turn increase surface tension in the alveolar space, allowing apposition and fusion of exposed basement membranes, and consequently causing, alveolar collapse and fibrosis.

Therefore, one of the critical events in the course of IPF is a profound defect in developing an appropriate re-epithelialization. Among other effects, correct formation of a continuous layer of alveolar epithelial cells is essential to limit and contain migration of fibroblasts into the alveolar space, as well as their increased synthesis of intra-alveolar extracellular matrix.

4.2. Epithelial cell apoptosis and alveolar re-epithelialization

Numerous microscopic areas of alveolar epithelial cell dropout often intercalated with hyperplastic cells are usually noted in IPF lungs (4). The reason for this observation is not clear, but it could be related to chronically induced epithelial cell death. Indeed, a number of studies performed both *in vitro* and *in vivo* support the notion that alveolar epithelial apoptosis and necrosis are highly increased in IPF lungs. Fibroblasts/myofibroblasts obtained from IPF patients release angiotensin peptides capable of inducing type 2 pneumocyte programmed cell death (46, 47). Furthermore, apoptotic epithelial cells *in vivo* are closely related to underlying foci of myofibroblasts (48). Apoptosis of type 2 pneumocytes occurs even in normal alveoli of IPF lungs whereas it is absent in normal lungs (49). Additionally, IPF patients exhibit upregulated Fas expression in bronchiolar and alveolar epithelial cells suggesting that the Fas-Fas ligand pathway may be also involved (50, 51). Taken together, these findings demonstrate that numerous alveolar epithelial cells of the fibrotic lung are dying, which may at least partially explain the areas of epithelial cell loss.

On the other hand, a severe impairment in re-epithelialization seems to occur in IPF where the capacity of type 2 alveolar cells to restore damaged type 1 cells is seriously affected (52). Actually, it has been found that high levels of circulating KL-6, a marker of regenerating type 2 pneumocytes and indirectly of the magnitude of the

death of type 1 pneumocytes, predict a rapidly progressive IPF with consequent poor outcome (53).

Epithelial dysfunction in IPF displays several faces. In the last 10 years it has become evident that altered alveolar type 2 cells synthesize a variety of cytokines, growth factors and enzymes involved in matrix cleavage, suggesting that the contribution of the epithelium in ECM remodeling is greater than expected (4).

Several lines of evidence support this notion. Thus for example, in experimental models of lung fibrosis it has been shown that impairment of type 2 cell proliferation after injury enhances the fibrotic response, while coverage of the denuded basement membrane by type 2 cell proliferation and migration prevents interstitial fibrosis (54, 55). Furthermore, some *in vivo*/*ex vivo* models have shown that pulmonary fibrosis can be induced after alveolar epithelial injury even in absence of inflammation (56).

4.3. Epithelial cells: The source of profibrotic cytokines/growth factors in IPF

It has been traditionally accepted that inflammatory cells, primarily alveolar macrophages, are the source for most of the soluble molecules implicated in fibrogenesis. However, there is increasing evidence clearly showing that injured/activated alveolar epithelial cells play an essential role in this process synthesizing a number of cytokines and growth factors involved in fibroblast migration, proliferation and inducing changes in their phenotype. Several studies of cell localization by *in situ* hibridization and immunohistochemistry have demonstrated that in IPF, alveolar epithelial cells are the main site of synthesis of platelet-derived growth factor (PDGF), transforming growth factor beta, and tumor necrosis factor alpha (TNF alpha), all of them essential for the development of pulmonary fibrosis (57-61). Likewise, endothelin-1, a vasoconstrictor and a fibroblast and smooth muscle cell mitogenic peptide is strongly up-regulated in type 2 pneumocytes of patients with fibrosing alveolitis, particularly in those lining areas of young granulation tissue (62). In response to hyperoxia exposure, epithelial cells also modulate lung fibroblasts migration and proliferation through insulin growth factor-I production (63). More recently, Pan *et al* (64) evaluated the expression of connective tissue growth factor (CTGF) in the lung tissue of patients with IPF. CTGF, a chemotactic and growth factor for fibroblasts encoded by an immediate early gene that is transcriptionally activated by TGF-beta, was strongly upregulated in IPF lungs, and its localization was confined predominantly to proliferating type 2 alveolar epithelial cells and activated fibroblasts.

During alveolar repair processes close contacts are established between fibroblasts and lung epithelial cells through gaps in the basement membrane. With this concept in mind, rat alveolar epithelial cells were co-cultured with human fibroblasts, which resulted in a several fold increase in fibroblasts secretion of collagen type I into the conditioned medium. This stimulation seemed to be caused by the secretion of insulin growth factor 1 (65).

The possible participation of alveolar epithelium in generating a type-2 like pattern in IPF has been also studied. In this context, the type 2 alveolar epithelial cell expression of IL-4 and INF-gamma was examined in lung specimens from patients with IPF, HP, and sarcoidosis. The results showed that while in HP and sarcoidosis --- two reversible ILD --- there was an up-regulation of both IL4 and INF-γ, in IPF only IL-4 was detectable (66). These results are consistent with a predominantly type 2 pattern of cytokine network in IPF and support a role for epithelial cells in the characteristic imbalance of pro-fibrogenic cytokines in the distal lung of patients with this disease.

It is important to emphasize that epithelial cells on the alveolar surface of the fibrotic microenvironment may contribute to fibrogenesis not only because they over-express profibrotic cytokines, but also because they might be unable to secrete some inhibitors of fibroblast growth. Epithelial and mesenchymal cells crosstalk bidirectionally and dynamically. Thus for example, epithelial basement membrane components are assembled to a lamina densa via a supply of soluble factors by pulmonary fibroblasts (67). Likewise, under physiological circumstances fibroblasts induce epithelial cell proliferation probably through secretion of a number of members of the fibroblast growth factor family, primarily keratinocyte growth factor (68, 69). Likewise, epithelial cells inhibit fibroblast proliferation probably via secretion of prostaglandin-E2 (70). This reciprocal control seems to be lost during lung fibrosis. Thus, in the fibrotic phase of bleomycin-induced lung injury, where excess fibroblast growth occurs, alveolar epithelial cells appear to be functionally deficient in producing fibroblast inhibitors (68).

4.4. Alveolar epithelial cells and intra-alveolar coagulation

During normal wound healing, the coagulation cascade is activated promoting the generation of a fibrin clot. Afterwards, epithelial cells have to dissolve the fibrin barrier to migrate throughout the denuded wound surface and this process occurs throughout the activation of the fibrinolytic system (71). In contrast, persistent activation of the coagulation cascade enhances a fibrotic response, and this situation appears to occur in a number of ILD including idiopathic pulmonary fibrosis (72-74). Most importantly, there is a growing body of evidence supporting that alveolar epithelial cells contribute to the increased procoagulant and antifibrinolytic activities in this disorder. In IPF lungs, tissue factor, a potent procoagulant factor, and plasminogen activator inhibitor (PAI)-1 and 2 are both strongly expressed by alveolar epithelial cells (73-75). It is likely that expression of these factors is one of the reactions that follows epithelial damage contributing to the repair of microinjuries affecting alveolar cells and septa.

Supporting the importance of the coagulation/fibrinolisis process in the development of lung fibrosis, transgenic mice overexpressing PAI-1 develop significantly more lung fibrosis than littermate controls after bleomycin injury (76). In contrast, PAI-1 deficient mice behave as wild type mice (76). Likewise, pulmonary fibrosis associated to the exposure to nickel dust is also

related to excessive fibrin formation. Nickel activates AP-1 through an oxidant-independent pathway and promotes lung fibrosis by transcriptionally activating PAI-1 and inhibiting fibrinolysis (77).

Decrease of local plasmin production may have several deleterious effects on the alveolar spaces. These include excessive fibrin deposition, lack of activation of some MMPs responsible for matrix degradation, impairment of epithelial cell migration, and a thrombin-mediated increase of fibroblast proliferation, thereby enhancing a fibrotic process (78-80).

4.5. Are epithelial cells directly participating in fibrilar collagens accumulation?

Maintenance and repair of the pulmonary alveolar basement membrane are critical processes for preserving normal alveolar structure and function. In this context, it is known that alveolar epithelial cells are able to produce a number of ECM molecules, i.e. type IV collagen, laminin, fibronectin, perlecan, entactin, and thrombospondin directly related to their own basement membrane formation (67, 81, 82).

However, although fetal type 2 pneumocytes are able to synthesize components corresponding to the chains present in types I, III, and V interstitial collagens (83), evidence with adult alveolar epithelial cells has been ambiguous. Recently it was reported that SV40-transformed human airway epithelial cells are able to internalize silica particles which in turns stimulate the production of matrix proteins such as collagens and fibronectin (84).

A provocative paper examined this possibility *in vivo* and suggested that in IPF, alveolar epithelial cells express not only type I procollagen but also heat shock protein 47 (HSP47), which regulates the synthesis and assembly of type I collagen (85). In this work, both proteins were examined in lungs of IPF and BOOP patients. Marked HSP47 staining was observed in myofibroblasts and most of the type 2 alveolar epithelial cells primarily around active fibrotic areas. In addition, immunoreactive type I procollagen expression was detected in most myofibroblasts and in around 25% of the type 2 pneumocytes, usually in areas of active fibrogenesis, but not in advanced honeycomb areas. In contrast, HSP47 and collagen type I were weakly detected in only few type 2 pneumocytes in BOOP, a usually reversible disease. This is the first evidence that regenerating alveolar epithelial cells synthesize type I collagen in human lung tissues, and arise the possibility that these cells might play a direct role in the development of fibrosis. Actually, this finding might indirectly suggest that some epithelial changes in pulmonary fibrosis might resemble to a certain degree embryonic development. Supporting this possibility, it has recently reported that phenotypic changes of alveolar epithelial cells during radiation-induced pulmonary fibrosis exhibit similarities with the expression profile of epithelial antigens during lung development (86).

5. FIBROBLASTS/MYOFIBROBLASTS: THE COMMON ROUTE FOR THE INFLAMMATORY AND EPITHELIAL PATHWAYS TO PULMONARY FIBROSIS

An essential feature in the development of pulmonary fibrosis is the activation of fibroblasts, which is followed by abnormal remodeling of the ECM and subsequent destruction of the lung architecture. Proliferation/activation of fibroblasts widely dispersed throughout the lung parenchyma usually precedes accumulation of ECM molecules in the damaged alveolus, and it is considered as a key step in the transformation of a potentially reversible disorder into a progressive and irreversible one. Indeed, the amount of fibroblast/myofibroblasts foci is considered a main prognostic factor in IPF patients [Figure 2, (87)].

5.1. Fibroblast heterogeneity

Strong evidence has demonstrated that there is a noteworthy phenotypic and behavioral heterogeneity among fibroblast subpopulations in normal and fibrotic tissues.

Classical fibroblasts express vimentin, but not alpha SMC, which is an almost universal marker of myofibroblasts. Using cytoskeletal markers, the presence of at least four fibroblastic phenotypes has been described. They can express vimentin (V-type), vimentin and desmin (VD-type), vimentin and alpha-SM actin (VA-type) and vimentin, desmin, and alpha-SM actin (VAD-type) (88). Other important markers of myofibroblastic differentiation are the smooth muscle myosin heavy chains that seem to better define different well-differentiated myofibroblastic phenotypes (88, 89).

In the interstitium of normal lungs there is a subset of mesenchymal cells known as alveolar myofibroblasts or contractile interstitial cells (CICs) involved in the regulation of alveolar size during breathing, thus participating in the control of the ventilation-perfusion ratio (90). These cells express myogenic intermediate filaments, including desmin and vimentin although not α-smooth muscle actin (alpha-SMA) (91). However, CICs exhibit a close phenotypic relationship with mesenchymal cells expressing alpha-SMA as pericytes --- the myofibroblasts associated with capillaries ---, and with the alveolar ring cells, that represent smooth muscle-like cells found at the corners of alveolar ducts (91). In general, it has been proposed the existence of a spectrum of fibroblastic differentiation which includes cells with phenotypic features similar to those of classical fibroblasts and of classical smooth muscle cells (88, 92).

In human lung fibroblasts, we have found differences in cell size among different subsets expressing and not expressing alpha-SMA. By unit gravity sedimentation and flow cytometric analyses we identified three sizes of cell subpopulations corresponding to young quiescent, rapidly proliferating, and large slow-growing groups. Under conditions of log-phase growth, only the last group, corresponding to larger slow-growing cells, expressed alpha-SMA (93).

Mesenchymal cells continually modify their interactions with the microenvironment, and a number of experimental systems begin to uncover details of this behavior. However, the *in vivo* sequence of events leading to activation of fibroblasts and formation of foci of myofibroblasts in the injured lung parenchyma is far to be elucidated.

According to current knowledge we can assume that fibroblasts acquire first a migratory phenotype, then a proliferative phenotype, and finally a myofibroblast profibrotic phenotype during which they produce abundant ECM components.

5.2. First call: Fibroblast migration to the site of lesion

Cellular motility plays a critical role in the development of fibrosis, and some evidence suggests that fibroblasts obtained from fibrotic lungs migrate faster than those obtained form normal lungs. Suganuma *et al* (94) evaluated the migratory activity of fibroblasts obtained from IPF and control lungs. Migration of IPF fibroblasts was increased in serum-free maintenance medium alone and was significantly enhanced when cells were stimulated by PDGF.

Actually, PDGF is among the most potent stimuli for fibroblast migration, and appears to be one of the factors implicated in fibroblast chemotaxis during lung fibrosis. PDGF is highly upregulated in fibrotic lungs and it is synthesized by inflammatory as well as alveolar epithelial cells (57). During experimental lung fibrogenesis, PDGF receptor alpha is rapidly induced, and it is expressed primarily by mesenchymal cells residing within fibrotic lesions (95). More recently, it was demonstrated that both alpha- and beta-PDGF receptors promote lung fibroblast cell migration, and more importantly, it was shown that their effects are additive (96). PDGF stimulates fibroblast chemotaxis in a concentration-dependent manner, and this stimulation seems to be augmented by some cytokines such as IL-1 beta and TNF-alpha (97).

KL-6, a molecule synthesized by alveolar epithelial cells, also promote the migration of human lung fibroblasts, and checkerboard analysis has revealed that it is chemotactic as well as chemokinetic, effects that are enhanced by fibronectin (98). Indeed, human plasma fibronectin alone is also able to selectively recruit fibroblasts (99).

More recently, it has been suggested that nerve growth factor (NGF), a polypeptide which, in addition to its effect on nerve cells seems to participate in inflammatory responses, might play a role in fibroblast migration (100). Thus, NGF significantly induced skin and lung fibroblast migration in an *in vitro* model of wounded fibroblast and skin migration, but did not influence fibroblast proliferation, collagen production, or metalloproteinase production or activation (100).

5.3. Second call: Fibroblast proliferation in the site of lesion

Either in lung interstitium or alveolar spaces, (often in both) the expansion of the fibroblast/myofibroblast population is a key characteristic of active fibrotic lesions. In this context, the analysis of the rate of proliferation of these cells derived from fibrotic lungs either in experimental models or in humans, have received special attention. However, reports in the literature are some how contradictory. Thus, there are data showing that primary fibroblasts derived from IPF patients proliferated faster than normal lung fibroblasts (101). In contrast, we found that IPF-derived fibroblasts with more than 60% of alpha SMC actin positive cells showed a marked decrease in growth rate when compared with lung fibroblasts derived from normal individuals (102). The apparent contradiction might be partially explained by the observation that different rates of proliferation can be detected if fibroblasts are derived from areas of early (higher proliferation) or dense fibrosis (slower proliferation) (103).

Studies of fibroblasts isolated from bleomycin-induced lung injury have shown evidence of increased intrinsic proliferative capacity (104). When the role of telomerase in regulating this capacity was investigated in the same model, significant telomerase activity was detected in fibroblasts and tissue extracts isolated from day 14 samples as compared to controls (105). However, an interesting observation was that telomerase expression localized mainly to non-myofibroblastic mesenchymal cells. These findings together with ours (100) would suggest that actively proliferating fibroblasts are a different subpopulation from myofibroblasts. Supporting this point of view it has been observed that cytokines implicated in the early events of myofibroblast precursor proliferation and migration do not induce expression of alpha-SMA (106).

Studies dealing with lung fibroblast mitogenic cytokines in pulmonary fibrosis suggest that some of the factors involved in chemotaxis may also participate in cell proliferation. Thus for example, it has been demonstrated that PDGF stimulates human lung fibroblast proliferation in a concentration-dependent manner, whereas IL-1 beta and TNF-alpha have no effect (97). Basic fibroblast growth factor may also act as a chemotactic and mitogenic agent, as described for dermal fibroblasts in wound healing (107).

Insulin-like growth factor-1 (IGF-1) is a highly mitogenic polypeptide for fibroblasts (63), and it has been demonstrated that fibrotic lung fibroblasts show increased constitutive and TGF-beta-stimulated IGF-1 expression, as compared to normal fibroblasts (108). Additionally, bronchoalveolar lavage fluid from a number of fibrotic lung disorders exhibit IGF-1-mediated increased fibroblast proliferation (109, 110). Increased expression of this growth factor has been documented in IPF lungs, primarily expressed by interstitial macrophages and alveolar epithelial cells (111).

Recent evidence suggests that alpha-1 antitrypsin, at physiologically relevant concentrations, promotes fibroblast proliferation, and also stimulates procollagen synthesis independently of its effects on cell proliferation (112). The mitogenic effect is related to the rapid activation of p42MAPK and p44MAPK since a specific MEK1

inhibitor totally blocked the action of the antiprotease (112). While evidence supports that alpha-1 antitrypsin is the major circulating serine protease inhibitor protecting tissues from neutrophil elastase degradation, these results suggest that additionally, it might influence tissue repair by stimulating fibroblast proliferation and ECM production via classical mitogen-activated signalling pathways.

5.4. Third and last call: Fibroblasts differentiate into myofibroblasts

The presence of an increased population of myofibroblasts is recognized as an essential element of the fibrotic response to injury in human and experimental lung fibrosis, as well as in liver and other organs (113-116). Actually, once fibroblasts have migrated and proliferated into the lung-injured microenvironment, they acquire a myofibroblast phenotype and gradually switch their major functions to protein synthesis and contractility.

The mechanisms involved in myofibroblastic differentiation as well as the origin of progenitor cells in fibrotic lungs are still unclear, but several cytokines have been implicated. Thus for example, an early event involved in the change to a myofibroblast phenotype during embriogenesis as well as in adult wounds is the transformation of fibroblasts expressing PDGF receptor to cells expressing PDGF (107, 117).

Likewise, TGF-beta1 has been implicated in the pathogenesis of fibrosis not only based on its capacity to enhance accumulation of ECM molecules but also because it is capable of promoting myofibroblasts differentiation, upregulating fibroblast alpha-SMA expression both *in vivo* and *in vitro* (118, 119). Similarly, lung overexpression of granulocyte-macrophage colony-stimulating factor (GM-CSF) also induced expression of alpha-SMA in fibroblasts (120). However, when the underlying mechanisms were investigated, it was found that the emergence of α-SMC actin-rich myofibroblasts appeared only after TGF-beta1 production. Similar results were obtained in bleomycin-induced lung fibrosis where induction of GM-CSF in alveolar macrophages precedes the increase in TGF-beta1 and TGF-beta type II receptor transcripts and the presence of myofibroblasts (121). These findings suggest that GM-CSF is an early upstream regulator of TGF-beta1 expression, and support a crucial role of TGF-beta1 in the acquisition of the myofibroblastic phenotype in pulmonary fibrosis.

Some evidence suggests that the fibronectin domain ED-A, an isoform de novo expressed during wound healing and fibrosis, is necessary for the induction of the myofibroblastic phenotype by TGF-beta1 (122). Retinoic acid regulates alpha-SMC actin expression as well (123). Furthermore, cellular retinol binding protein-1, known to be a retinoic acid-responsive gene, is also controlled at the transcriptional level by TGF-beta although throughout distinct pathway (123).

5.5. Myofibroblast persistence in the active fibrotic site

During the resolution of normal wound healing, there is a striking decrease in cellularity, including the disappearance of myofibroblasts through apoptosis. Indeed, the finding of markers of apoptosis within myofibroblast populations *in vivo* has led to the speculation that fibroblast differentiation to the myofibroblast phenotype might represent a terminal pathway leading to apoptosis.

The question that arises is why this process does not seem to occur during the development of fibrotic disorders. A descriptive study in IPF lungs has showed that the apoptotic activity *in vivo* is remarkable lower in the fibroblastic foci as compared to the fibromyxoid lesions of BOOP, a reversible disease, suggesting that in IPF fibroblasts/myofibroblasts display a longer survival (124). In contrast, we have noticed that fibroblasts derived from IPF lungs, in addition to be enriched with a myofibroblasts phenotype, exhibit an increased rate of spontaneous apoptosis (102). The reason for this paradox is unknown. It can be speculated that antiapoptotic factors that may influence the fibroblast behavior *in vivo*, are lost when cells are cultured *in vitro*. Another possible explanation is that cells that are dying by apoptosis *in vitro* are not the "*in vivo* resistant" ones. In this context, it has recently been reported that some experimental conditions assumed to occur *in vivo*, i.e. TGF-beta1 production, might lead to selection and propagation of certain apoptosis-resistant or apoptosis-susceptible fibroblast subpopulations at least *in vitro* (125). On this regard, the balance of a number of cytokines may enhance or inhibit cell death, and can be different *in vivo* and *in vitro*. Thus for example, IL-1 beta selectively induces apoptosis in myofibroblasts via induction of nitric oxide synthase, while TGF-beta1 strongly inhibits apoptosis in lung myofibroblasts (126). Likewise, skin fibroblasts from systemic sclerosis are specifically resistant to apoptosis induced by Fas receptor stimulation, but are susceptibility to apoptosis induced by nonspecific stimuli, such as protein kinase inhibition or serum withdrawal (127).

5.6. Myofibroblasts and lung fibrogenesis

The increase of myofibroblasts in injured lungs has several deleterious effects. This cell subset is responsible for most of the increased lung collagen gene expression *in vitro* and *in vivo*, thus actively contributing to excessive ECM deposition in the lung parenchyma during the development of pulmonary fibrosis (102, 128).

One of the major roles of myofibroblasts in wound healing, facilitated by their contractile phenotype, is the size reduction of denuded surface area in the wounded tissue conducting the wound margins toward one another. Thus, in addition of being a marker of myofibroblast differentiation, filamentous α-SMA is important for cell contraction, and this is probably one of the mechanisms involved in the characteristic decrease of lung compliance observed in patients with pulmonary fibrosis.

As mentioned, myofibroblasts from IPF lungs induce alveolar epithelial cell death, and at least in part, the failure in reepithelialization appears to be provoked by this mechanism (46, 47).

Myofibroblasts seem to be also implicated in the degradation of basement membrane. Migration of

fibroblasts/myofibroblasts into the alveolar spaces occurs through partially disrupted and denuded epithelial basement membranes (113, 129, 130). This pathological process may also contribute to the failure of an orderly repair of the damaged alveolar type I epithelial cells. Although the mechanisms involved in the disruption of the basement membrane remain unknown it has been demonstrated that subepithelial myofibroblasts express gelatinases A and B occasionally coinciding with some areas with denuded alveolar basement membranes (129-131). Likewise, in bleomycin-induced pulmonary fibrosis, an increase of gelatinase B activity and disruption of the alveolar epithelial basement membrane have been found (132).

Additionally, it should be considered that basement membrane carry signals for cell survival, and its degradation such as that occurring in IPF, could lead to the loss of these signals resulting in epithelial cell death, as occur in the evoluting mammary gland (133).

6. MATRIX REMODELING: A CRUCIAL ROLE FOR THE MATRIX METALLOPROTEINASE FAMILY

Abnormal accumulation of ECM is the final common feature of any fibrotic disorder. In this context, some recent work has explored the possible role of MMPs during ECM remodeling, the MMPs. These represent a large family of over 20 enzymes that are collectively capable of cleaving the numerous components of the ECM, as well as many other non matrix substrates. MMPs can be classified according to their structural domains and substrate affinity into several subfamilies including: collagenases, gelatinases, stromelysins, matrilysins, membrane type MMPs, and other MMPs that do not appear to fall into any of these subgroups (134).

The regulation of these enzymes is controlled at several levels including gene transcription, activation of latent enzyme and through binding to a specific family of homologous proteins referred to as TIMPs. TIMP-1 to -4 are a family of two domain proteins that besides to its common MMP inhibitory action, differ in expression patterns, and other properties such as pro-MMP activation, cell growth-promoting activity, matrix binding, inhibition of angiogenesis, cell survival promoting activity, and induction of apoptosis (134, 135).

6.1. MMPs/TIMPs relationships in pulmonary fibrosis

The possible role of both, MMPs and TIMPs in the development of lung fibrosis has been analyzed in humans and in experimental models, where in general an overexpression of MMPs has been found (102, 129-131, 136-140).

This observation in fibrotic diseases where the main characteristic is the exaggerated deposit of ECM has been considered as a paradox, since MMPs are mainly viewed as enzymes contributing to pathological extracellular matrix destruction. However, we are far from understanding the biological consequences of cleavage of matrix and non matrix substrates that result in abnormal repair. Furthermore, location of MMPs expression, as well as TIMPs behavior are critical for ECM degradation.

This complex process can be exemplified with our recent findings regarding the collagenases subfamily (130). In this study, although MMP-1 was highly expressed in IPF lung tissue, the localization of the enzyme was noticed in alveolar macrophages, reactive alveolar epithelial cells as well as in bronchiolar epithelial cells lining honeycomb cystic spaces, but it was practically absent in the interstitial compartment. Collagenase-2 was revealed in neutrophils, which were not very abundant in IPF sections, and collagenase-3 was not found (130). Considering that one of the main substrates of collagenases are fibrillar collagens, the lack of the expression of this enzyme in interstitial fibroblasts might explain in a simplistic way the presence of scars that do not undergo resorption.

Moreover, the observation that fibroblasts did not show MMP-1 *in vivo* is in agreement with our results obtained *in vitro* where no differences in MMP-1 mRNA and protein expression have been noticed between IPF and control fibroblasts (102, 136).

On the other hand, its high expression in injured epithelium is intriguing, and might suggest a possible role in alveolar epithelial cells migration. This interpretation is suggested by analogy with injured skin where MMP-1 is induced in migrating keratinocytes by binding through alpha-2 beta-1 integrin to native type I collagen. Degradation of collagen in this system initiates keratinocyte migration during reepithelialization (137).

Gelatinases are the subgroup of MMPs most extensively studied in interstitial lung diseases. MMP-2 and MMP-9 have a broad matrix substrate specificity including laminin, fibronectin, elastin, and type IV collagen, but also of non-matrix substrates influencing the activation of a number of growth factors, such as pro-TGF beta, pro-TNF alpha and IL1 beta (134, 139). Its over-expression in fibrotic diseases has been mainly associated with its capacity to degrade components of the basement membranes. MMP-2 and MMP-9 have been observed in subepithelial myofibroblasts, occasionally in areas of denuded alveolar basement membranes, suggesting that they may play a role in the migration of these cells to the alveolar spaces (129-131). Furthermore, IPF fibroblasts express MMP-9 transcript *in vitro*, and its expression is related with the percent of myofibroblasts (102).

A recent work using cDNA microarray analysis has shown that one of the genes highly expressed in IPF lungs is matrilysin (MMP-7). The immunoreactive enzyme was localized primary in reactive epithelial cells and bound to ECM (141). This enzyme has a strong affinity for heparin and is able to degrade several matrix substrates, such as proteoglycans, laminin, fibrin/fibrinogen, and others (134).

Expression and localization of TIMPs have also been studied in fibrotic lung disorders, both *in vitro* and *in*

vivo. Cultured fibroblasts/myofibroblasts obtained from IPF lungs exhibited a marked upregulation of all 4 TIMPs as compared with fibroblasts from normal lung (102). TIMPs expression and localization has been also studied *in vivo* (129, 130). TIMP-1 is expressed by interstitial cells associated to fibrous tissue, and by alveolar epithelial cells. TIMP-2 is specifically expressed by myofibroblasts within fibroblast foci. TIMP-3 is the only TIMP that binds to the ECM and was primarily localized to the elastic lamina of vessels. TIMP-4 was found in interstitial macrophages and plasma cells. It is important to emphasize that increased TIMP-1 and -2 expression may induce mesenchymal cell proliferation, while TIMP-3 may induce apoptosis (134, 135). Interestingly, TIMP-3 is also capable of inhibiting members of the ADAMs (a disintegrin and a metalloprotease domain) family-like TACE (TNF-alpha cleaving enzyme ADAM 17) (142). Recently, it was reported that TIMP-3 null mice present remarkable lung changes characterized by enlarged air spaces, with no signs of inflammation or fibrosis (143).

Overall, the higher interstitial expression of the 4 TIMPs as compared to interstitial collagenases, supports the notion that a non-degrading fibrillar collagen microenvironment is present in IPF **(**130).

7. GENETIC SUSCEPTIBILITY AND PULMONARY FIBROSIS

Abnormal expression of a number of genes controlling the inflammatory response to injury, and/or epithelial and fibroblast activities could potentially predispose the development of pulmonary fibrosis. In this context, pulmonary fibrosis should be considered a complex disorder involving multiple genes and environmental factors. Moreover, since a variety of interstitial lung diseases eventually evolve to lung fibrosis, it is important to determine if an individual genetic susceptibility is related to the specific disease, i.e. sarcoidosis, or to the exaggerated deposit of ECM, i.e. fibrosis.

There are numerous observations supporting the notion that genetic factors may determine susceptibility or resistance to acquire pulmonary fibrosis. Thus for example, substantial variability exists in the development of lung inflammation/fibrosis among individuals similarly exposed to organic particles (i.e. hypersensitivity pneumonitis), inorganic particles (i.e. asbestosis), or drugs (i.e. amiodarone) (144-146). Similar observations have been reported in bleomycin- or radiation-induced pulmonary fibrosis in different animal strains (147, 148).

Likewise, familial forms of pulmonary fibrosis have been described elsewhere, and the disorder has also been found in monozygotic twins who were separated at early age (149-152). Moreover, lung fibrosis often occurs in a number of pleiotropic inherited syndromes such as Neimann-Pick disease, infantile Gaucher's disease, neurofibromatosis, tuberous sclerosis, and Hermansky-Pudlak syndrome (153).

However, studies regarding genetics factors conferring either susceptibility or resistance to develop pulmonary fibrosis are scanty. Two analytical methods have been suggested to approach complex disease genes, linkage analysis and association mapping. The former tests for co-segregation of a gene marker and a disease phenotype within a family to determine whether a disease-predisposing gene and a genetic marker are in close physical proximity to each other.

So far no reports have been published using linkage analysis regarding familial pulmonary fibrosis, although a protocol is currently in progress in the USA (D. Schwartz, Duke University Medical Center). On the other hand, some studies have suggested that genes located on chromosome 14 encoding immunoglobulin heavy chain G allotypes or α-1 protease inhibitor may be implicated in familial pulmonary fibrosis (154, 155). However, the linkage of these genes to the disease is rather weak. More recently, a mutation in one allele of the surfactant protein C gene, consistent with an autosomal dominant pattern of inheritance, was found in two familial patients with two different forms of idiopathic interstitial pneumonias (156).

7.1. Genetic polymorphisms and pulmonary fibrosis

Association studies designed to explore the co-occurrence of a genetic marker and a specific disease at the population level are widely used for discovering susceptibility loci. However, few studies have been done in pulmonary fibrosis. Avila *et al.*, analyzed the possible association of fibronectin gene polymorphisms and lung fibrosis in patients with systemic sclerosis (SSc), a generalized connective tissue disorder (157). They assessed restriction fragment length polymorphisms in 161 patients with SSc and 253 healthy controls. Fibronectin was selected because it acts in the lung as chemoattractant and adhesive substrate for fibroblasts, and because some splice variants appear to be implicated in inflammation. A significant increase and co-association of the genotypes AB and CD was observed in SSc patients who developed pulmonary fibrosis, suggesting that fibronectin gene polymorphims might predict SSc individuals likely to develop pulmonary fibrosis.

Whyte *et al* (158) examined the TNF-alpha and IL-1 receptor antagonist (Il-1ra) gene polymorphisms in patients with IPF. Two different ethnic populations were mapped, English and Italian cases and controls. The authors found that IL-1ra (+2018) allele 2, and TNF-A (-308) allele 2 seemed to confer increased risk of developing fibrosing alveolitis. Similar results were reported in silicosis by Yucesoy *et al* (159) who found that regardless of disease severity, the odd ratios of disease for carriers of the IL-1ra (+2018) or TNF-alpha (-308) variants were elevated.

However, different results, at least regarding TNF-alpha gene polymorphisms were found in a recent case-control sample from the United Kingdom (160). In this work, Pantelidis *et al* also tested lymphotoxin-alpha, TNF receptor II and IL-6 gene polymorphisms in 74 patients with IPF, and found no significant association with genotype, allele, or haplotype frequencies when compared with 201 healthy individuals. Interestingly, an increased frequency of cocarriage of the IL-6 intron 4G and the TNF-RII 1690C alleles was observed in the IPF population.

Possible genotypic variations in the TGF-beta1 gene, a potent profibrogenic cytokine, was evaluated by Awad *et al* in patients with pulmonary fibrosis (161). Five polymorphisms were identified in the TGF-beta1 gene between position –1321 and +966 relative to the first major transcription start site. One of them consists of a single base substitution (G/C) at position +915 in the signal sequence, which changes codon 25 (arginine/proline). Interestingly, cells from individuals with the arginine/arginine homozygous genotype displayed higher production of the protein *in vitro*. An increase of this polymorphism was found in patients with pre-transplant lung fibrotic pathology when compared with controls and patients with pre-transplant nonfibrotic pathology. More recently, the same group found that this polymorphism also predicts the development of post-transplant lung fibrosis (162). Thus, 39 of 91 lung transplanted patients developed allograft fibrosis, and more than 90% of them (36 of 39 recipients) were of the homozygous codon 25 arginine/arginine high TGF-beta1-producer genotype.

Taken together, these studies suggest that several polymorphisms might increase the risk to develop pulmonary fibrosis. However, several considerations are important to discuss. First, in many of the polymorphisms studied, it is not clear whether they are neutral or non-neutral in relation to the disease or an intermediate phenotype. Second, population stratification, sample size and precise diagnosis are crucial, and some contradictory results may be due to the design of more or less rigorous studies. Most disturbingly, positive association may arise as an artifact of population admixture. Finally, it is important to keep in mind that the association might not be causal but rather be due to linkage disequilibrium of the marker with a susceptibility gene.

7.2. Gene-gene and gene-gene-environment interactions

The genetic dissection of complex disorders such as pulmonary fibrosis should take into account that the risk to develop the pathological condition might be conferred jointly by the interaction of multiple genes. Moreover, different gene alterations may involve a number of molecules produced by distinct resident or inflammatory cell types, and implicate a variety of pathways.

Thus for example, it has been recently described that alveolar epithelial cells from some patients with IPF exhibit microsatellite instability in the TGF-beta1 type II receptor gene (163). A deletion in the polyadenine tract in exon 3 was detected in epithelial cells isolated by microdissection, and coincidentally, low TGFbeta1-II receptor was observed by immunohistochemical staining. The putative pathogenic effect, if any, of this deletion mutation is still unclear, although hyporesponsiveness to TGF-beta may induce increased expression of the cytokine and consequently promote fibrosis.

To date, it is unknown if fibroblasts from patients who develop pulmonary fibrosis have genetic susceptibility to respond abnormally after stimulation. Interestingly, lung fibroblasts isolated from IPF patients have shown a defect in cyclooxygenase-2 expression, and a failure in their capacity to synthesize PGE2 that has, among other functions, an antifibrogenic effect (164). Likewise, it has been demonstrated that basal and TGF-beta1-induced PGE-2 synthesis is limited in fibroblasts from fibrotic lung, and correlated with a loss of the anti-proliferative response to this cytokine. This failure to induce PGE-2 synthesis was due to an inability to up-regulate COX-2 mRNA levels in these fibroblasts. Furthermore, mice deficient in COX-2 exhibited an enhanced fibrotic response to bleomycin. Therefore, it seems that a reduced capacity to up-regulate COX-2 expression and COX-2-derived PGE-2 synthesis may lead to unopposed fibroblast proliferation and collagen synthesis and contribute to the pathogenesis of pulmonary fibrosis (165).

Environmental components are also important, although unknown in many cases. Thus for example, in the case of IPF it can be speculated that some inciting factor(s), i.e. viral infections or gastroesophageal reflux and acid exposure, injure lung parenchyma. Then, activated epithelial cells secrete profibrotic molecules, which in turn act on genetically defective fibroblasts provoking a continuous fibrotic response.

Gene-environment interactions are clearly exemplified in some ILD of known etiology such as hypersensitivity pneumonitis where an increased frequency of the HLA haplotype DRB1*1305-DQB1*0301, and of TNF-2 (-308) allele significantly increase the risk of developing the disease in individuals exposed to avian antigens (166). However, this association is related to the inflammatory response, but not with the fibrotic response since this association was observed regardless the outcome.

7.3. Functional genomics and gene-expression profile

cDNA microarray technology represents a powerful approach to identify genes specifically expressed in different cell or tissue types, as well as to study their up- or down-regulation during pathological processes. To better understand the mechanisms involved in experimental lung inflammation and fibrosis, Kaminski and coworkers analyzed the lung transcription programs by using oligonucleotide microarrays (167). Gene expression patterns were examined in mice injured with bleomycin, as well as in mice carrying a null mutation in the epithelial-restricted integrin beta-6 subunit (beta 6-/-) which develop inflammation but are protected from pulmonary fibrosis. The results of this study identified several clusters of genes that were each expressed with a distinct temporal pattern. Some early upregulated genes were known to be induced by DNA damage, and others were related to the inflammatory response. Interestingly, one cluster of genes was dramatically induced by bleomycin in wild-type mice, but in a lesser degree in β6-/- mice. These genes included a number of genes involved in ECM metabolism, in the regulation of cellular responses to the ECM, and most of the known TGF-beta inducible genes. Therefore, these genes could provide some clues to the development of pulmonary fibrosis, at least in mice. Interestingly, osteopontin, a molecule that mediates various functions, including cell attachment and migration by interacting with alpha v integrin, represented one of the most increased

genes. More recently, it was demonstrated that osteopontin is strongly expressed in alveolar macrophages accumulating in bleomycin-induced fibrotic area of the lung, and that the development of the fibrotic process in mice is associated with an increase in the expression of osteopontin mRNA and protein (168). Furthermore, treatment of mice with antimouse alpha v integrin monoclonal antibody repressed the extent of pulmonary fibrosis in this model.

A study in humans has also corroborated a noteworthy increase of osteopontin gene expression (141). In addition, many genes encoding for proteins associated with ECM turnover, primarily MMP-1 and MMP-7, as well as proteins expressed in smooth muscle cells, were significantly increased in these human fibrotic lungs. However, further work will be necessary to better understand the implications of this methodology, as well as to separate real pathogenic events from epiphenomena.

8. PERSPECTIVE

Recent evidence challenges the conventional dogma related to the pathogenesis of pulmonary fibrosis. From a new perspective, two routes are visualized, the inflammatory pathway and the epithelial cell pathway, the latter one represented by idiopathic pulmonary fibrosis, one of the most frequent and by far the most aggressive fibrotic lung disorder. This is an important distinction because while lung fibrosis preceded and triggered by inflammation may be controlled by anti-inflammatory therapy, mainly if the lung disorder is diagnosed in an early phase, IPF do not respond to treatment and should be faced with new different therapeutic approaches. Research and design of pharmacological protocols should consider pathogenic mechanisms related to epithelial cell activation, and should tows on epithelial-mesenchymal cell relationships. Likewise, a better understand of the gene-environment interactions in the lungs will contribute to improve our comprehension of the mechanisms involved in the development of pulmonary fibrosis.

9. REFERENCES

1. M.I. Schwarz: Approach to the understanding, diagnosis, and management of interstitial lung disease. In: Interstitial Lung Disease. Eds: Schwarz M.I., King T.E., B.C. Decker Inc, Hamilton, Ontario, 3-30 (1998)

2. Basset F, V.J. Ferrans, P. Soler, T. Takemura, Y. Fukuda & R.G. Crystal: Intraluminal fibrosis in interstitial lung disorders. *Am J Pathol* 122, 443-461 (1986)

3. Katzenstein A.L.A., & J.L. Myers: Idiopathic pulmonary fibrosis. Clinical relevance of pathologic classification. *Am J Respir Crit Care Med* 157, 1301-1315 (1998)

4. Selman M, T.E. King & A. Pardo: Idiopathic pulmonary fibrosis: Prevailing and evolving hypotheses about its pathogenesis and implications for therapy. *Ann Intern Med* 134, 136-151 (2001)

5. Selman M, & A. Pardo: Idiopathic pulmonary fibrosis: an epithelial/fibroblastic crosstalk disorder. *Resp Research* 3, 1-8, (2002).

6. Kourilsky P, & P. Truffa-Bachi: Cytokine fields and the polarization of the immune response. *TRENDS Immunol* 22, 502-509 (2001)

7. Mohrs M, C.M. Blankespoor, Z.E. Wang, G.G. Loots, V. Afzal, H. Hadeiba, K. Shinkai, E.M. Rubin & R.M. Locksley: Deletion of a coordinate regulator of type 2 cytokine expression in mice. *Nat Immunol* 2, 842-847 (2001)

8. Weiss D.L, & M.A. Brown: Regulation of IL-4 production in mast cells: a paradigm for cell-type-specific gene expression. *Immunol Rev* 179, 35-47 (2001)

9. Duncan M.R, & B. Berman: γ interferon is the lymphokine and b interferon the monokine responsible for inhibition of fibroblast collagen production and late but not early fibroblast proliferation. *J Exp Med* 162, 516-527 (1985)

10. Okada T, I. Sugie & K. Aisaka: Effects of gamma-interferon on collagen and histamine content in bleomycin-induced lung fibrosis in rats. *Lymphokine Cytokine Res* 12, 87-91 (1993).

11. Yokozeki M, Baba Y, Shimokawa H, Moriyama K, Kuruda T. Interferon-gamma inhibits the myofibroblastic phenotype of rat palatal fibroblasts induced by transforming growth factor-beta1 *in vitro*. *FEBS Lett* 442, 61-64 (1999)

12. Ziesche R, E. Hofbauer, K. Wittmann, V. Petkov & L.H. Block: A preliminary study of long-term treatment with interferon Gamma-1b and low-dose prednisolone in patients with idiopathic pulmonary fibrosis. *N Engl J Med* 341, 1264-1269 (1999)

13. Postlewaite A.E, M.A. Holness, H. Katai & R. Raghow: Human fibroblasts synthesize elevated levels of extracellular matrix proteins in response to interleukin-4. *J Clin Invest* 90, 1479-1485 (1992)

14. Oriente A, N.S. Fedarko, S.E. Pacocha, S.K. Huang, L.M. Lichtenstein & D.M. Essa: Interleukin-13 modulates collagen homeostasis in human skin keloid fibroblasts. *J Pharmacol Exp Ther* 292, 988, 994 (2000)

15. Hashimoto S, Y. Gon, I. Takeshita, S. Maruoka & T. Horie. IL-4 and IL-13 induce myofibroblastic phenotype of human lung fibroblasts through c-Jun NH2-terminal kinase-dependant pathway. *J Allergy Clin Immunol* 107, 1001-1008 (2001)

16. Westermann W, R. Schobl, E.P. Rieber & K.H. Frank: Th2 cells as effectors in postirradiation pulmonary damage preceding fibrosis in the rat. *Int J Radiat Biol* 75, 629-638 (1999)

17. Gharaee-Kermani M, B. McGarry, N. Lukacs, G. Huffnagle, R.W. Egan & S.H. Phan: The role of IL-5 in bleomycin-induced pulmonary fibrosis. *J Leukoc Biol* 64, 657-666 (1998)

18. Gharaee-Kermani M, Y. Nozaki, K. Hatano & S.H. Phan: Lung interleukin-4 gene expression in a murine model of bleomycin-induced pulmonary fibrosis. *Cytokine* 15, 138-147 (2001)

19. Wallace W.A, E.A. Ramage, D. Lamb & S.E. Howie: A type 2 (Th2-like) pattern of immune response predominates in the pulmonary interstitium of patients with cryptogenic fibrosing alveolitis (CFA). *Clin Exp Immunol* 101, 436-441 (1995)

20. Atamas S.P, V.V. Yurovsky, R. Wise, F.M. Wigley, C.J.G. Robinson, P. Henry, W.J. Alms & B. White:

Production of type 2 cytokines by CD8+ lung cells is associated with greater decline in pulmonary function in patients with systemic sclerosis. *Arthritis Rheum* 42, 1168-1178, (1999)
21. Prior C, & P.L. Haslam: *In vivo* levels and *in vitro* production of interferon-gamma in fibrosing interstitial lung diseases. *Clin Exp Immunol* 88, 280-287 (1992)
22. Yamasaki H, M. Ando, W. Brazer, D.M. Center & CW.W: Cruikshank: Polarized type 1 cytokine profile in bronchoalveolar lavage T cells of patients with hypersensitivity pneumonitis. *J Immunol* 163, 3516-3523 (1999)
23. Pérez-Padilla R, J. Salas, R. Chapela, M. Sánchez, G. Carrillo, R. Pérez, R. Sansores, M. Gaxiola & M. Selman: Mortality in Mexican patients with chronic pigeon breeders lung compared to those with usual interstitial pneumonia. *Am Rev Respir Dis* 148, 49-53 (1993)
24. Pardo A, R. Barrios, M. Gaxiola, L. Segura, G. Carrillo, A. Estrada, M. Mejía & M. Selman: Increase of lung neutrophils in hypersensitivity pneumonitis is associated with lung fibrosis. *Am J Respir Crit Care Med* 161, 1698-1704 (2000)
25. Zlotnik A, & O. Yoshie: Chemokines: a new classification system and their role in immunity. *Immunity* 12, 121-127 (2000)
26. Smith R.E: Chemotactic cytokines mediate leukocyte recruitment in fibrotic lung disease. *Biol Signals* 5, 223-231 (1996)
27. Hasegawa M, S. Sato & K. Takehara: Augmented production of chemokines (monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein-1alpha (MIP-1alpha) and MIP-1beta) in patients with systemic sclerosis: MCP-1 and MIP-1alpha may be involved in the development of pulmonary fibrosis. *Clin Exp Immunol* 117, 159-165 (1999)
28. Ziegenhagen M.W, S. Schrum, G. Zissel, P.F. Zipfel, M. Schlaak & J. Muller-Quernheim: Increased expression of proinflammatory chemokines in bronchoalveolar lavage cells of patients with progressing idiopathic pulmonary fibrosis and sarcoidosis. *J Investig Med* 46, 223-231 (1998)
29. Petrek M, P. Pantelidis, A.M. Southcott, P. Lympany, P. Safranek, C.M. Black, V. Kolek, E. Weigl & R.M. du Bois: The source and role of RANTES in interstitial lung disease. *Eur Respir J* 10, 1207-1216 (1997)
30. Pardo A, K.M. Smith, J. Abrams, R. Coffman, M. Bustos, T. K. McClanahan, J. Grein, E.E. Murphy, A. Zlotnik & M. Selman: CCL18/DC-CK-1/PARC upregulation in hypersensitivity pneumonitis. *J Leukoc Biol* 70, 610-619 (2001)
31. Belperio J.A, M.P. Keane, D.A. Arenberg, C.L. Addison, J.E. Ehlert, M.D. Burdick & R.M. Strieter: CXC chemokines in angiogenesis. *J Leukoc Biol* 68, 1-8 (2000)
32. Turner-Warwick M: Precapillary systemic-pulmonary anastomoses. *Thorax* 18, 225-237 (1963)
33. Fukuda Y, M. Ishizaki, S. Kudoh, M. Kitaichi & N.Yamanaka: Localization of matrix metalloproteinases-1, -2, and -9, and tissue inhibitor of metalloproteinase-2 in interstitial lung diseases. *Lab Invest* 78, 687-698 (1998)
34. Beer T.W, H.C. Baldwin, J.R. Goddard, P.J. Gallagher & D.H. Wright: Angiogenesis in pathological and surgical scars. *Hum Pathol* 29, 1273-1278 (1998)
35. Keane M.P, J.A. Belperio, D.A. Arenberg, M.D. Burdick, Z.J. Xu, Y.Y. Xue & R.M. Strieter: IFN-γ inducible protein-10 attenuates bleomycin-induced pulmonary fibrosis via inhibition of angiogenesis. *J Immunol* 163, 5686-5692 (1999)
36. Keane M.P, J.A. Belperio, T.A. Moore, B.B. Moore, D.A. Arenberg, R.E. Smith, M.D. Burdick, S.L. Kunkel & R.M. Strieter: Neutralization of the CXC chemokine, macrophage inflammatory protein-2, attenuates bleomycin-induced pulmonary fibrosis. *J Immunol* 162, 5511-5518 (1999)
37. Zingoni A, H. Soto, J.A. Hedrick, A. Stoppaciaro, C:T: Storlazzi, F. Sinigaglia, D. D´Ambrosio, A. O´Garra, D. Robinson, M. Rocchi, A. Santoni, A. Zlotnik & M. Napolitano: The chemokine receptor CCR8 is preferentially expressed in Th2 but not Th1 cells. *J Immunol* 161, 547-551 (1998)
38. Imai T, M. Nagira, S. Takagi, M. Kakizaki, M. Nishimura, J. Wang, P.W. Gray, K. Matsushima & O. Yoshie: Selective recruitment of CCR4-bearing Th2 cells toward antigen-presenting cells by the CC-chemokines thymus and activation-regulated chemokine and macrophage-derived chemokine. *Int Immunol* 11, 81-88 (1999)
39. Chensue S.W, N.W. Lukacs, T.Y. Yang, X. Shang, K.A. Frait, S.L. Kunkel, T. Kung, M.T. Wiekowski, J.A. Hedrick, D.N. Cook, A. Zingoni, S.K. Narula, A. Zlotnik, F.J. Barrat, A. O'Garra, M. Napolitano & S.A. Lira: Aberrant *in vivo* T helper type 2 cell response and impaired eosinophil recruitment in CC chemokine receptor 8 knockout mice. *J Exp Med* 193, 573-584 (2001)
40. Annunziato F, L. Cosmi, G. Galli, C. Beltrame, P. Romagnini, R. Manetti, S. Romagnini & E. Maggi: Assessment of chemokine receptor expression by human Th1 and Th2 cells *in vitro* and *in vivo. J Leukoc Biol* 65, 691-699 (1999)
41. Springer T.A: Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 76, 301-314 (1994)
42. Petruzzelli L, M. Takami & H.D. Humes: Structure and function of cell adhesion molecules. *Am J Med* 106, 467-476 (1999)
43. Paine R, & P.A. Ward: Cell adhesion molecules and pulmonary fibrosis. *Am J Med* 107, 268-279 (1999)
44. Hamacher J, & T. Schaberg: Adhesion molecules in lung diseases. *Lung* 172, 189-213 (1994)
45. Iyonaga K, M. Miyajima, M. Suga, N. Saita & M. Ando: Alterations in cytokeratin expression by the alveolar lining epithelial cells in lung tissues from patients with idiopathic pulmonary fibrosis. *J Pathol* 182, 217-224 (1997)
46. Uhal B.D, I. Joshi, A.L. True, S. Mundle, A. Raza, A. Pardo & M. Selman: Fibroblasts isolated after fibrotic lung injury induce apoptosis of alveolar epithelial cells *in vitro*. *Am J Physiol* 269, L819-828 (1995)
47. Wang R, C. Ramos, I. Joshi, A. Zagariya, A. Pardo, M. Selman & B. Uhal: Human lung myofibroblast-derived inducers of alveolar epithelial apoptosis identified as angiotensin peptides. *Am J Physiol* 277, L1158-L1164 (1999)
48. Uhal B.D, I. Joshi, W.F. Hughes, C. Ramos, A. Pardo & M. Selman: Alveolar epithelial cell death adjacent to underlying myofibroblasts in advanced fibrotic human lung. *Am J Physiol* 275, L1192-L1199 (1998)

49. Barbas-Filho J.V, M.A. Ferreira, A. Sesso, R.A. Kairalla, C.R. Carvalho & V.L. Capelozzi: Evidence of type II pneumocyte apoptosis in the pathogenesis of idiopathic pulmonary fibrosis (IFP)/usual interstitial pneumonia (UIP). *J Clin Pathol* 54, 132-138 (2001)
50. Kuwano K, H. Miyazaki, N. Hagimoto, M. Kawasaki, M. Fujita, R. Kunitake, Y. Kaneko & N. Hara: The involvement of Fas-Fas ligand pathway in fibrosing lung diseases. *Am J Respir Cell Mol Biol* 20, 53-60 (1999).
51. Maeyama T, K. Kuwano, M. Kawasaki, R. Kunitake, N. Hagimoto, T. Matsuba, M. Yoshimi, I. Inoshima, K. Yoshida & N. Hara: Upregulation of Fas-signalling molecules in lung epithelial cells from patients with idiopathic pulmonary fibrosis. *Eur Respir J* 17, 180-189 (2001)
52. Kasper M, & G. Haroske: Alterations in the alveolar epithelium after injury leading to pulmonary fibrosis. *Histol Histopathol* 11, 463-483 (1996)
53. Yokoyama A, N. Kohno, H. Hamada, M. Sakatani, E. Ueda, K. Kondo, Y. Hirasawa & K. Hiwada: Circulating KL-6 predicts the outcome of rapidly progressive idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 158, 1680-1684 (1998)
54. Selman M, M. Montaño, I. Montfort & R. Pérez-Tamayo: The duration of the pulmonary paraquat toxicity-enhancement effect of oxygen in the rat. *Exp Molec Pathol* 43, 388-396 (1985)
55. Woods L.W, D.W. Wilson & H.J. Segall: Manipulation of injury and repair of the alveolar epithelium using two pneumotoxicants: 3-methylindole and monocrotaline. *Exp Lung Res* 25, 165-181 (1999)
56. Adamson I.Y, L. Young & D.H. Bowden: Relationship of alveolar epithelial injury and repair to the induction of pulmonary fibrosis. *Am J Pathol* 130, 377-383 (1988)
57. Antoniades H.N, M.A. Bravo, R.E. Avila, T. Galanopoulus, J. Neville & M. Selman: Platelet-derived growth factor in idiopathic pulmonary fibrosis. *J Clin Invest* 86, 1055-1064 (1990)
58. Kapanci Y, A. Desmouliere, J.C. Pache, M. Redard & G. Gabbiani: Cytoskeletal protein modulation in pulmonary alveolar myofibroblasts during idiopathic pulmonary fibrosis. Possible role of transforming growth factor beta and tumor necrosis factor alpha. *Am J Respir Crit Care Med* 152, 2163-2169 (1995)
59. Nash J.R, P.J. McLaughlin, D. Butcher & B. Corrin: Expression of tumour necrosis factor-alpha in cryptogenic fibrosing alveolitis. *Histopathology* 22, 343-347 (1993)
60. Khalil N, R.N. O'Connor, H.W. Unruh, P.W. Warren, K.C. Flanders, A. Kemp, O.H. Bereznay & A.H. Greenberg: Increased production and immunohistochemical localization of transforming growth factor-beta in idiopathic pulmonary fibrosis. *Am J Respir Cell Mol Biol* 5, 155-162 (1991)
61. Khalil N, R.N. O'Connor, K.C. Flanders & H. Unruh: TGF-beta 1, but not TGF-beta 2 or TGF-beta 3, is differentially present in epithelial cells of advanced pulmonary fibrosis: an immunohistochemical study. *Am J Respir Cell Mol Biol* 14, 131-138 (1996)
62. Giaid A, R.P. Michel, D.J. Stewart, M. Sheppard, B. Corrin & Q. Hamid: Expression of endothelin-1 in lung of patients with cryptogenic fibrosing alveolitis. *Lancet* 341, 1550-1554 (1993)
63. Chetty A, S. Faber & H.C. Nielsen: Epithelial-mesenchymal interaction and insulin-like growth factors in hyperoxic lung injury. *Exp Lung Res* 25, 701-718 (1999)
64. Pan L.H, K. Yamauchi, M. Uzuki, T. Nakanishi, M. Takigawa, H. Inoue & T. Sawai: Type II alveolar epithelial cells and interstitial fibroblasts express connective tissue growth factor in IPF. *Eur Respir J* 17, 1220-1227 (2001)
65. Griffin, M., R. Bhandari, G. Hamilton, Y.C. Chan & J.T. Powell: Alveolar type II cell-fibroblast interactions, synthesis and secretion of surfactant and type I collagen. J Cell Sci 105, 423-432 (1993)
66. Wallace W.A, & S.E. Howie: Immunoreactive interleukin 4 and interferon-gamma expression by type II alveolar epithelial cells in interstitial lung disease. *J Pathol* 187, 475-480 (1999)
67. Furuyama A, K. Kimata & K. Mochitate: Assembly of basement membrane *in vitro* by cooperation between alveolar epithelial cells and pulmonary fibroblasts. *Cell Struct Funct* 22, 603-614 (1997)
68. Young L, & I.Y.R. Adamson: Epithelial-fibroblast interactions in bleomycin-induced lung injury and repair. *Environm Health Perspect* 101, 56-61 (1993)
69. Finch P.W, S.S. Rubin, T. Miki, D. Ron & S.A. Aaronson: Human KGF is FGF-related with properties of a paracrine effector of epithelial cell growth. *Science* 245, 752-755 (1989)
70. Klein J.H, & I.Y.R. Adamson: Fibroblast inhibition and prostaglandin secretion by alveolar epithelial cells exposed to silica. *Lab Invest* 60, 808-813 (1989)
71. Martin P: Wound healing. Aiming for perfect skin regeneration. *Science* 276, 75-81 (1997)
72. Chapman H.A, C. L. Allen & O.L. Stone: Abnormalities in pathways of alveolar fibrin turnover among patients with interstitial lung disease. *Am Rev Respir Dis* 133, 437-443 (1986)
73. Kotani I, A. Sato, H. Hayakawa, T. Urano, Y. Takada & A. Takada: Increased procoagulant and antifibrinolytic activities in the lungs with idiopathic pulmonary fibrosis. *Thromb Res* 77, 493-504 (1995)
74. Imokawa S, A. Sato, H. Hayakawa, M. Kotani, T. Urano & A. Takada: Tissue factor expression and fibrin deposition in the lungs of patients with idiopathic pulmonary fibrosis and systemic sclerosis. *Am J Respir Crit Care Med* 156, 631-636 (1997)
75. Fujii M, H. Hayakawa, T. Urano, A. Sato, K. Chida, H. Nakamura & A. Takada: Relevance of tissue factor and tissue factor pathway inhibitor for hypercoagulable state in the lungs of patients with idiopathic pulmonary fibrosis. *Thromb Res* 99, 111-117 (2000)
76. Eitzman DT, McCoy RD, Zheng X, Fay WP, Shen T, Ginsburg D & R.H. Simon: Bleomycin-induced pulmonary fibrosis in transgenic mice that either lack or overexpress the murine plasminogen activator inhibitor-1 gene. *J Clin Invest* 97, 232-237 (1996)
77. Andrew A.S, L.R. Klei & A. Barchowsky: AP-1-dependent induction of plasminogen activator inhibitor-1 by nickel does not require reactive oxygen. *Am J Physiol* 281, L616-L623 (2001)
78. Murphy G, H. Stanton, S. Cowell, G. Butler, V. Knauper, S. Atkinson & J. Gavrilovic: Mechanisms for pro matrix metalloproteinase activation. *APMIS* 107, 38-44 (1999)

79. Legrand C, M. Polette, J.M. Tournier, S. De Bentzmann, E. Huet C. Monteau & P. Birembaut: uPa/plasmin system-mediated MMP-9 activation is implicated in bronchial epithelial cell migration. *Exp Cell Res* 264, 326-336 (2001)
80. Gandossi E, C. Lunven & C.N. Berry: Role of clot-associated (-derived) thrombin in cell proliferation induced by fibrin clots *in vitro*. *Br J Pharmacol* 129, 1021-1027 (2000)
81. Simon R.H, M.J. Scott, M.M. Reza & P.D. Killen: Type IV collagen production by rat pulmonary alveolar epithelial cells. *Am J Respir Cell Mol Biol* 8, 640-646 (1993)
82. Sage H, F.M. Farin, G.E. Striker & A.B. Fisher: Granular pneumocytes in primary culture secrete several major components of the extracellular matrix. *Biochemistry* 22, 2148-2155 (1983)
83. Leheup B.P, S.J. Federspiel, M.L. Guerry-Force, N.T. Wetherall, P.A. Commers, S.J. DiMari & M.A. Haralson: Extracellular matrix biosynthesis by cultured fetal rat lung epithelial cells. I. Characterization of the clone and the major genetic types of collagen produced. *Lab Invest* 60, 791-807 (1989)
84. Bodo M, T. Baroni, S. Bellocchio, M. Calvitti, C. Lilli, A. D'Alessandro, G. Muzi, A. Lumare & G. Abbritti: Bronchial epithelial cell matrix production in response to silica and basic fibroblast growth factor. *Mol Med* 7, 83-92 (2001)
85. Iwashita T, J. Kadota, S. Naito, H. Kaida, Y. Ishimatsu, M. Miyazaki, Y. Ozono Y & S. Kohno: Involvement of collagen-binding heat shock protein 47 and procollagen type I synthesis in idiopathic pulmonary fibrosis: contribution of type II pneumocytes to fibrosis. *Hum Pathol* 31, 1498-1505 (2000)
86. Kasper M, & H. Fehrenbach: Immunohistochemical evidence for the occurrence of similar epithelial phenotypes during lung development and radiation-induced fibrogenesis. *Int J Radiat Biol* 76, 493-501 (2000)
87. King T.E, M.I. Schwarz, K. Brown, J.A. Tooze, T.V. Colby, J.A. Waldron, A. Flint, W. Thurlbeck & R.M. Cherniak: Extent of fibroblast foci predict mortality in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 163, A982 (2001)
88. A. Desmouliere, & G. Gabbiani: The role of the myofibroblast in wound healing and fibrocontractive diseases. In: The molecular and cellular biology of wound repair. Ed: Clark R.A.F., Plenum Press, NY 391-423 (1996)
89. Chiavegato A, M.L. Bochaton-Piallat, E. D'Amore, S. Sartore & G. Gabbiani: Expression of myosin heavy chain isoforms in mammary epithelial cells and in myofibroblasts from different fibrotic settings during neoplasia. *Virchows Arch* 426, 77-86 (1995)
90. Kapanci Y, A. Assimacopoulos, C. Irle, A. Zwahlen & G. Gabbiani: "Contractile interstitial cells" in pulmonary alveolar septa: a possible regulator of ventilation-perfusion ratio?. Ultrastructural, immunofluorescence, and *in vitro* studies. *J Cell Biol* 60, 375-392 (1974),
91. Vyalov S.L, G. Gabbiani, & Y. Kapanci: Rat alveolar myofibroblasts acquire alpha-smooth muscle actin expression during bleomycin-induced pulmonary fibrosis. *Am J Pathol* 143, 1754-1765 (1993)
92. Sappino A. P, W. Schurch, & G. Gabbiani: Differentiation repertoire of fibroblastic cells: Expression of cytoskeletal proteins as marker of phenotypic modulations. *Lab Invest* 63,144-161 (1990)
93. Uhal B.D, Ramos C., Joshi I., Bifero A., Pardo A., & M. Selman: Cell size, cell cycle, and -smooth muscle actin expression by primary human lung fibroblasts. *Am J Physiol* 275, L998-L1005 (1998)
94. Suganuma H, A. Sato, R. Tamura & K. Chida: Enhanced migration of fibroblasts derived from lungs with fibrotic lesions. *Thorax* 50, 984-989 (1995)
95. Bonner J.C, P.M. Lindroos, A.B. Rice, C.R. Moomaw & D.L. Morgan: Induction of PDGF receptor-alpha in rat myofibroblasts during pulmonary fibrogenesis *in vivo. Am J Physiol* 274, L72-L80 (1998)
96. Yu J, A. Moon & H.R. Kim: Both platelet-derived growth factor receptor (PDGFR)-alpha and PDGFR-beta promote murine fibroblast cell migration. *Biochem Biophys Res Commun* 282, 697-700 (2001)
97. Sasaki M, M. Kashima, T. Ito, A. Watanabe, N. Izumiyama, M. Sano, M. Kagaya, T. Shioya & M. Miura: Differential regulation of metalloproteinase production, proliferation and chemotaxis of human lung fibroblasts by PDGF, interleukin-1beta and TNF-alpha. *Mediators Inflamm* 9, 155-160 (2000)
98. Hirasawa Y, N. Kohno, A. Yokoyama, Y. Inoue, M. Abe & K. Hiwada: KL-6, a human MUC1 mucin, is chemotactic for human fibroblasts. *Am J Respir Cell Mol Biol* 17, 501-507 (1997)
99. Kawamoto M, Matsunami T, Ertl RF, Fukuda Y, Ogawa M, Spurzem JR, Yamanaka N, Rennard SI. Selective migration of alpha-smooth muscle actin-positive myofibroblasts toward fibronectin in the Boyden's blindwell chamber. *Clin Sci* 93, 355-362 (1997)
100. Micera A, E. Vigneti, D. Pickholtz, R. Reich, O. Pappo, S. Bonini, F.X. Maquart, L. Aloe, & F. Levi-Schaffer: Nerve growth factor displays stimulatory effects on human skin and lung fibroblasts, demonstrating a direct role for this factor in tissue repair. *Proc Natl Acad Sci USA* 98, 6162-6167 (2001)
101. Jordana M, J. Schulman, C. McSharry, L. B. Irving, M. T. Newhouse, G. Jordana, & J. Gauldie: Heterogeneous proliferative characteristics of human adult lung fibroblast lines and clonally derived fibroblasts from control and fibrotic Tissue. *Am Rev Respir Dis* 137, 579- 584 (1988)
102. Ramos C, M. Montaño, J. García-Alvarez, V. Ruiz, B.D. Uhal, M. Selman, & A. Pardo: Fibroblasts from idiopathic pulmonary fibrosis and normal lungs differ in growth rate, apoptosis, and TIMP's expression. *Am J Respir Cell Mol Biol* 24, 591-598 (2001)
103. Raghu G, Y.Y. Chen, V. Rusch, & P. S. Rabinovitch: Differential proliferation of fibroblasts cultured from normal and fibrotic human lung. *Am Rev Respir Dis* 138: 703-708 (1988)
104. Phan S.H, J. Varani, & D. Smith: Rat lung fibroblast collagen metabolism in bleomycin-induced pulmonary fibrosis. *J Clin Invest* 76, 241-247 (1985)
105. Nozaki Y, T. Liu, K. Hatano, M. Gharaee-Kermani, & S. H. Phan: Induction of Telomerase Activity in Fibroblasts from Bleomycin-Injured Lungs. *Am J Respir Cell Mol Biol* 23, 460-465 (2000)

106. Ronnov-Jessen L, & O.W. Petersen: A function for filamentous alpha-smooth muscle actin: retardation of motility in fibroblasts *J Cell Biol* 134, 67-80 (1996)
107. P. Martin: Wound healing--aiming for perfect skin regeneration. *Science* 276, 75-81 (1997)
108. Bloor C.A, R.A. Knight, R.K. Kedia, M.A. Spiteri & J.T. Allen: Differential mRNA expression of insulin-like growth factor-1 splice variants in patients with idiopathic pulmonary fibrosis and pulmonary sarcoidosis. *Am J Respir Crit Care Med* 164, 265-272 (2001)
109. Harrison N.K, A.D. Cambrey, A.R. Myers, A.M. Southcott, C.M. Black, R.M. DuBois, G.J. Laurent & R.J. McAnulty: Insulin-like growth factor-1 is partially responsible for fibroblast proliferation induced by bronchoalveolar lavage fluid from patients with systemic sclerosis. *Clin Sci* 86, 141-148 (1994)
110. Allen T.J, C.A. Bloor, R.A. Knight & M.A. Spiteri: Expression of insulin-like growth factor binding proteins in bronchoalveolar lavage fluid of patients with pulmonary sarcoidosis. *Am J Respir Crit Care Med* 99, 250-258 (1998)
111. Uh S.T, Y. Inoue, T.E. King Jr, E.D. Chan, L.S. Newman & D.W. Riches DW: Morphometric analysis of insulin-like growth factor-I localization in lung tissues of patients with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 158, 1626-1635 (1998)
112. Dabbagh K, G.J. Laurent, A. Shock, P. Leoni, J. Papakrivopoulou & R.C. Chambers: Alpha-1-antitrypsin stimulates fibroblast proliferation and procollagen production and activates classical MAP kinase signalling pathways. *J Cell Physiol* 186, 73-81 (2001)
113. Kuhn C, & J.A. McDonald: The roles of the myofibroblast in idiopathic pulmonary fibrosis. Ultrastructural and immunohistochemical features of sites of active extracellular matrix synthesis. *Am J Pathol* 138, 1257-1265 (1991)
114. Adler K.B, L.M. Callahan, & J.N. Evans: Cellular alterations in the alveolar wall in bleomycin-induced pulmonary fibrosis in rats. An ultrastructural morphometric study. *Am Rev Respir Dis* 133, 1043–1048 (1986)
115. Gressner A.M: Transdifferentiation of hepatic stellate cells (Ito cells) to myofibroblasts: a key event in hepatic fibrogenesis. *Kidney Int* suppl 54, S39-S45 (1996)
116. Bachem M.G, E. Scneider, H. Gross, H. Weidenbach, R. M. Schmid, A. Menke, M. Siech, H. Beger, & A. Grunert. Identification, culture, and characterization of pancreatic stellate cells in rats and humans. *Gastroenterology* 115, 421-432 (1998)
117. Serini G, & G. Gabbiani: Mechanisms of myofibroblast activity and phenotypic modulation. *Exp Cell Res* 250,273-283 (1999)
118. Desmouliere A, A. Geinoz, F. Gabiani, & G. Gabbiani: Transforming growth factor-β1 induces a smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. *J Cell Biol* 122, 103-11 (1993)
119. Sime P.J, Z. Xing, F.L. Graham, K.G. Csaky, & J. Gauldie: Adenovector-mediated gene transfer of active transforming growth factor-beta1 induces prolonged severe fibrosis in rat lung. *J Clin Invest* 100, 768-76 (1997)
120. Xing Z, G.M. Tremblay, P.J. Sime, & J. Gauldie. Overexpression of granulocyte-macrophage colony-stimulating factor induces pulmonary granulation tissue formation and fibrosis by induction of transforming growth factor-beta 1 and myofibroblast accumulation. *Am J Pathol* 150:59-66 (1997)
121. Andreutti D, G. Gabbiani, & P. Neuville: Early granulocyte-macrophage colony-stimulating factor expression by alveolar inflammatory cells during bleomycin-induced rat lung fibrosis. *Lab Invest* 78, 1493-502 (1998)
122. Serini G, M.L. Bochaton-Piallat, P. Ropraz, A. Geinoz, L. Borsi, L. Zardi & G. Gabbiani: The fibronectin domain ED-A is crucial for myofibroblastic phenotype induction by transforming growth factor-β1. *J Cell Biol* 142, 873-881 (1998)
123. Xu G, M.L. Bochaton-Piallat, D. Andreutti, R.B. Low, G. Gabbiani & P. Neuville: Regulation of alpha-smooth muscle actin and CRBP-1 expression by retinoic acid and TGF-beta in cultured fibroblasts. *J Cell Physiol* 187, 315-325 (2001).
124. Lappi-Blanco E, Y. Soini & P. Paakko: Apoptotic activity is increased in the newly formed fibromyxoid connective tissue in bronchiolitis obliterans organizing pneumonia. *Lung* 177:367-376 (1999)
125. Jelaska A, & J.H. Korn: Role of apoptosis and transforming growth factor beta1 in fibroblast selection and activation in systemic sclerosis. *Arthritis Rheum* 43, 2230-2239 (2000)
126. Zhang H.Y, & S.H. Phan:. Inhibition of myofibroblast apoptosis by transforming growth factor beta (1). *Am J Respir Cell Mol Biol* 21, 658-665 (1999)
127. Santiago B, M. Galindo, M. Rivero & J.L. Pablos: Decreased susceptibility to Fas-induced apoptosis of systemic sclerosis dermal fibroblasts. *Arthritis Rheum* 44, 1667-1676 (2001)
128. Zhang K, M. D. Rekhter, D. Gordon, & S.H. Phan: Myofibroblasts and their role in lung collagen gene expression during pulmonary fibrosis. *Am J Pathol* 145, 114-125 (1994)
129. Fukuda Y, M. Ishizaki, S. Kudoh, M. Kitaichi & N. Yamanaka: Localization of matrix metalloproteinases-1, -2, and -9 and tissue inhibitor of metalloproteinase-2 in interstitial lung diseases. *Lab Invest* 78, 687-698 (1998)
130. Selman M, V. Ruiz, S. Cabrera, L. Segura, R. Ramírez, R. Barrios & A. Pardo: Localization of tissue inhibitor of metalloproteinases (TIMPs) -1, -2, -3, and -4 in idiopathic pulmonary fibrosis. TIMPs/collagenases imbalance in the fibrotic lung microenvironment. *Am J Physiol* 279, L562-L574 (2000)
131. Hayashi T, W.G. Stetler-Stevenson, M.V. Fleming, N. Fishback, M.N. Koss, L.A. Liotta, V.J. Ferrans & W.D. Travis: Immunohistochemical study of metalloproteinases and their tissue inhibitors in the lungs of patients with diffuse alveolar damage and idiopathic pulmonary fibrosis. *Am J Pathol* 149, 1241-1256 (1996)
132. Yaguchi T, Y. Fukuda, M. Ishizaki & N. Yamanaka: Immunohistochemical and gelatin zymography studies for matrix metalloproteinases in bleomycin-induced pulmonary fibrosis. *Pathol Int* 48, 954-963 (1998)
133. Alexander C.M, E.W. Howard, M.J. Bissell & Z. Werb: Rescue of mammary epithelial cell apoptosis and entactin degradadtion by a tissue inhibitor of metalloproteinase-1 transgene. *J Cell Biol* 135:1669-1677 (1996)

134. Woessner J. F, & H. Nagase: Matrix metalloproteinases and TIMPs, New York: Oxford University Press; (2000)
135. Brew K, D. Dinakarpandian, & H. Nagase: Tissue inhibitors of metalloproteinases: evolution, structure and function. *Biochim Biophys Acta* 1477, 267-283 (2000)
136. Pardo A, M. Selman, R. Ramírez, C. Ramos, M. Montaño, G. Stricklin, & G. Raghu: Production of collagenase and tissue inhibitor of metalloproteinases by fibroblasts derived from normal and fibrotic human lungs. *Chest* 102, 1085-1089, (1992)
137. Dumin J. A, S.K. Dickeson, T.P. Stricker, M. Bhattacharyya-Pakrasi, J.D. Roby, S.A. Santoro, W.C. Parks: Pro-collagenase-1 (matrix metalloproteinase-1) binds the alpha(2)beta(1) integrin upon release from keratinocytes migrating on type I collagen. *J Biol Chem* 276, 29368-29374 (2001)
138. Pérez-Ramos J, L. Segura, R. Ramírez, B. Vanda, M. Selman, & A. Pardo: Matrix metalloproteinases 2, 9, and 13 and tissue inhibitor of metalloproteinases 1 and 2 in early and late lesions of experimental lung silicosis. *Am J Respir Crit Care Med* 160, 1274-1282 (1999)
139. McCawley L.J, & L.M. Matrisian. Matrix metalloproteinases: they're not just for matrix anymore, *Curr Op Cell Biol* 13, 534-540 (2001)
140. Swiderski R.E, J.E. Dencoff, C.S. Floerchinger, S.D. Shapiro, & G. W. Hunninghake: Differential expression of extracellular matrix remodeling genes in a murine model ofbleomycin-induced pulmonary fibrosis. *Am J Pathol* 152, 821-828 (1998)
141. Zuo F, N. Kaminski, E. Eugui, J. Allard, Z. Yakhini, A. Ben-Dor, L. Lollini, D. Morris, Y. Kim, B. DeLustro, D. Sheppard, A. Pardo, M. Selman, & R.A. Heller: Gene expression analysis reveals matrilysin as a key regulator of pulmonary fibrosis in mice and humans. *Proc Natl Acad Sci USA* 99, 6292-6297 (2002)
142. Amour A, P.M. Slocombe, A. Webster, M. Butler, C.G. Knight, B.J. Smith, P.E. Stephens, C. Shelley, M. Hutton, V. Knauper, A.J. Docherty, & G. Murphy: TNF-alpha converting enzyme (TACE) is inhibited by TIMP-3. *FEBS Lett* 435, 39-44 (1998).
143. Leco K.J, P. Waterhouse, O.H. Sanchez, K.L. Gowing, A.R. Poole, A. Wakeham , T.W. Mak, & Khokha R: Spontaneous air space enlargement in the lungs of mice lacking tissue inhibitor of metalloproteinases-3 (TIMP-3). *J Clin Invest* 108, 817-829.
(2001)
144. M Selman: Hypersensitivity Pneumonitis. In: Interstitial Lung Disease. Eds: Schwarz M, King T, B.C. Decker Inc., Hamilton Ontario, 393-422 (1998)
145. Hirvonen A, S.T. Saarikoski, K. Linnainmaa, K. Koskinen, K. Husgafvel-Pursiainen, K. Mattson & H. Vainio: Glutathione S-transferase and N-acetyltransferase genotypes and asbestos-associated pulmonary disorders. *J Natl Cancer Inst* 88, 1853-1856 (1996)
146. Tisdale J.E, S.L, Follin, A. Ordelova & C.R. Webb: Risk factors for the development of specific noncardiovascular adverse effects associate with amiodarone. *J Clin Pharmacol* 35, 351-356 (1995)
147. Schrier D.J, R.G Kunkel & S.H. Phan: The role of strain variation in murine bleomycin-induced pulmonary fibrosis. *Am Rev Respir Dis* 127, 63-66 (1993)
148. Haston C.K, & E.L. Travis: Murine susceptibility to radiation-induced pulmonary fibrosis is influenced by a genetic factor implicated in susceptibility to bleomycin-induced pulmonary fibrosis. *Cancer Res* 57, 5286-5291 (1997)
149. Bitterman P.B, S.I. Rennard, B.A. Keogh, M.D. Wewers, S. Adelberg & R.G. Crystal: Familial idiopathic pulmonary fibrosis. Evidence of lung inflammation in unaffected family members. *N Engl J Med* 314, 1343-1347 (1986)
150. Marshall R.P, A. Puddicombe, W.O.C. Cookson & G.J. Laurent: Adult familial cryptogenic fibrosing alveolitis in the United Kingdom. *Thorax* 55, 143-146 (2000)
151. Javaheri S, D.H. Lederer, J.A. Pella, G.J. Mark & B.W. Levine: Idiopathic pulmonary fibrosis in monozygotic twins. The importance of genetic predisposition. *Chest* 78, 591-594 (1980)
152. Thomas H, & U. Costabel: Progressive course of idiopathic pulmonary fibrosis in 2 monozygotic twin sisters. *Pneumologie* 50, 679-682 (1996)
153. Mageto Y.N, & G. Raghu: Genetic predisposition of idiopathic pulmonary fibrosis. *Curr Op Pulm Med* 3, 336-340 (1997)
154. Musk A.W, P.J. Zitko, P. Manners, P.H. Kay & M.I. Kamboh: Genetic studies in familial fibrosing alveolitis. Possible linkage with immunoglobulin allotypes (Gm). *Chest* 89, 206-210 (1986)
155. Lane K. B, A. Marney, J.A. Phillips, E. Loyd, R. Gaddipati & J. Loyd: Familial interstitil pulmonary fibrosis and linkage to chromosome 14. *Chest* 120, Suppl 75S-76S (2001)
156. Nogee L.M, A.E. Dunbar, S.E. Wert, F. Askin, A. Hamvas & J.A. Whitsett: A mutation in the surfactant protein C gene associated with familial interstitial lung disease. *N Eng J Med* 344, 573-579 (2001)
157. Avila J.J, P.A. Lympany, P. Pantelidis, K.I. Welsh, C.M. Black & R.M. du Bois: Fibronectin gene polymorphisms associated with fibrosing alveolitis in systemic sclerosis. *Am J Respir Cell Mol Biol* 20, 106-112 (1999)
158. Whyte M, R. Hubbard, R. Meliconi, M. Whidborne, V. Eaton, C. Bingle, J. Timms, G. Duff, A. Facchini, A. Pacilli, M. Fabbri, I. Hall, J. Britton, I. Johnston & F. Di Giovine: Increased risk of fibrosing alveolitis associated with interleukin-1 receptor antagonist and tumor necrosis factor-α gene polymorphisms. *Am J Respir Crit Care Med* 162, 755-758 (2000)
159. Yucesoy B, V. Vallyathan, D.P. Landsittel, D.S. Sharp, A. Weston, G.R. Burleson, P. Simeonova, M. McKinstry & M.I. Luster: Association of tumor necrosis factor-alpha and interleukin-1 gene polymorphisms with silicosis. *Toxicol Appl Pharmacol* 172, 75-82 (2001).
160. Pantelidis P, G.C. Fanning, A.U. Wells, K.I. Welsh & R.M. du Bois: Analysis of tumor necrosis factor-α, lymphotoxin-α, tumor necrosis factor receptor II, and interleukin-6 polymorphisms in patients with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 163, 1432-1436 (2001)
161. Awad M.R, A. El-Gamel, P. Hasleton, D.M. Turner, P.J. Sinnott & I.V. Hutchinson: Genotypic variation in the transforming growth factor-β1 gene. Association with transforming growth factor-β1 production, fibrotic lung

disease, and graft fibrosis after lung transplantation. *Transplantation* 66, 1014-1020 (1998).
162. El-Gamel A, M.R. Awad, P.S. Hasleton, N.A. Yonan, J.A. Hutchinson, C.S. Campbell, A.H. Rahman, A.K. Deiraniya, P.J. Sinnott & I.V. Hutchinson IV: Transforming growth factor-beta (TGF-beta1) genotype and lung allograft fibrosis. *J Heart Lung Transplant* 18, 517-523 (1999)
163. Mori M, H. Kida, H. Morishita, S. Goya, H. Matsuoka, T. Arai, T. Osaki, I. Tachibana, S. Yamamoto, M. Sakatani, M. Ito, T. Ogura & S. Hayashi: Microsatellite instability in transforming growth factor β-1 type II receptor gene in alveolar lining epithelial cells in idiopathic pulmonary fibrosis. *Am J Respir Cell Mol Biol* 24, 398-404 (2001)
164. Wilborn J, L.J. Crofford, M.D. Burdick, S.L. Kunkel, R.M. Strieter & M. Peters-Golden: Cultured lung fibroblasts isolated from patients with idiopathic pulmonary fibrosis have a diminished capacity to synthesize prostaglandin E2 and to express cyclooxygenase-2. *J Clin Invest* 95, 1861-1868 (1995)
165. Keerthisingam C.B, R.G. Jenkins, N.K. Harrison, N.A. Hernandez-Rodriguez, H. Booth, G.J. Laurent, S.L. Hart, M.L. Foster & R.J. McAnulty: Cyclooxygenase-2 deficiency results in a loss of the anti-proliferative response to transforming growth factor-beta in human fibrotic lung fibroblasts and promotes bleomycin-induced pulmonary fibrosis in mice. *Am J Pathol* 158, 1411-1422 (2001)
166. Camarena A, A. Juárez, M. Mejía, A. Estrada, G. Carrillo, R. Falfán, J. Zúñiga, C. Navarro, J. Granados & M. Selman: Major histocompatibility complex and TNF-α gene polymorphisms in pigeon breeder´s disease. *Am J Respir Crit Care Med* 163, 1528-1533 (2001)
167. Kaminski N, J.D. Allard, J.F. Pittet, F. Zuo, M.J.D. Griffiths, D. Morris, X. Huang, D. Sheppard & R.A. Heller: Global analysis of gene expression in pulmonary fibrosis reveals distinct programs regulating lung inflammation and fibrosis. *Proc Natl Acad Sci (USA)* 97, 1778-1783 (2000)
168. Takahashi F, K. Takahashi, T. Okazaki, K. Maeda, H. Ienaga, M. Maeda, S. Kon, T. Uede & Y. Fukuchi: Role of osteopontin in the pathogenesis of bleomycin-induced pulmonary fibrosis. *Am J Respir Cell Mol Biol* 24, 264-271 (2001)

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