Tissue repair in lung disorders

Andersson Sjöland, Annika

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From the Department of Clinical Sciences, Lund
and
From the Department of Experimental Medical Science
Lund University

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Annika Andersson Sjöland

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Annika Andersson Sjöland

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Abstract

The remodeling phase of tissue repair in lung disorders such as idiopathic pulmonary disease, asthma, obliterative bronchiolitis, and after lung transplantation is not well understood. One of the key players in fibrosis is the fibroblast and its progenitor, the fibrocyte. The fibroblast is the main producer of extracellular matrix molecules such as collagen, versican, perlecan, biglycan, and decorin. Fibrocytes are recruited from the bone marrow to the site of injury. It is possible that the stromal cell-derived factor-1/CXCL12 builds up in a gradient to recruit CXCR4 expressing fibrocytes, at least in the case of idiopathic pulmonary fibrosis. Patients with obliterative bronchilitis have an enlarged vessel lumen and a larger endothelial layer area, which has been shown to be correlated to more fibrocytes in the tissue. A correlation was found between the numbers of fibrocytes identified in tissue and structural changes in the lung. In idiopathic pulmonary fibrosis, a correlation was found between numbers of fibrocytes and numbers of fibroblastic foci, and in patients with obliterative bronchiolitis there was a correlation between fibrocyte numbers and thickening of the alveolar parenchyma. Eventually the fibrocyte enters the tissue and can differentiate into a fibroblast.

Matrix production by fibroblasts in patients who have undergone lung transplantation and in asthmatic subjects involves both the central and the distal parts of the lung. In lung-transplanted patients, the production of proteoglycans in the distal part was found to be double that in the central part. Versican production was particularly elevated compared to controls. The production of proteoglycans was further correlated with proliferation rate. A common feature of fibroblasts in patients after lung transplantation and asthmatic subjects was that these cells proliferated more slowly than in control subjects. Distally-derived fibroblasts from asthmatic subjects produced more veriscan, were immobile, and had many protrusions. The distal parts of the lungs were also rich in collagen.

A better understanding of fibrocyte and fibroblast function will help us to characterise the mechanisms behind idiopathic pulmonary disease, asthma, obliterative bronchiolitis. This knowledge can then be applied to other types of fibrotic disorders.

Key words: fibrocyte, fibroblast, lung transplantation, idiopathic pulmonary fibrosis, asthma, obliterians bronchiolitis, bronchial obliterians syndome, remodelling, vessels

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Annika Andersson Sjöland

LUND
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Additional papers

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Abbreviations

BAL	bronchoalveolar lavage
BOS	bronchiolitis obliterans syndrome
CF	cystic fibrosis
CMV	cytomegalovirus
CS	chondriotin sulphate
COPD	chronic obstructive pulmonary disease
DAPI	4',6-diamidino-2-phenylindole
DMEM	Dulbecco's Eagle's minimal essential medium
DS	dermatan sulphate
ECM	extracellular matrix
EGF	epidermal growth factor
EMT	epithelial-mesenchymal transition
FGF	fibroblast growth factor
FEV_1	forced expiratory volume in 1 second
HIF	hypoxia-induced factor
HS	heparin sulphate
GAG	glycosaminoglycan
IFN-γ	interferon-γ
IL	interleukin
IPF	idiopathic pulmonary fibrosis
MAPK	mitogen-activated protein kinases
MHC	major histocompatibility complex
MMP	matrix metalloproteinase
OB	obliterative bronchiolitis
PDGF	platelet-derived growth factor
SAPK/JNK	stress-activated protein kinase / jun N-terminal kinases
SDF-1 / CXCL12	stromal cell-derived factor-1 / chemokine ligand 12
α-SMA	α-smooth muscle cell
TGF-β	transforming growth factor-β
TNF-α	tumor necrosis factor-α
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor
vWF	von Willebrand factor
ZO	zona occludens

Introduction

Tissue repair processes and remodelling are ongoing processes in all types of wound healing. In healthy subjects, the primary role of the extracellular matrix (ECM) is to provide tissues with specific mechanical properties, and to serve as a structural framework for cell attachment and migration. An ongoing tissue repair can result in fibrosis, regarded as an abnormal wound-healing process. In the lung the fibrosis can be localised in the central part of the lung, or in the distal alveolar parenchymal part, or something in-between, in the small airways. In this thesis, we have included three different patient groups believed to differ somewhat in primary site of fibrotic deposition. In asthma, the basement membrane, which is located below the epithelial layer, is thickened because of accumulation of collagens and proteoglycans. Idiopathic pulmonary fibrosis (IPF) is characterised by fibroblastic foci that are in demarcated areas rich in ECM and proteoglycans but with few cells. In obliterative bronchiolitis (OB), the small airways are obliterated with ECM where the proteoglycans function as staples to attach the connective tissue. In OB, the parenchymal part of the lung is also involved with a thickening of the alveolar septa. The structural changes will be discussed in more detail later in this thesis.

The above-mentioned disorders are chronic diseases with remodelling of both the airways and the pulmonary vessels. The remodelling processes have many differences but, surprisingly, also many similarities even though the underlying pathophysiologies are different. Remodelling usually starts with an epithelial injury that later gives rise to structural changes in the airways and in the lung architecture, featuring lung function decrease and chronic airway symptoms.

Tissue repair and inflammation often interact in a dynamic and parallel manner. The players at both the cellular and the molecular levels are dependent on both the type of disease and the disease state of the patient. Inflammation is a process that can be preceded by infections. Depending of what kind of stimuli triggered the inflammation, the response of the immune system is regulated in different ways. The immune system can be divided into an *innate part* on the one hand, including barriers to the surroundings, eosinophils, neutrophils, macrophages, and natural killer cells, and an *adaptive part* on the other, including T-lymphocytes and B-lymphocytes.

One of the diseases that has both inflammatory and remodelling features is asthma. Asthma is a widespread disease; today, 8% of the adult population in Sweden suffers from asthma (1). Another condition in which both inflammation and

remodelling are important characteristics is chronic rejection after organ transplantation. The first lung transplantation was done in 1983 in Toronto, Canada (2) and today around 40 lung transplantations are performed annually in Sweden (about 15 at the University hospital in Lund and about 25 at Sahlgrenska University Hospital in Göteborg). Unfortunately, up to 60% of the transplanted patients develop bronchiolitis obliterans syndrome (BOS), as a sign of chronic rejection. IPF is a disease where the cause still is unclear and the possibilities of treatments are limited. Some patients with IPF will eventually be candidates for lung transplantation. However, the most common disorders today, leading to transplantations are chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF) (3).

The aims of the work for this thesis were to determine whether fibrocytes, which are progenitors of fibroblasts, are of importance in tissue remodeling. A second aim was to further characterise factors involved in the recruitment of fibrocytes from the bloodstream to specific areas of the lung. A third aim was to identify phenotypes of fibroblasts that are associated with tissue repair.

Background

Lung Disorders

This thesis concentrates on tissue repair, and remodelling in lung disorders such as IPF, OB after lung or bone marrow transplantation, and asthma is discussed. The origins of these disorders are different, but they have the common denominator that ECM deposition changes the lung structure and causes deterioration of the tissue, and thereby of lung function.

Idiopathic Pulmonary Fibrosis

IPF is a chronic disease that is usually lethal, is progressive, and is of unknown cause (4). The disease is by definition grouped as an idiopathic interstitial pneumonia, but is distinguished histologically from other diseases in the group because of its characteristic fibroblast foci of proliferative and matrix-producing fibroblasts and myofibroblasts. The diagnosis of IPF can be established after surgical lung biopsies with preoperative high-resolution computed tomography to show specific abnormal regions of the lung (5). IPF is more common in men than in women, and the age at onset of the disease is around 60 years. The treatments for IPF are focused on inflammation, fibrosis, and the immune response but unfortunately the effects of pharmacological treatment are limited. Lung transplantation has been shown to be a good treatment for some IPF patients, even though this solution is only possible if the patients, excluding lung status are healthy enough to undergo transplantation (6).

Even though the cause of the fibrosis is unknown, there are some substances that have been shown to be associated with the disease, such as asbestos, substances associated with agriculture and livestock, metal dust, and cigarette smoke (7). Cigarette smoke, which reduces the length of telomeres, could be an important pathogenetic factor in IPF (8). In families with IPF, 8% of individuals have a mutation in genes that result in short telomeres (9).

Many cell types are of importance for the pathology behind IPF, but fibroblasts with their ability to produce matrix molecules are of special interest. In tissues from patients with IPF, fibroblastic foci have been identified as discrete areas rich in ECM but with few cells (Figure 1). The cells of the fibroblast foci are arranged in an outstretched and parallel arrangement relative to the other cells and to the alveolar septa (10). Fibroblasts in the lungs of IPF patients could come from epithelial cells, from fibrocytes recruited from the bone marrow, or from proliferation of residual fibroblasts. For the origin of fibroblasts derived from

epithelial cells the level of TGF-β, a stimulator of epithelial-mesenchymal transition (EMT), is known to be increased in IPF lungs (11). Furthermore, EMT is regulated by Gremlin, which is an inhibitor of bone morphogenetic proteins, and is up-regulated in IPF so that epithelial cells are more sensitive to TGF-β (12). Another possible origin of the fibroblasts in IPF is recruitment of fibrocytes from the bone marrow. Fibrocytes as a possible source of fibroblasts are discussed in detail below, but it needs to be mentioned that the chemokine stromal cell-derived factor-1/chemokine ligand 12 (SDF-1/CXCL12) is up-regulated in both plasma and bronchoalveolar lavage (BAL) fluid in patients with IPF (Paper 1). Concerning vessel remodelling in IPF, it is of interest that the concentration of VEGF, a growth factor involved in vessel formation, is reduced in BAL fluid and it is not expressed in fibroblastic foci. In an *in vitro* study, it has also been shown that endothelial tubule formation is suppressed in the presence of IPF lung homogenates from IPF lung (13).

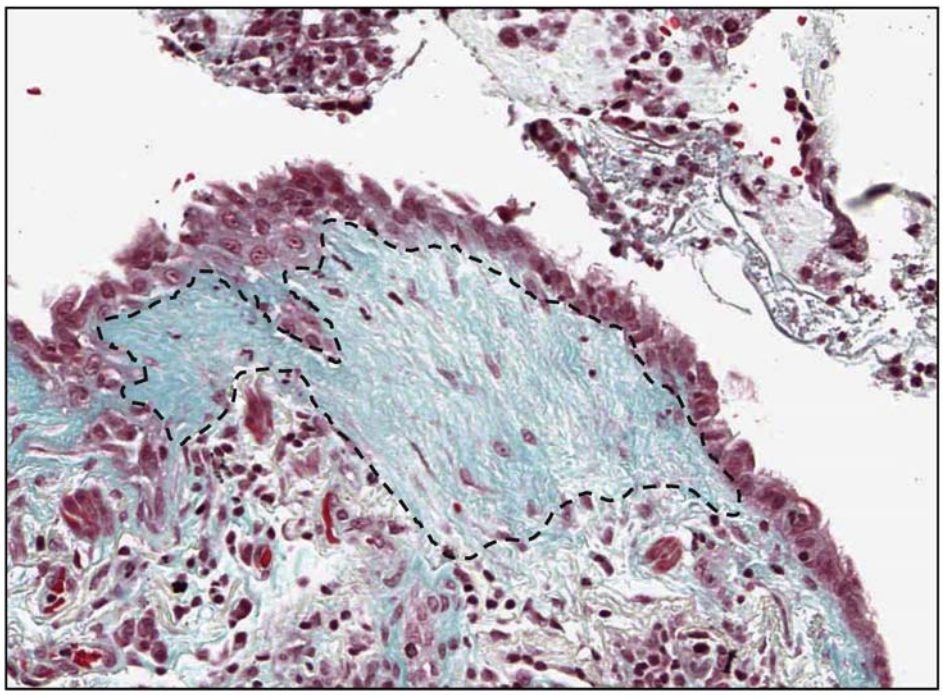

Figure 1. Fibroblastic foci are a characteristic feature of tissue from patients with idiopathic pulmonary fibrosis. The foci are identified as demarcated areas rich in ECM but with few cells. The cells in the fibroblast foci are outstretched and aligned parallel to the other cells and to the alveolar septa. A fibroblast focus can be seen here after Gomori's trichrome staining (see dashed line). Original magnification: 20×.

Lung Transplantation and Fibro-obliteration

Lung transplantation is a treatment available for end-stage lung disease in patients with COPD, CF, lymphangioleiomyomatosis, and fibrosing interstitial lung disorders (ILD) such as IPF. Depending on the diagnosis, single or bilateral lung transplantation is performed. The number of transplantations is still limited by the number of donors available. At the time of transplantation, immunosuppressant treatment is started and continues for the rest of the patient's life.

During the operation, the bronchial circulation is not restored for technical reasons. A Danish study has, however, shown that restoration of bronchial artery revascularization delays the onset of BOS (14). Also, the vagal nerve is often injured during the transplantation and results in deterioration in the patterns of breathing and coughing, which can give rise to an increased risk of pneumonia and aspiration of gastroesophageal secretions, and of developing BOS. However, the cough reflex is restored 12 months after the transplantation (15).

Without pharmacological immunosuppressant treatment, transplanted lungs should be rejected in a few days in an alloimmune rejection. The treatment today often combines immune-suppressive drugs such as cyclosporine A and azathioprine with steorids.

Chronic rejection is a common consequence of lung transplantation (affecting 60% of those transplanted) (3) (Figure 2). The tissue process starts with lymphocyte infiltration in the submucosa and injury of the mucosa and epithelial cell layer, which results in recruitment of ECM-producing fibroblasts or the progenitor cells, fibrocytes. Histologically, the rejection is seen as an ECM plug with few fibroblasts in the bronchioles (16-18). Clinically, the rejection is apparent as reduced lung function, and cough and dyspnea are common symptoms. Table 1 covers the criteria for the diagnosis of BOS (19). For recommendations concerning choice of spirometric equipment, confounding conditions, definition of baseline, and BOS stages, see reference (16).

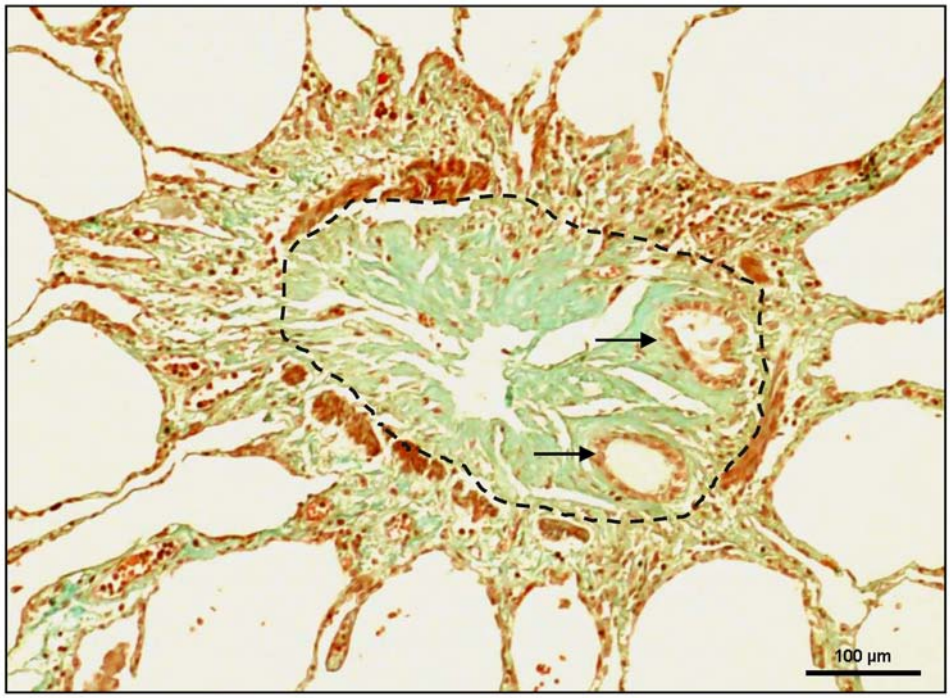

Figure 2. Histologically, obliterative bronchiolitis can be seen as fibro-proliferative intraluminal plugs with few fibroblasts in the terminal respiratory bronchioles. The dashed line indicates the plug, while the two arrows show neo-lumina. Original magnification: 20×.

BOS 0	$FEV_1 > 90\%$ of baseline and $FEF_{25–75} > 75\%$ of baseline
BOS 0p	FEV_1 81–90% of baseline and/or $FEF_{25–75} \leq 75\%$ of baseline
BOS 1	FEV_1 66–80% of baseline
BOS 2	FEV_1 51–65% of baseline
BOS 3	$FEV_1 \leq 50\%$ of baseline

BOS = bronchiolitis obliterans syndrome, FEF = forced expiratory flow, FEV1 = forced expiratory volume in 1 second

Table 1. Classification system for bronchiolitis obliterans. Adapted from Estenne and Hertz 2002.

There have been a number of articles addressing risk factors for BOS, most commonly mentioned are acute cellular rejection, human leukocyte antigen (HLA) mismatch, infections by cytomegalovirus (CMV) or lymphocytic bronchitis, and female donor to male recipient (16;20). The pathogenesis of BOS involves both non-alloimmune mechanisms such as infections, ischaemia, and gastro-oesophageal reflux and alloimmune mechanisms such as acute rejection and lymphocytic bronchiolitis (19). Which of the immune responses (type 1, the cell-mediated response; type 2, driven by cytotoxic T-lymphocytes (3); or type 17, the autoimmune response (21)) causes the rejection is still under debate. The growth factors involved in the fibro-proliferative phase of the chronic rejection in particular are platelet-derived growth factor (PDGF) (22) and TGF-β (23), which are known to up-regulate ECM deposition. Also, the size of vessels is increased in patients with BOS (20) (Paper 2) and this could be the result of local hypoxia, which promotes angiogenesis**.**

To prevent BOS attempts with a special double transplantation has shown promising results. At the same time as the lung transplantation occurs, it is combined with infusion of bone marrow from the same donor as the lung came from. The theory behind the double transplantation is that the recipient's immune cells are modulated, and the results show a lower incidence of OB compared to lung transplantation alone. Interestingly, the numbers of acute cellular rejections were found to be the same in a group of doubly transplanted subjects as in a group of subjects who only underwent lung transplantation. The outcomes of the studies in the field are difficult to interpret because of the small size of patient groups and the lack of matched control groups (24).

The treatment of BOS is unfortunately limited to changes in the immunosuppressive medication, both concerning dose and type of therapy (3). Re-transplantation is an alternative treatment for BOS, even though re-transplantation is associated with a higher mortality than initial transplantation (25). A five-year follow-up study of patients with BOS after initial transplantation showed that over 60% were alive even though this patient group had an elevated risk of developing BOS once again (25;26).

Bone Marrow / Haematopoietic Stem Cell Transplantation

A number of late complications such as pulmonary, cardiac, and renal complications are known to occur after bone marrow transplantation (27). The pulmonary complications occur at a frequency of 2–11% of the adult cases and 8% of the paediatric cases (28-31). As with OB after lung transplantation, it is mainly the small airways that are obliterated; and the risk factors for development of fibrosis are infections, smoking, cytotoxic therapy, irradiation, and chronic graft-versus-host disease (32;33). The histological characteristics of OB after bone marrow/haematopoietic stem cell transplantation are similar to those after lung

transplantation (Figure 2). The symptoms are cough, dyspnea, and wheezing. The clinical OB is defined as forced expiratory volume in 1 second (FEV_1)/ forced vital capacity ratio < 0.7, FEV_1 < 75% of predicted value, evidence of air trapping or small airway thickening, and absence of infection (34). The treatments are limited to immunosuppressive drugs and corticosteroids or lung transplantation (31;35-37). For OB after lung transplantation, the outcome is poor (33).

Asthma

Also in asthma remodelling and accumulation of ECM are histological features. While asthma historically was regarded as a single disease it has during the last years been clear that asthma is a very heterogeneous disorder, rather a syndrome, including different clinical phenotypes (38). The diagnosis of asthma is often based on symptoms such as wheezing, coughing, shortness of breath, and chest tightness with objective measures as variable airflow obstruction and bronchial hyperresponsiveness (39). The different phenotypes of asthma are based on: *(1)* clinical observations such as frequency of exacerbations, air flow restriction, resistance to pharmaceuticals (corticosteroids), age at onset (40-42); *(2)* determining the trigger-factor such as allergens or exercise (43;44); and *(3)* determining the number and localisation of inflammatory cells such as eosinophils and neutrophils (45;46). There may also be some overlap between the different phenotypes of asthma.

The complexity of the disease and phenotypes has made it difficult to implicate genes in asthma. However, one gene (*ADAM33*) with polymorphism in asthma patients has been identified; it codes for a Zn^+-dependent matrix metalloproteinase (MMP). *ADAM33* is expressed in mesenchymal cells such as fibroblasts and smooth muscle cells (47;48).

Many cell types with different features are involved in asthma. The structural cells that are involved in asthma are for example epithelial cells, smooth muscle cells, and (myo)fibroblasts. The epithelial layer forms a structural barrier between the air space and the tissue of the lung. Some of the known risk factors in asthma, such as indoor and outdoor allergens, tobacco smoke, and air pollution, have their first contact with the patient by way of the epithelium (49). In studies of biopsies from asthmatic patients, a correlation has been found between the degree of epithelial loss and the degree of airway reactivity (50). The main function of the muscle cells are to contract tissue. Both the smooth muscle area and the size of the smooth muscle cells increase with the severity of asthma (51). Smooth muscle cells are capable of proliferating, migrating, producing pro-inflammatory cytokines, and forming new muscle with change of phenotype and function (52-54). Even though other cell types are capable of producing ECM molecules, the fibroblast is the main producer. Fibroblasts, myofibroblasts, the ECM, and collagen are described below in more detail.

In asthma patients, the inflammatory response leads to release of cytokines and interleukins which results in narrowing of the airway lumen, secretion of mucus, accumulation of ECM molecules, increased number and size of smooth muscle cells, and thickening of the bronchial wall (55) (Figure 3). Mast cells and basophils have granulas containing chymase and tryptase, eosinophils can release a number of cytokines, leukotrienes, prostaglandins and the number of all these cell type is associated with a more severe asthma phenotype. Other immune cells that are involved in asthma are neutrophils that attract inflammatory cells, T-cells and macrophages and dendritic cells which act as antigen presenting cells (56-59).

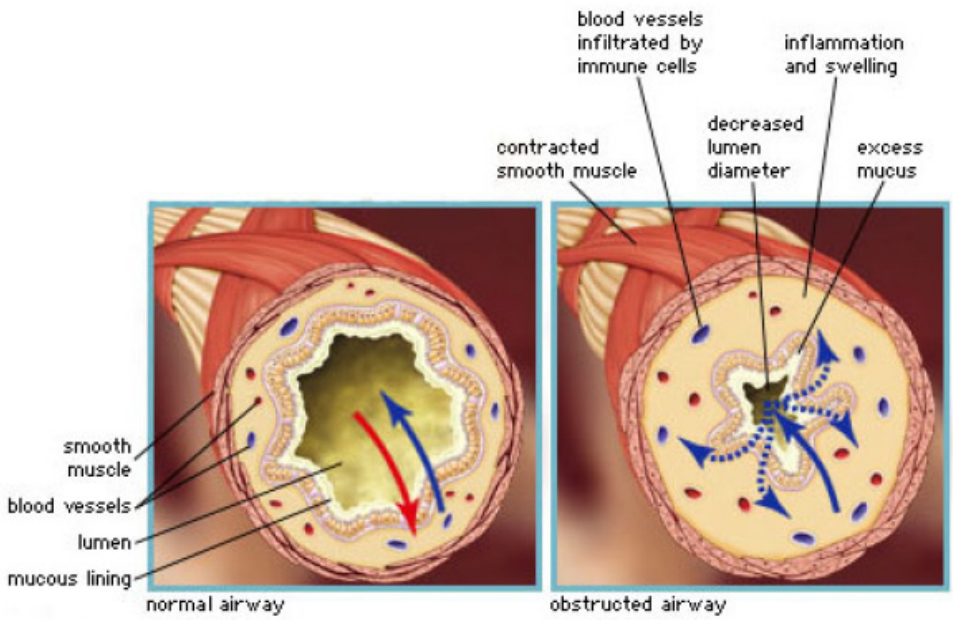

Figure 3. During an asthma attack, smooth muscles around the airways contract which gives a tightening of the lumen. This, together with swelling and inflammation with infiltration of immune cells and excessive secretion of mucus into the airways, gives an obstructed airway. Adapted from: Encyclopædia Britannica.

Asthma therapy has been based on bronchodilator agents, often combined with anti-inflammatory treatment. The bronchodilators, β2-agonists, act by binding to β2-adenoreceptors located on smooth muscle cells, epithelial cells, and immune cells and cause smooth muscle cells in particular to relax (60;61). The anti-inflammatory drugs, glucocorticoids, act by binding to the glucocorticoid receptor

in the cytoplasm and regulate gene transcription by activating anti-inflammatory genes (62).

Structural Cells Involved in Tissue Repair

Nearly all types of cells are involved in tissue repair. As mentioned at the beginning of this thesis, inflammation and remodelling work together in the disorders described here. In the following paragraphs, the main structural cell types that are involved in the remodelling process in tissue repair are presented.

Fibroblasts

Cytologically, fibroblasts are defined as elongated cells (Paper 4) with a typical marked rough endoplasmic reticulum and a typical Golgi apparatus that are common in cells that produce ECM and collagen (63) (Figure 4). The number of ECM molecules produced by fibroblasts are almost countless. The connective tissue from the fibroblasts surrounds the cells and provides a lattice to which the cells can bind and move along. Fibroblasts are in many cases a heterogeneous cell population and their behaviour is strongly dependent on and modulated by the surrounding environment, growth factors, and chemokines bound to the ECM.

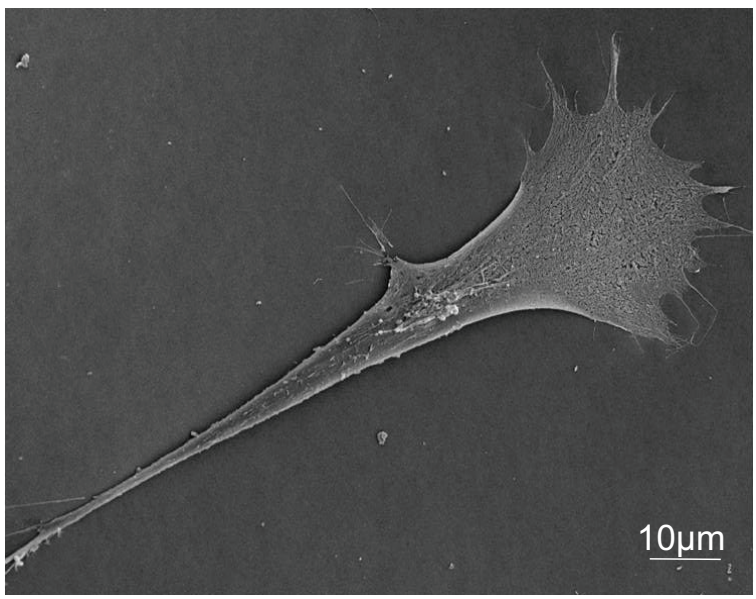

Figure 4. One of the structural cell types involved in tissue repair is the fibroblast. Fibroblasts are defined as elongated, cells with a typically marked rough endoplasmic reticulum. They can produce a number of extracellular matrix molecules. This cultured fibroblast was visualized by electron microscope.

Fibroblasts are involved in tissue repair and wound healing because of their ability to produce ECM and collagen. The fibroblast can also use its contractile forces to diminish the area of the wound, and this property is enhanced if the cells are primed by α-smooth muscle cells (α-SMA). Sub-populations of fibroblasts seem to be more or less primed for specific functions; for example, Westergren-Thorsson *et al.* have shown how fibroblast clones are negatively correlated to proliferation and synthesis of decorin (64) and in fibroblasts from lung-transplanted patients there is a negative correlation between proteoglycan production and proliferation / migration (Paper 3).

Fibroblasts can be characterised using antibodies to fibroblast markers. Many of these markers overlap with those of macrophages and smooth muscle cells. However, instead combinations of markers can be used to identify these cells (Papers 3 and 4).

Myofibroblasts

A myofibroblast is a fibroblast phenotype that is activated, is more contractile, and has a higher production of ECM and collagen than fibroblasts. The shape of the cells is described as spindle-shaped. The phenotype was first observed by Gabbiani *et al.* in 1971 (65). Today, the myofibroblasts are well-characterised as α-SMA-expressing cells. However, the pattern of expression is more complex than in smooth muscle cells, however, concerning both conserning enhancers and inhibitors in the downstream regulation of intracellular signalling (66;67).

There are two distinct populations of myofibroblasts, proto-myofibroblasts and differentiated myofibroblasts. Proto-myofibroblasts are formed after mechanical stress in fibroblasts. The characteristics of proto-myofibroblasts are that they express intracellular stress fibers and cell-surface fibronectin, which can both generate contractile forces. The differentiated myofibroblast develops from the proto-myofibroblast after TGF-β stimulation, which increases the amount of fibronectin at the cell surface to form fibrils; after further mechanical stress, α-SMA is formed intracellularly and focal adhesion at the cell surface (68).

Myofibroblasts are often located in areas of fibrosis, or in areas close to fibroblastic foci in IPF patients (69). They are capable of destroying overlying epithelial cells by secreting H_2O_2. The end result of the wound healing is determined by whether or not the myofibroblast is capable of apoptosis. Without apoptosis, fibrosis develops and this process is driven by TGF-β (70). One possible treatment for TGF-β-induced activation of myofibroblasts is interferon γ (IFN-γ), which regulates TGF-β by up-regulation of Smad7 through phosphorylation of signal transducers and activator of transcription (STAT) (71).

The Origin of the Fibroblast/Myofibroblast

A few years ago, tissue-resident fibroblasts were thought to be the only possible origin of fibroblasts. In this thesis, both paper 1 and paper 2 focuses on fibrocytes; these are one of the more recently discovered origins of fibroblasts. Epithelial-mesenchymal transition and endothelial-mesenchymal transition are also known to be possible sources of fibroblasts. In bleomycin-induced lung fibrosis, one-third of the fibroblasts are derived from epithelium and one-fifth are derived from bone marrow. In this study no other origins were investigated, and conclusions concerning endothelial-mesenchymal transition and other origins are still unclear (72).

Resident Fibroblasts

As early as 1990, Darby *et al.* showed how resident fibroblasts proliferate and differentiate into myofibroblasts in an open scar wound healing model in the rat. The processes started 6 days after scar formation, with the highest level of fibroblasts 15 days later, and ended with apoptosis on days 20–25 after scar formation (73). Furthermore, fibroblasts from fibrotic patients have a higher proliferation capacity than those from healthy controls (64). Proliferation of the resident fibroblasts is probably driven both by factors in the tissue such as ECM molecules and by the inflammation.

Fibrocytes

Fibrocytes are progenitor cells that originate in the bone marrow. The fibrocytes have many characteristics that make them a discrete cell population. They are mostly known to have an important role in fibrotic lung diseases such as asthma (74;75), COPD, IPF (76) (Paper 1), and OB (Paper 2), but also in skin wound healing (77) and kidney fibrosis (78). Unfortunately, we do not have any specific marker for fibrocytes; instead, a combination of markers for different cell types is being used such as combining haematopoietic markers with mesenchymal markers. For example, there are molecules specific for leukocytes (CD45), monocytes (CD11a, CD11b, CD13), and stem cells (CD34), and also chemokine receptors (CXCR4), major histocompatibility complex (MHC) molecules, and mesenchymal markers (prolyl 4-hydroxylase, α-SMA) (77;79). One of the most potent markers is CXCR4, which is expressed by 90% of the circulating pool of fibrocytes (80). The expression of these specific proteins changes as the fibrocytes are released from the bone marrow and recruited to the tissue. Mori *et al.* isolated circulating fibrocytes from mice and analysed the cells regarding their CD13, CD34, CD45, collagen I, and α-SMA expression for one week in serum-free medium or in medium supplemented with TGF-β, a factor involved in wound healing. The expression of CD13, CD34, and CD45 became reduced while the expression of collagen I was constantly high and the expression of α-SMA increased. The differences were even higher when TGF-β was present (81).

Fibrocytes have a role in many steps of angiogenesis. For example, fibrocytes express MMP-9, which helps these cells to penetrate the basement membrane during formation of new vessels. Isolated fibrocytes produce a number of pro-angiogenic factors such as bFGF, VEGF, granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-1 (IL-1), IL-8, and macrophage colony-stimulating factor (M-CSF). These factors induce migration, proliferation, and alignment of endothelial cells into tube-like structures (82).

Fibrocytes differ from fibroblasts in many ways. An immunologically important feature is antigen presentation. They express both MHC class I and class II antigens and co-factors CD80 and CD86. Furthermore, fibrocytes can migrate to lymphatic organs and sensitise naive T-cells. Previously, this feature was only thought to be a task of dendritic cells (83).

Recruitment of Fibrocytes

Fibrocytes have to be recruited from the bone marrow to the injured tissue, and one of the possibilities for recruitment is the CXCR4 – SDF-1 / CXCL12 axis. SDF-1 / CXCL12 belongs to the CXC family and has a length of 68 amino acids (8 kDa). The only receptor for SDF-1 / CXCL12 is the G-protein-coupled seven-span transmembrane receptor CXCR4 (84), which is present on its target cell. Binding causes changes to the cell: increased secretion of MMPs, VEGF, and NO, and also cytoskeletal rearrangements, which give increased mortality and chemotaxis. Some molecules involved in inflammation (hyaluronan, fibronectin, and fibrinogen) appear to increase the sensitivity to SDF-1 / CXCL12 (85).

The expression of CXCR4 and its ligand SDF-1 / CXCL12 is known to be up-regulated under hypoxic conditions by hypoxia-induced factor 1α (HIF-1α) (86;87). The bone marrow is hypoxic compared to the surrounding vessels, and bone marrow has expression of SDF-1 / CXCL12. An injury in the lung leads to increased levels of SDF-1 / CXCL12 in the plasma (Paper 1), and fibrocytes are released from the bone marrow to migrate over a chemotactic gradient to the injured lung, where SDF-1 / CXCL12 is being expressed (88).

The importance of the CXCR4 – SDF-1 / CXCL12 axis has been shown by Phillips *et al*. using an animal model of lung fibrosis. In a bleomycin-induced model of lung fibrosis mice were treated with anti-CXCL12 antibodies and there was a significantly lower level of collagen and α-SMA compared to the animals treated with control antibodies (89) (Figure 5). Another possible way of recruitment is a gradient of the chemokine secondary lymphoid tissue chemokine / chemokine ligand 21 (SLC / CCL21), which is expressed in lymphoid organs and also in lung tissue under inflammatory conditions. The receptor is CCR7, but it is only expressed by less than 10% of circulating fibrocytes (80). This way of

recruitment has been mentioned most in papers on renal fibrosis (77;78). The third way of recruitment of fibrocytes, the CCR2 / CCL2 axis, is only present in animals and has been shown to occur in lung tissue after injury (90).

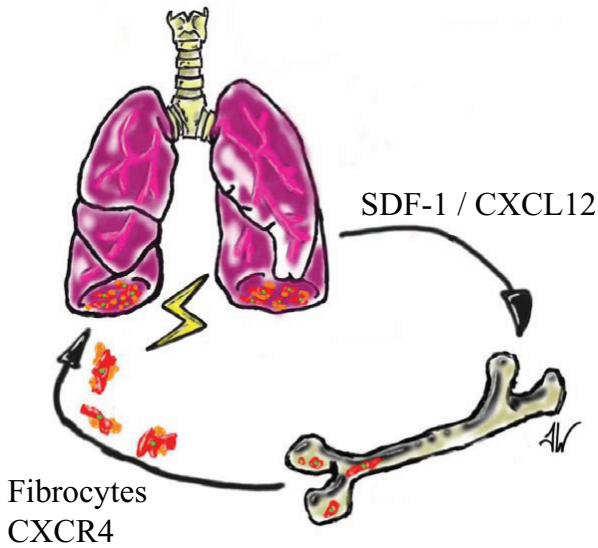

Figure 5. One possible way of recruitment of fibrocytes is by the CXCR4 – SDF-1 / CXCL12 axis. Fibrocytes that express CXCR4 originate in the bone marrow. A lung injury results in increased levels of SDF-1 / CXCL12, thus causing build-up of a gradient to recruit CXCR4-positive fibrocytes to the location of injury of the tissue.

Fibrocyte Differentiation

When fibrocytes have entered an injured tissue, they move through a matrix where many cytokines are bound. These cytokines are known to influence the behaviour of the fibrocytes; as mentioned previously, SDF-1 induces migration by interacting with CXCR4. Mori *et al.* showed how TGF-β is capable of inducing fibrocytes to differentiate into α-SMA-positive myofibroblasts (81). The pathways that are involved in this differentiation are activation of Smad 2/3 and stress-activated protein kinase / jun N-terminal kinases mitogen-activated protein kinases (SAPK/JNK MAPK) (91). The markers on the fibrocytes change during recruitment to the injured tissue. The expression of mesenchymal markers increases while the expression of haematopoietic markers decreases (92).

Another possible way of differentiation of fibrocytes is to adipocytes, which is driven by specific adipogenetic hormones and cytokines and which follows activation of specific adipocyte genes. On the other hand, TGF-β inhibits this

differentiation by activating SAPK/JNK MAPK, which is normally suppressed during differentiation to adipocytes (91).

Differentiation of monocytes to fibrocytes has been shown by Shao *et al*. (93). Monocytes cultured together with the pro-fibrotic cytokines IL-4 and IL-13 differentiated into fibrocytes, while the anti-fibrotic cytokines IFN-γ and IL-12 inhibited fibrocyte differentiation of monocytes to fibrocytes.

Epithelial-Mesenchymal Transition and Endothelial-Mesenchymal Transition

Different types of epithelial cells cover the whole airway tree. In the trachea, there is a pseudo-stratified layer where ciliated cells, goblet cells, and basal cells predominate; in the small airways, there are also Clara cells. In these airways, the epithelial layer is simple cuboidal. The cells that cover the alveolar duct are mainly alveolar type I and type II cells. Alveolar type I cells are flat and enable gas exchange; type II cells are progenitors of type I cells, are mucus producing, and have immunological functions (94).

The epithelial-mesenchymal transition, where fully differentiated alveolar type II cells become transformed into a mesenchymal cell phenotype, has been identified in many disorders—for example, in lung transplantation, OB, and in renal and lens fibrosis (95-97). TGF-β has been recognised as the main inducer of EMT. The process is mainly mediated by the Smad pathway but an alternative pathway in which the *ras* homolog gene family; RhoA, *ras,* MAPK, and P13 are involved has also identified. Also, cross-talk between the pathways appears to be involved in most cases (11;98).

In a recent study by Borthwick *et al.* (95), EMT has been identified in patients with BOS. EMT was identified by a combination of analyses: cell morphology, mesenchymal proteins, epithelial proteins, secretion of ECM, and functional analysis of invasive and migratory properties. The patients with BOS had significantly more EMT than lung-transplanted patients without BOS and healthy controls (95).

In addition, endothelial cells are capable of undergoing transition to cells with features of mesenchymal cells. Of the endothelial cells in an adult, 0.01–0.03% are capable of undergoing endothelial-to-smooth muscle transdifferentiation (99).

Endothelial Cells

Endothelial cells make up the tunica intima in both arteries and veins. The cells are connected to each other by tight junctions with zona occludens-1 (ZO-1) and ZO-2 at the cytoplasm side (100;101). Vessels and endothelial cells are particularly involved in remodelling; these cells are able to proliferate and migrate in response

to stimulation. Endothelial cells respond to different types of stimuli, such as vasodilation after stimulation with NO and prostacyclin PGI_2, and proliferation after stimulation with PDGF, VEGF, and FGF. Endothelial cells react to prolonged hypoxia by proliferation.

Recruitment and Differentiation of Endothelial Progenitor Cells

Progenitor cells with the capacity to differentiate into endothelial cells can be recruited from the bone marrow and become endothelial cells with characteristic expression of von Willebrand factor (vWF) (102). The immature progenitor cells express CXCR2 and CD133 (103) and vascular endothelial growth factor receptor 2 (VEGFR2). When the progenitor cell has reached its mature state, it also expresses CD34. CXCR2 and VEGFR2 have been suggested to be of special importance in the recruitment of endothelial progenitor cells (104). The progenitor cells are of importance both in re-endothelialisation and in neo-visualisation (101;102). The numbers of endothelial progenitor cells and the degree of incorporation into endothelial cell tubes is elevated in patients with asthma. Furthermore, there is a correlation between the number of endothelial progenitor cells and the number of bronchial vessels in this patient group (105).

Extracellular Matrix Molecules in Tissue Repair

In a previous section on structural cells involved in tissue repair, fibroblasts were described as the main producers of ECM molecules. The ECM forms an extracellular environment for cells to attach to, and also forms the basement membrane located under epithelial and endothelial cells. The main components of ECM are collagens, proteoglycans, hyaluronan, and other glycoproteins. The ECM functions as a reservoir for growth factors and chemokines, and it is also a water-absorbent gel mass that gives the tissue its specific features. (Figure 6) Under physiological conditions, the ECM is built up and degradated at same rate, while under pathological conditions such as during tissue repair there is a higher building rate and/or lower degradation rate. The degradation of ECM is mainly regulated by proteases. This thesis deals particularly with the ECM molecules versican, perlecan, biglycan, and decorin.

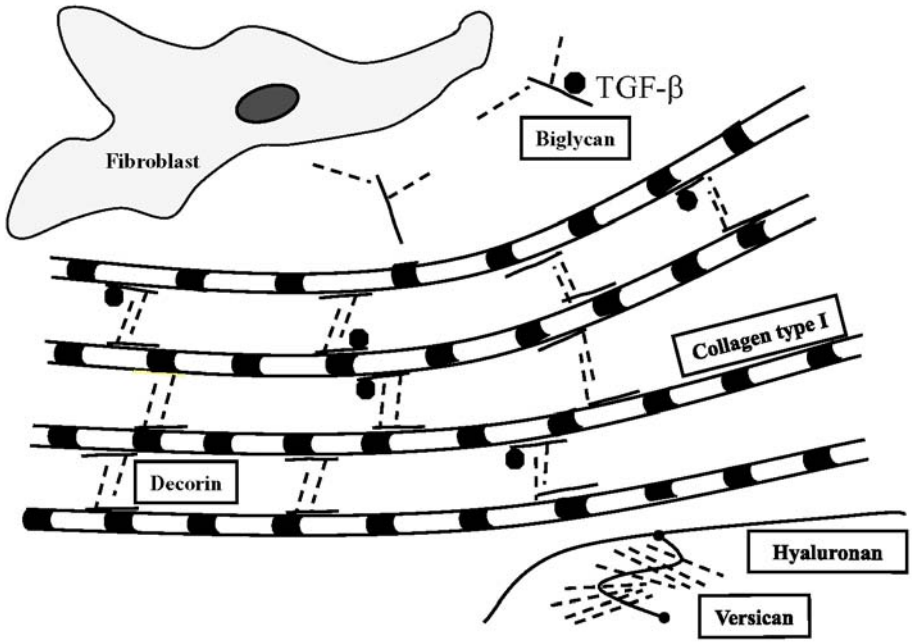

Figure 6. Schematic figure over the extracellular matrix components collagens, proteoglycans, and hyaluronan.

The remodelling is closely associated with inflammatory processes. The inflammatory cells are recruited to the site of injury by a number of reactive mediators. These mediators are part of the defense system, but they also destroy the tissue. An example of the relationship between inflammation and remodelling is featured in an article by Schultz *et al.* (106). After the injury monocytes are recruited to the destroyed tissue. After binding to fibronectin the monocytes differentiate into macrophages. Macrophages produce growth factors such as transforming growth factor β (TGF-β), fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF). TGF-β stimulates fibroblasts to produce ECM molecules. FGF form complexes with heparan sulphate (HS) proteoglycans and the complex then can bind to FGF receptors (107) on fibroblasts or endothelial cells which cause the fibroblasts to migrate and endothelial cells to proliferate. In the presence of VEGF angiogenesis is initiated (106).

Collagen

The two first articles on the triple-helix structure of collagen were published in Nature in 1955 (108;109). Today, 27 distinct types of human collagens have been

identified, where the compositions of the trimers differ from each other (110). Most collagens are defined as fibril-forming (types I, II, III, and minor types V and XI) and are located in skin, tendon, and other tissues. The other kinds of collagen are known as fibril-associated, network-forming, and transmembrane collagens (111). Type I especially, but also type III, is important for lung structure. Type I is the collagen type that is most important and up-regulated in asthma (110;112;113), IPF (114), and in OB after lung transplantation (95). The functions of a collagen depend on the type and where it is located in tissues.

Triple-helix formation by the three polypeptide chains occurs mainly intracellularly, where prolyl 4-hydroxylase located in the endoplasmic reticulum is one of the key enzymes involved (115). The expression of collagen is enhanced by TGF-β, tumour necrosis factor-α (TNF-α), IL-13, and PDGF, while IFN-γ inhibits the synthesis (116-119).

Proteoglycans

Proteoglycans is a family name for a group of macromolecules that function as an anchorage between, for example, laminins, collagens, and cells (120). The fascinating aspect of proteoglycans is their ability to regulate the surrounding cells by changing between binding and release of growth factors and cytokines etc. depending on the molecular setting in the local milieu of the tissue as well as on physiological and pathological conditions. The proteoglycans are composed of a core protein containing domains with specific functions such as binding ability. The prominent feature of the proteoglycans is their ability to absorb water; a figure of 1,000 times their own volume has been mentioned. The capacity to absorb water is conferred by the polysaccharide chains, glycosaminoglycan GAGs that are attached to the core protein when the proteoglycans are synthesised intracellularly. Depending of what kind of disaccharides the GAGs are composed of, the proteoglycans get their specific features. Currently, five types of GAGs are known: chondriotin sulphate (CS), dermatan sulphate (DS), HS, keratan sulphate, and hyaluronan (the latter are not attached to a core protein). CS and DS proteoglycans are mostly located in the ECM, while HS proteoglycans are mostly located at the cell surface of the producing cell (except perlecan, which is an ECM proteoglycan) (Figure 7).

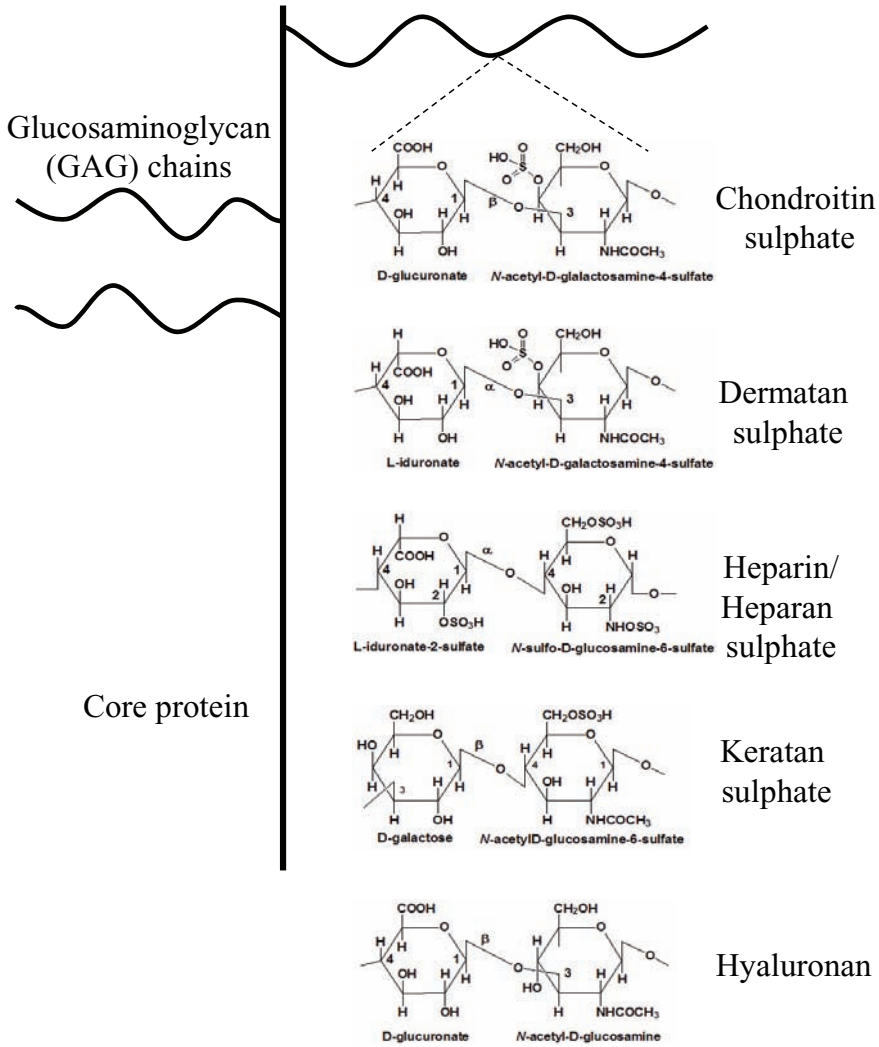

Figure 7. The proteoglycans are composed of a protein core with chains of glycosaminoglycan attached, such as chondriotin sulphate, dermatan sulphate, heparan sulphate, and keratin sulphate. Hyaluronan is a non sulphated GAG, devoid of a core protein. Dermatan sulphate is distinguished from chondriotin sulphate by the presence of iduronic acid.

Even though the main producers of ECM molecules are fibroblasts, HS is produced by all cell types. As previously mentioned, HS proteoglycans can be located both at the cell surface and in ECM. The negatively charged region of HS can interact with different types of proteins, which can then be transported across

the cell membrane and basement membrane (121;122). The properties of HS make it possible to use the HS-protein complex as a therapeutic alternative in drug discovery. Lindahl *et al.* have shown ways in which the interaction between HS and proteins can be used in the design of possible drugs. They described how a long-lasting stable drug displaces endogenous HS on its binding receptor. Furthermore, it is also possible to inhibit the receptor signal with a competitive ligand or to block the binding site. A general inhibition of production of HS could also be a possible therapeutic strategy, but this is only possible if the HS proteoglycans on the cell surface have enough capacity for the essential functions of cell survival (123).

Versican

Versican is one of the larger proteoglycans, with a protein core of 200 kDa and 10–30 CS or DS chains attached. One of the characteristics of versican is its ability to absorb water. It can also bind hyaluronan, which is why versican is included in the group of hyalectans. All the members of this group also have a region of the protein core that binds to lectins, and a central domain binds to the GAG chains (124). Versican can also bind to CD44 on the cell surface and to several other proteins; together, these form a lattice that stabilises the surrounding matrix.

The expression of versican is elevated by a number of cytokines such as TGF-β, PDGF, epidermal growth factor (EGF), and IL-1β, and also after mechanical stimulation (125-127). The mRNA levels of versican in macrophages have been found to be increased after hypoxia, and in the same study HIF1α and HIF2α were also mentioned as co-regulators of versican expression (128).

In the airways versican is known to be localised in the sub-epithelial layer, with a higher expression in asthmatic patients than in healthy individuals (129). In a study of fibroblasts obtained from asthmatic subjects, a correlation was found between high production of versican and hyper-responsive airways (130). Another function of versican is to inhibit cells from binding to elastic fibres. In chronic obstructive pulmonary disease (COPD), loss of elastin in the alveolar walls is a main pathological feature and patients with mild-to-moderate COPD show progressively increased expression of versican (131;132).

Perlecan

Perlecan is mostly located in the basement membrane, but it has also been found pericellularly. The basement membrane is also composed of collagen IV, laminin-1, and nidogen; together with perlecan, they make up the highly organised structure of the membrane (111). There are three binding sites for GAG chains but in some case there are five sites (133;134) and the GAG chains are mainly of HS type but also CS chains are found. Because of the structure of perlecan, it is capable of binding to many types of molecules: collagens IV, XIII, and XVIII,

fibrillin, fibronectin, laminin, fibronectin, thrombospondin, FGF, PDGF, VEGF, and low-density lipoprotein (LDL) (135;136). Binding to these molecules stabilises the ECM. Perlecan is both pro- and anti-angiogenic as a result of its ability to bind to angiopoietins (Ang)-3, progranulin, and ECM-1 (which are pro-factors) and endorepellin (which is an anti-factor) (136). Basement membrane thickening is a well-known histological characteristic of asthma (112); also, lung-transplanted patients have a thickening of the basement membrane after transplantation, but this thickening usually reverts to normal by 300 days after surgery (137).

Biglycan and Decorin

The protein cores of biglycan and decorin consist of more than 70% leucine-rich repeats and they have a size of around 40 kDa. They are both members of the group of small leucine-rich proteoglycans. The GAG chains of biglycan and decorin are of CS/DS type. Biglycan has two GAG chains while decorin only has one. Apart from their function as part of the ECM lattice, biglycan and decorin also function in binding to cytokines such as TGF-β and TNF-α (138;139). The production of biglycan is stimulated by both TNF-α and TGF-β while the production of decorin is reduced by TNF-α (125).

Decorin is known to regulate both cell survival and differentiation in epithelial and endothelial cells through signaling of EGF surface receptors (140). In an animal model of fibrosis, biglycan was found to be increased in the early phase of the fibrosis while decorin was produced at higher levels when the fibrotic lesions had built up (141). Decorin functions as a collagen decorator, hence its name (142). The decoration of collagen has importance in the fibril formation of collagen molecules. Animal models of mice without the decorin gene show a phenotype with fusion of the collagen fibrils which cause for example skin fragility (143). It is known that biglycan is expressed in almost all organs of the human body but the location—e.g. the cell surface *vs.* extracellularly—varies. The functions of biglycan appear to depend on the microenvironment (144), and it has been shown, for example, to stimulate migration of fibroblasts through the Rho / Ras pathway (145).

Aims

The overall aim of this thesis was to define the fibroblast phenotypes and factors involved in tissue repair and in the remodelling process in fibrotic disorders of the lung such as IPF, OB, asthma, and also after lung transplantation.

The specific aims were as follows:

- To analyse tissue alterations that are associated with alterations in the numbers of fibrocytes in tissue, and also how tissue and lung function changes in relation to an up-regulation of the fibrocyte population.

- To identify factors involved in the recruitment of fibrocytes from the bloodstream to specific locations in lung tissue.

- To investigate whether specific fibroblast phenotypes (regarding location in the lung, ECM production, proliferation, and migration) are involved in asthma or are associated with tissue alterations and repair after lung transplantation.

Methods

The materials included in this thesis were all obtained from humans and the methods were based on cell biological and histological characterizations of cell phenotypes (Figure 8).

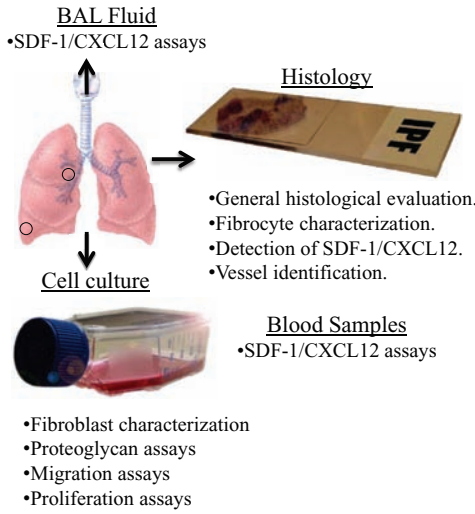

Figure 8. Schematic figure of the method set-up.

Subjects

Patients with Idiopathic Pulmonary Fibrosis (Paper I)

The patient group consisted of 42 patients suffering from IPF. All of the patients underwent operations to obtain open lung biopsies and the diagnosis was established based on microscopic findings consistent with usual interstitial pneumonia. The mean age of the group was 65.7 ± 10.3 years and there were 27 males and 15 females. Characterization of the patients was further established after lung function tests such as oxygen saturation during exercise, blood sampling, and cell profile in BAL fluid. The patients had been recently diagnosed before they were included in the study and they had not started on any treatment.

Patients with Oblitertive Bronchiolitis (Paper II)

The autopsy material was obtained from a patient group consisting of 5 patients who had been lung transplanted and 3 patients who had been bone marrow

transplanted. The mean age at transplantation was 34.4 years (range: 10–65). Both types of transplantation had led to OB, defined as bronchioles with a narrowed lumen and characteristic fibrous scarring. The background diagnoses that had led to lung transplantation were bronchiolitis, emphysema, or CF and for the bone marrow transplantation the patients had been diagnosed with aplastic anaemia or lymphatic leukaemia.

Lung-Transplanted Patients (Paper III)

The patient group consisted of 17 single or bilateral lung-transplanted subjects aged between 23 and 66 years (mean 54.4). The reasons for the transplantation differed between the patients, but the most common background diagnosis was COPD followed by CF. The biopsies were obtained both from central and distal parts of the lungs at fixed time points after transplantation. If there were clinical indications, biopsies were also obtained at other time points after transplantation.

Patients with asthma (Paper IV)

Thirteen atopic asthmatic subjects with a clinical diagnose of mild persistent asthma were included. All had confirmed bronchial hyper responsiveness to methacholine defined as FEV $PD_{20} < 2{,}000$ μg. Bronchial biopsies from the central airways were collected by direct vision through the bronchoscope. Thereafter were distal airway biopsies collected under fluoroscopic guidance. None of the subjects had a respiratory tract infection three weeks prior to the investigation and all were steroid naïve or had withheld inhaled corticosteroid therapy 3 month prior to the bronchoscopy.

Healthy Controls

The control subjects in the papers (I–IV) were as follows.

Paper I: The control specimens for part of the immunofluorescence and confocal microscopy in Paper I were obtained from patients who died from causes other than pulmonary disease. The 23 controls used for the definition of normal plasma levels of SDF-1/CXCL12 and the 5 controls for definition of normal SDF-1/CXCL12 levels in BAL fluid were all healthy volunteers.

Paper II: The control biopsies were collected during surgery from patients aged between 50 and 73 years (mean 65.3) with well-defined lung tumours, and none of the controls had ever smoked.

Papers III and IV: The control biopsies were obtained both from central and distal parts of the lung in 16 healthy volunteers aged between 22 and 40 years (mean 28). The control subjects were non-atopic, non-smokers and were free from respiratory tract infections during the last 6 weeks. They all denied respiratory

symptoms and had a negative bronchial challenge test defined as FEV PD20 > 2000μg methacholine.

General Histopathological Evaluation

To analyse general histological properties, haematoxylin and eosin staining (Paper I), Gomori's trichrome staining (Paper II), or Masson's trichrome staining (Paper IV) was used.

The haematoxylin and eosin staining was used to identify the number of fibroblastic foci in tissue from IPF patients. The staining gave the following pattern: collagen, pale pink; muscle, deep pink; nuclei, blue; acidophilic cytoplasm, red; basophilic cytoplasm, purple.

The actual tissue in proportion to air was determined in patients with OB after staining with Gomori's trichrome. Areas of 1.20 mm^2 without any bronchioles or vessels were analysed using ImageJ software after threshold values were selected for each parameter.

The collagen-containing tissue from asthmatic and control subjects was analysed after Masson's trichrome staining. Threshold values were selected for the blue staining of collagen and the total area of the tissue was determined in ImageJ to obtain results expressed as percentage collagen tissue of total tissue area.

Fibrocyte Characterization

Fibrocytes were identified in lung tissue after immunohistochemistry and following fluorescence microscopy. Combinations of mesenchymal markers such as α-SMA and prolyl 4-hydroxylase, haematopoetic markers such as CD34, leukocyte markers such as CD45, and chemokine receptors such as CXCR4 were used together with 4',6-diamidino-2-phenylindole (DAPI) to identify nuclei of fibrocytes. By combining markers, we could identify subtypes of fibrocytes.

The staining procedure started with heat-induced antigen retrieval and blocking with bovine serum albumin (BSA) in Tris-buffered saline (TBS), incubation in serum from the same species as that used for preparation of the biotinylated antibody, avidin/biotin blocking, and overnight incubation with one of the primary antibodies to CD34, CD45, or CXCR4. The signals of the primary antibody were amplified by first using biotinylated antibody and then with a corresponding biotinylated anti-immunoglobulin antibody. Next, we used primary antibodies directed against prolyl 4-hydroxylase, α-SMA, procollagen I (paper *I*), or prolyl 4-hydroxylase (paper *II*) and then used a corresponding secondary antibody conjugated with Alexa fluorochrome. The nuclei were visualised with DAPI. To

quantify lung fibrocytes, five (paper *I*) or six (paper *II*) randomly selected fields of fixed area were analysed for triple-stained cells.

Control sections were included for all combinations of straining, both among the patients and the normal tissues. The control staining involved staining without primary and/or secondary antibodies, and gave the opportunity to correct for background fluorescence.

Detection of Stromal Derived Factor 1/CXCL12

SDF-1/CXCL12 was detected in tissue, BAL fluid, and blood plasma. The ligand was identified in tissue by immunohistology using primary antibodies to SDF-1/CXCL12. The staining was visualised with secondary biotinylated anti-immunoglobulin followed by horseradish peroxidase-conjugated streptavidin. The levels of SDF-1/CXCL12 in BAL fluid and plasma were determined by enzyme-linked immunosorbent assay (ELISA).

Vessel Identification

The tissue sections were incubated with antibodies to vWF overnight and visualised with secondary fluorescent antibody. The nuclei were visualised with DAPI.

The parenchymal pulmonary vessels (located more than 500 μm from bronchioles and with an area of between 400 and 180,000 μm^2) were analysed by computerised image analysis both concerning inner area of the lumen and area of the endothelial layer. These parameters also gave the density of vessels, the percentage of total lung tissue that made up the luminal area of pulmonary vessels, and the percentage of total lung tissue that made up the pulmonary vessel endothelium.

Fibroblast Culture

Biopsies, obtained either centrally or distally in the lung, were placed at the bottom of a cell culture flask. If possible, the biopsies were divided into 2 or more pieces. After 4 h, when the biopsy had become attached, cell medium (Dulbecco's Eagle's minimal essential medium (DMEM) containing 10% foetal clone III serum, gentamycin, and amphotericin B) was used to cover the tissue pieces. Fibroblasts were allowed to grow out for up to 4 months. When the cultures were confluent the cells were passaged in the ratio 1:2 and all experiments were done at passages 3–5.

Fibroblast Characterization

To ensure that true fibroblasts were being cultured, the cells were analysed concerning expression of vimentin, prolyl 4-hydroxylase, and α-SMA as positive

controls and SM22, a smooth muscle cell marker, as negative control. The expression was detected by fluorescence microscopy, after the secondary antibodies had been added. Nuclei were visualised with DAPI.

Proteoglycan Assays

Fibroblasts from lung-transplanted asthmatic patients and healthy subjects were cultured to confluence in 6-well plates. The culture medium was changed after 24 h to the same type of cell medium as the fibroblasts were cultured in (DMEM containing 10% foetal clone III serum, gentamycin and amphotericin B) but without sulphate. Instead, radioactively-labelled sulphate (^{35}S) was added. Cell medium was added to a diethyl-aminoethyl (DEAE)-52 cellulose anion exchange column equilibrated with 6 M urea, 50 mM sodium acetate, 5 mM N-ethylmalimide, 1 mM ethylenediaminetetraacetic acid (EDTA), and 0.5% ovalbumin, pH 5.8. Unincorporated sulphates were removed by extensive washing. Hyaluronan was removed from the column by increasing the acetate concentration to 0.5 mM and the proteoglycans were eluted with 4 M guanidinium hydrogen chloride, 50 mM acetate, and 0.5% ovalbumin, pH 5.8. The total amount of (^{35}S)-labelled proteoglycans was quantified by scintillation counting. Further separations of the proteoglycan were done using 3–8% (Paper III) or 3–12% (Paper IV) sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) after precipitation with 3 volumes of 96% ethanol containing 0.4% sodium acetate, dextran, and CS 6. The PAGE gels were analysed with a FUJI image analyser and adjusted for protein concentration.

Migration Assays

Fibroblasts were cultured in cylinders of 2 mm diameter that were placed in a 12-well plate for 6 h. The cylinders were lifted up and the fibroblasts were allowed to migrate for 24 h. After fixation with glutarealdehyde in Hanks' balanced salt solution (HBSS), the cells were stained with crystal violet and the length of migration was determined.

Proliferation Assays

Five thousand cells were seeded in a 96-well plate and cultured for 6, 24, and 48 h in 10% foetal clone III serum. The cells were fixated with 1% glutaraldehyde for 30 min and stained with crystal violet for 30 min. To remove unincorporated crystal violet the plates were gently washed with water, and before measurement of absorbance at 595 nm the cells were made permeable with 1% Triton-X 100 overnight. The first plate were fixated after 6 h and were used as a as reference plate. After 6 h the cells have stuck to the bottom of the wells but have not started to divide yet.

Results

Fibrocytes are a Potential Source of Lung Fibroblasts in Idiopathic Pulmonary Fibrosis (Paper I)

IPF is a lung disorder associated with extensive fibrosis, and it is associated with characteristic fibroblast foci. The main producers of the ECM seen both in the general fibrosis and in fibroblast foci are myofibroblasts and fibroblasts. One of the possible origins of this myofibroblast/fibroblast population is fibrocytes that are recruited from the bone marrow to the injured lung.

By combining markers, the following subtypes were identified (in order, more fibrocytes identified by the combination, mentioned first): CXCR4/procollagen-I, CXCR4/prolyl-4-hydroxylase (Figure 9), CD34/procollagen-I, CD34/α-SMA, CD45RO/prolyl-4-hydroxylase. There was a correlation between numbers of fibrocytes expressing CXCR4/prolyl-4-hydroxylase and numbers of fibroblast foci (Figure 10). The possible recruitment axes for fibrocytes CXCR4 / SDF-1 were identified after having identified the ligand SDF-1 at elevated levels in plasma and BAL fluid. The ligand SDF-1 was also found to be expressed in alveolar epithelial cells in patients with IPF (Figure 11). The lung function, measured as diffusing capacity of the lung for carbon monoxide (DLCO) and oxygen saturation on exercise (Figure 12), was reduced with more SDF-1 in BAL fluid.

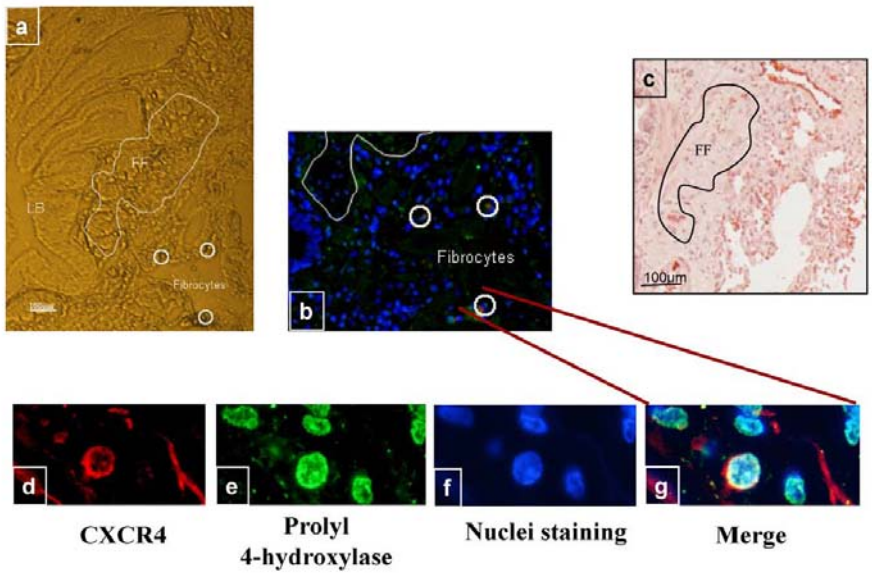

Figure 9. Fibrocytes were identified in tissue from patients with idiopathic pulmonary fibrosis using a combination of CXCR4 and prolyl-4-hydroxylase. Panel (a) (light microscopy) and panel (b) (fluorescence microscopy) show three fibrocytes located 150–400 μm from the fibroblastic focus (FF). The lumen of the bronchiole is indicated (LB). Panel (c) shows a consecutive lung section stained with haematoxylin and eosin. In panels (d)–(f) the separate markers for CXCR4, prolyl-4-hydroxylase, and nuclear staining are shown, while panel (g) represents a merged picture of the fibrocyte. Original magnification: 20× for (a)–(c) and 40× for (d)–(g).

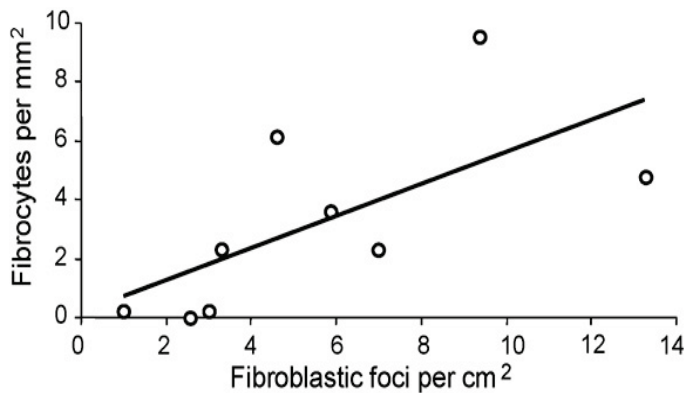

Figure 10. There was an association between the numbers of CXCR4/prolyl-4-hydroxylase fibrocytes and the number of fibroblastic foci identified per cm2 ($r = 0.79$; $p < 0.02$).

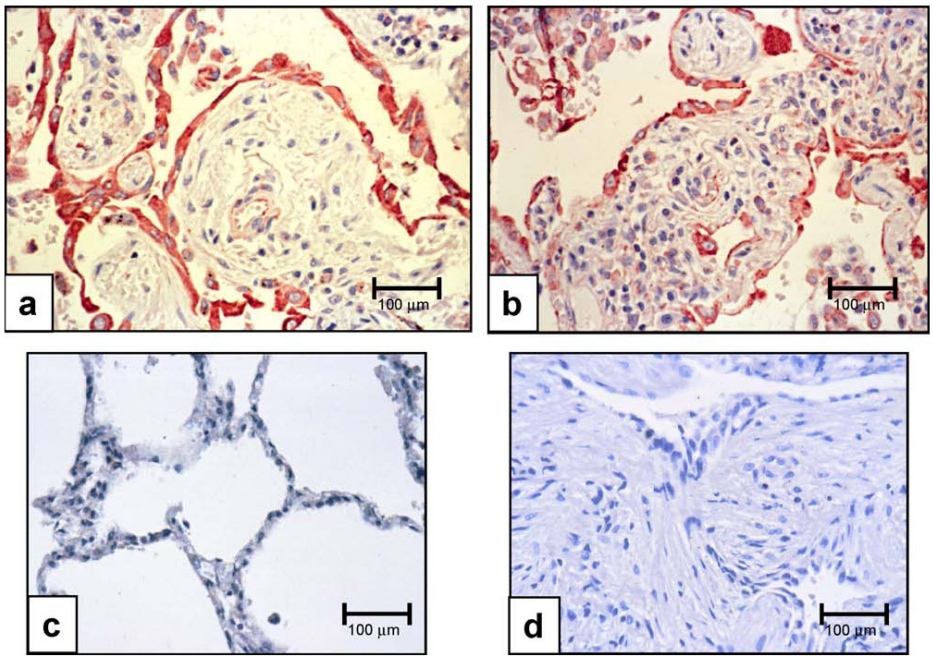

Figure 11. Panels (a) and (b) show SDF-1/CXCL12 protein that was mainly found in alveolar epithelial cells in patients with idiopathic pulmonary fibrosis. Panel (c) shows lung tissue from a healthy control that was negative for SDF-1/CXCL12. Panel (d) shows a negative control where the primary antibody was replaced with non-immune serum. Original magnifications: 40×.

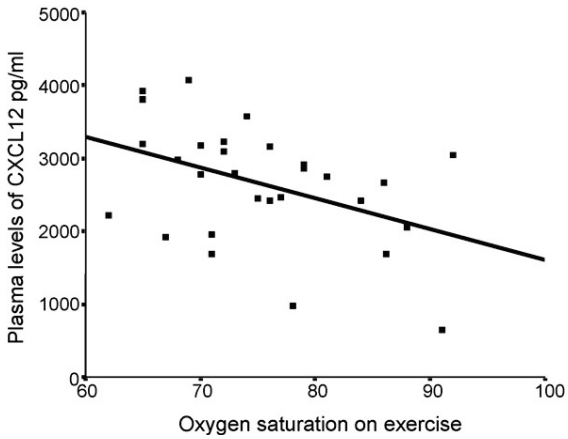

Figure 12. There was a negative correlation between plasma concentration of SDF-1/CXCL12 and oxygen saturation on exercise ($r = -0.41$; $p < 0.04$).

Fibrocytes are Associated with Vascular and Parenchymal Remodelling in Patients with Obliterative Bronchiolitis (Paper II)

The results of this paper show parameters of tissue remodelling that are associated with the number of fibrocytes in patients with OB, after lung or bone marrow transplantation. The proportion of tissue (as opposed to air) was higher in patients with OB (Figure 13). Moreover, the transplanted group had a higher proportion of vessel lumen and endothelial layer than the control group.

The combinations to identified fibrocytes were as follows (in order, more fibrocytes identified by the combination, mentioned first): CXCR4/prolyl 4-hydroxylase, CD45R0/prolyl 4-hydroxylase, and CD34/prolyl 4-hydroxylase (Figure 14). The numbers of fibrocytes were correlated to proportion of tissue (as opposed to air), proportion of vessel lumen, and proportion of endothelial layer. The two patient groups of OB (lung-transplanted and bone marrow-transplanted) could not be differentiated concerning the parameters analysed in this study. However, the bone marrow-transplanted patients were more homogenous than the lung-transplanted patients concerning number of fibrocytes, remodelling of vessels, and thickening of parenchyma.

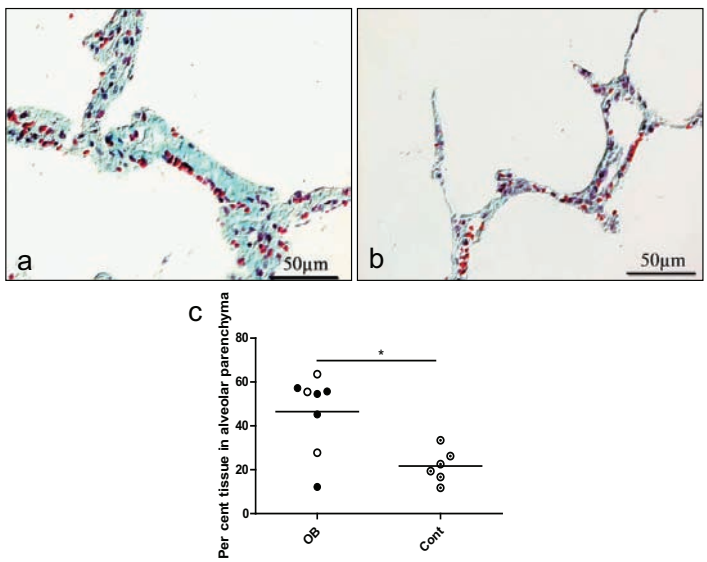

Figure 13. Structural changes in bronchioles were characterized by Gomori's trichrome staining. Panel (a) and (b) represent tissues from patients with thickened (a) and normal (b) alveolar parenchyma, respectively. Original magnification: 20×. Panel (c) shows changes in tissue (in per cent) in alveolar parenchyma in patients with obliterative bronchiolitis (OB). The amount of parenchyma was significantly greater in the OB group than in the controls. Closed circles, LTP; open circles, BMT; circles with dots, control.

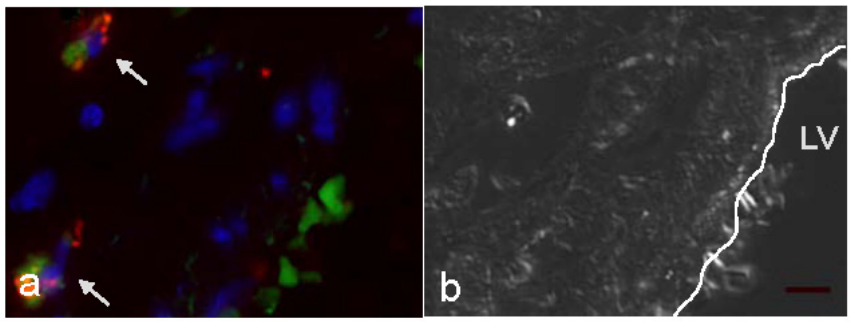

Figure 14. Fibrocytes were identified in patients with obliterative bronchiolitis and controls. Panel (a) shows two fibrocytes identified with a combination of anti-CD34, anti-prolyl 4-hydroxylase, and nuclear staining (indicated with arrows), which were situated 32 µm and 45 µm from the endothelial layer. Panel (b) is a differential interference-contrast image with the lumen of the vessel (labelled LV).

Fibroblasts from Lung-transplanted Patients have Altered Proteoglycan and Proliferation Profiles Compared to Controls (Paper III)

Still today up to 60% of lung-transplanted patients develop BOS within 5 years of transplantation. The rejection is histologically defined as matrix granulation of the airways.

Analysis of the main producers of ECM, fibroblasts, showed that in lung-transplanted patients, distally-derived fibroblasts produced 4.30, 3.91, 2.52, and 1.87 times that produced by the centrally derived fibroblasts of versican, biglycan, biglycan, and decorin, respectively (Figure 15). Fibroblasts obtained from patients 6 months after transplantation produced more versican and they proliferated at a lower rate than the controls (Figure 16). Three of the four patients with the highest production of versican had a poor prognosis with early signs of development or possible development of BOS. The correlation analysis between proteoglycan production and migratory and proliferative properties showed that proteoglycan production by distally derived fibroblasts was negatively associated with migratory rate and proliferative rate. On the other hand, there was a negative association between proteoglycan production by centrally derived fibroblasts and proliferative rate (Figure 17).

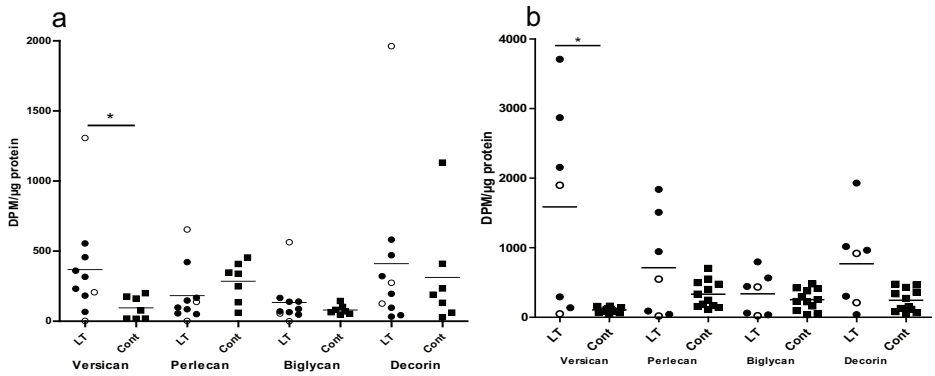

Figure 15. Both centrally-derived (a) and distally-derived (b) fibroblasts from transplanted patients produced significantly more versican than the controls ($p < 0.05$). There was a tendency for the distally-derived fibroblasts to produce more decorin than the controls ($p = 0.11$). Closed circles (●) represent lung-transplanted patients without BOS (bronchiolitis obliterans syndrome), open circles (○) represent lung-transplanted patients with BOS, and closed squares (■) represent controls.

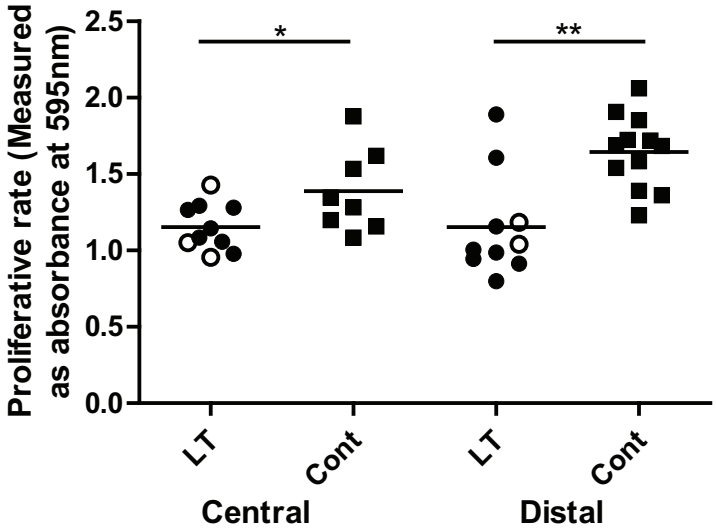

Figure 16. Both centrally- and distally-derived fibroblasts from lung-transplanted patients had significantly lower proliferative rates than the controls ($p < 0.05$ and $p < 0.01$, respectively). Closed circles (●) represent lung-transplanted patients without OB (obliterative bronchiolitis), open circles (○) represent lung-transplanted patients with OB, and closed squares (■) represent controls.

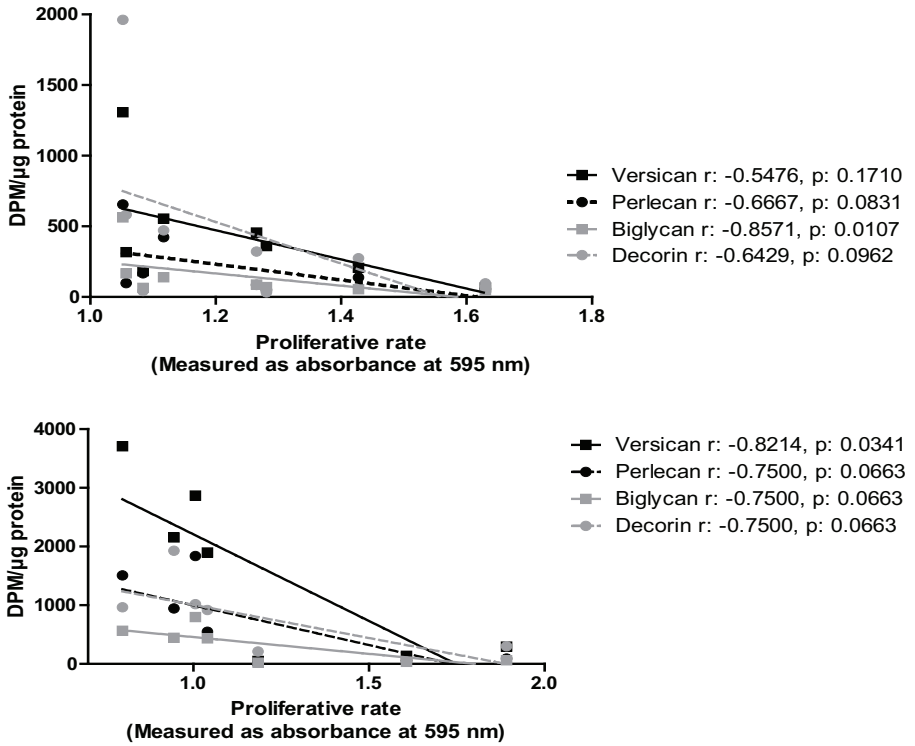

Figure 17. Panel (a) represents correlations in centrally-derived fibroblasts from transplanted subjects between proliferative rate and versican, perlecan, biblycan and decorin production, respectively. Panel (b) represents the same kinds of correlations, but regarding distally-derived fibroblasts.

In two of the patients, biopsies were obtained at different time points 3–12 months after transplantation. One of the patients who developed BOS had a history of CMV infection and elevated C-reactive protein (CRP) levels in the first months after transplantation. These episodes were followed by increased proteoglycan production and reduced lung function. The lung function parameters increased again after changing the immunosuppressive drugs. The other patient did not have any episodes of infection, and the lung function parameters were continuously increasing. In this patient, proteoglycan production was stable over time, except for biglycan, which was elevated shortly after transplantation but decreased afterwards.

Altered Matrix Production in the Distal Airways of Asthmatic and Atopic Individuals (Paper IV)

Modern asthma treatment, as inhaled corticosteroids (ICS), aims to treat the inflammatory processes usually reflected by increased number of eosinophils in the airways. However, ICS do not seem to change the natural course of the disease and do not prevent the ongoing ECM remodelling. This study included atopic patients with and without asthma and healthy controls. The study shows that both central and peripheral airways are involved with activated fibroblasts expressing various proteoglycans.

Expression of one of the proteoglycans, versican, was significantly elevated in fibroblasts derived from the distal part of the lung in asthma patients (Figure 18). The same type of fibroblast also had reduced proliferative properties (Figure 19) and an immobile, scattered, and protrusion-rich appearance. Interestingly, also in these patients with mild asthma, in contrast to the atopic non-asthmatic subjects and the healthy controls, we found increased deposition of collagen in the tissue in the alveolar region (Figure 20).

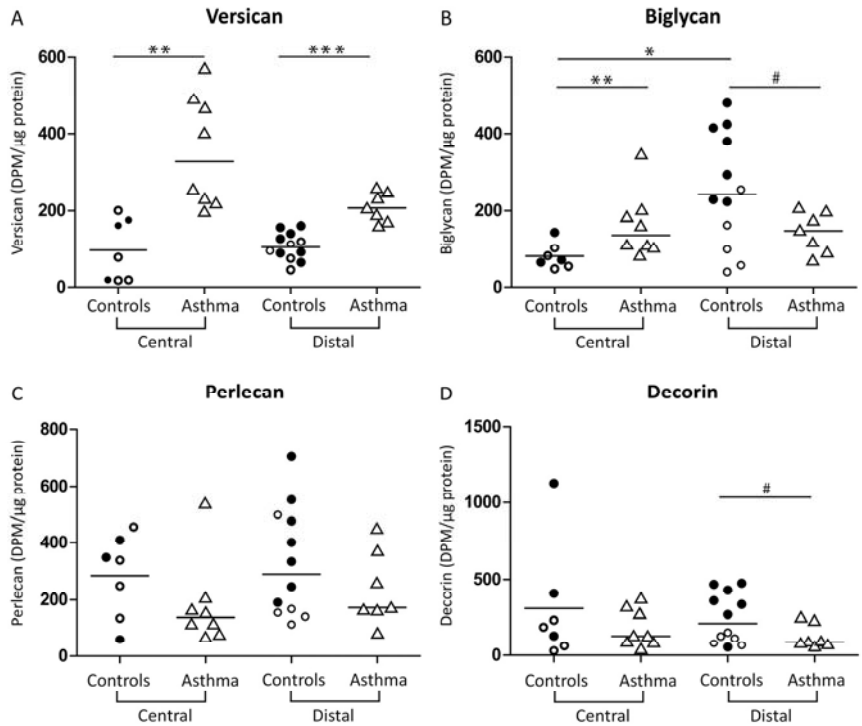

Figure 18. Proteglycan production in centrally- and distally-derived fibroblasts was analysed. Closed circles represent non-atopic controls and open circles represent atopic controls, and open triangles represent asthma subjects.

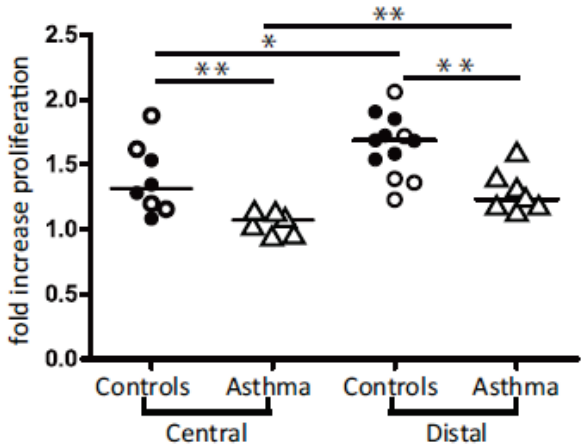

Figure 19. Both centrally- and distally-derived fibroblasts from asthmatic patients had significantly lower proliferative rate than controls ($p < 0.01$). Closed circles represent non-atopic controls and open circles represent atopic controls, and open triangles represent asthma subjects.

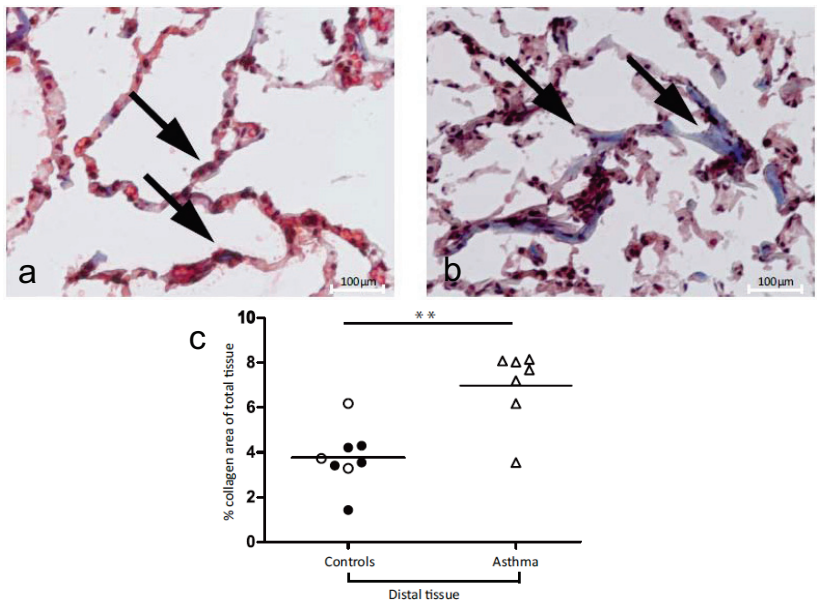

Figure 20. Collagen deposition in control subjects (panel a) and patients with asthma (panel b) as visualised with Masson trichrome staining at 400× magnification. Black arrows show collagen. Panel (c) shows the percentage of the total tissue area that was collagen-positive.

In most cases, fibroblasts obtained from atopic subjects had the same properties as healthy controls, but the production of decorin and biglycan was reduced compared to the controls, and was more similar to that in asthmatic subjects.

TGF-β stimulation increased the general production of versican, perlecan, and biglycan. The centrally-derived fibroblasts from asthmatic subjects did not, however, respond to TGF-β stimulation.

General Discussion

This thesis has focused on tissue repair with special focus on fibrocytes/fibroblasts and tissue alterations involving vessels and parenchyma in IPF, OB, and asthma, and after lung transplantation. Originally, fibroblasts were thought to be immobile cells, their only function being to produce ECM—and that ECM was an inert substance that prevented disintegration. Today, and based on the papers included in this thesis, fibrocytes and fibroblasts are known to be of importance in wound healing as ECM-producing cells that function in response to injury, and we also know that they can release cytokines and growth factors.

The structural changes that are characteristic of IPF are fibroblast foci, for asthma they are basement membrane thickening (74), and for OB occlusion of small airways due to fibrous vascular lesions together with thickening of the alveolar parenchyma. Interestingly, the numbers of fibrocytes can be correlated to structural changes in all three diseases (Figure 21). In IPF, the amounts of fibroblast/myofibroblast foci are a prognostic factor (the more the foci, the worse the prognosis) (146). After normal wound healing, the fibroblasts and myofibroblasts should be diminishes by apoptosis but in IPF, and especially in fibroblast foci, the numbers of fibroblasts and myofibroblasts remain constant (147). It has been speculated that fibroblastic foci with their specific milieu have a composition of cytokines, growth factors, and tissue inhibitor of metalloproteinases (TIMP) such that fibroblasts and myofibroblasts become apoptosis-resistant and ECM is produced in excess. The fibrocytes that were identified in tissue of patients with IPF were however not located inside the foci, but they were located in close proximity to the foci instead, in areas with ongoing signs of inflammation, preceding later development of fibroblastic foci. Fibrocytes that have been recruited to the fibroblast foci could already have been differentiated to fibroblasts or myofibroblasts while they were migrating to the foci.

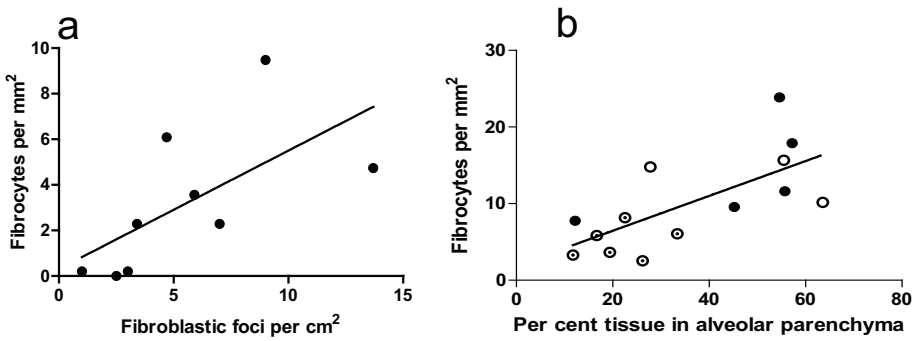

Figure 21. The numbers of fibrocytes identified in tissue in idiopathic pulmonary fibrosis (panel a) were correlated with numbers of fibroblast foci. In obliterative bronchiolites (panel b) the numbers of fibrocytes were correlated with thickening of the alveolar parenchyma.

Patients with OB after lung or bone marrow transplantation had a thickening of the alveolar parenchyma; there was a correlation between this and greater numbers of fibrocytes identified in the tissue. Thickening of the parenchyma could give reduced lung function, which is the criterion of OB. The structural changes observed in this patient group were also that the vessels were remodelled in terms of increased amounts of both endothelial layer and lumen. The common denominators of the remodelling of lung tissue after lung or bone marrow transplantation are fibrocytes—and, more speculatively, local hypoxia.

The cross-talk between HIF-1α – HIF-1β, SDF-1 – CXCR4, VEGF – VEGFR, angiogenesis, and hypoxia is known to be of importance in many diseases, including fibrotic disorders. The two sub units HIF-1α – HIF-1β is together forming a transcription factor that regulate expression of around 100 genes that are of importance in mechanisms such as anaerobic metabolism, angiogenesis, and apoptosis (148). Under normal oxygen levels, HIF-1α is degraded and the complex with HIF-1β does not occur. Hypoxia increases the expression of SDF-1 in endothelial and epithelial cells, and in cells that are in stress after an injury, for example. Furthermore, expression of its receptor CXCR4 (149) is also elevated. A number of cells are known to express CXCR4 on their surfaces: fibrocytes, lymphocytes, muscle cells, and endothelial progenitor cells. Likewise, the expression of VEGF and its receptor VEGFR is also up-regulated, to promote angiogenesis. The remodelled vessels with enlarged lumen and endothelial cell area identified in patients with OB after lung or bone marrow transplantation could be a result of local hypoxia. Furthermore, an enlarged vessel gives a larger entrance area for the fibrocyte. The number of cells that co-express prolyl 4-hydroxylase and VEGFR2 is higher in patients with OB than in control individuals, and furthermore there is a correlation with the number of fibrocytes

identified in tissue (Figure 22). Even though asthma and IPF were not studied in this thesis concerning vessel remodelling, it has been shown that both diseases involve angiogenesis that could be driven by hypoxic forces. In asthma, the vessels located in the bronchia and in the small airways are increased in number. In IPF the angiogenesis is dependent on an imbalance between IL-8 which is angiogenic, and IFN-γ which is angiostatic (150-152).

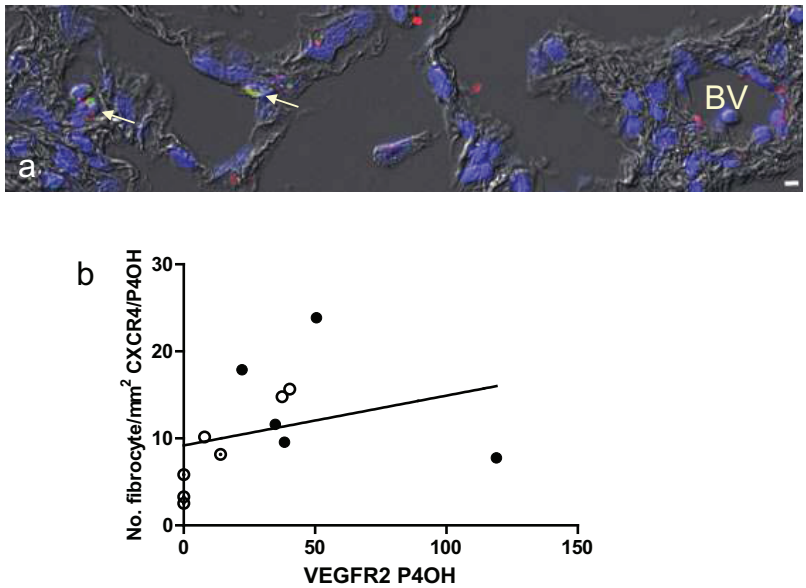

Figure 22. VEGFR2–prolyl 4-hydroxylase positive cells were identified in tissue from patients with oblitertive bronchiolitis. Panel (a) shows two VEGFR2–prolyl 4-hydroxylase positive cells (indicated with arrows), identified 560 µm and 320 µm from the blood vessel (indicated as BV). In the differential interference-contrast picture, VEGFR2 appeared in red, prolyl 4-hydroxylase appeared in green, and nuclear staining was visualised in blue. Original magnifications: 20×. Scale bars of 10 µm are indicated. Panel (b) shows the correlation between the number of VEGFR2–prolyl 4- hydroxylase positive cells and the number of fibrocytes stained with markers CXCR4 / prolyl 4-hydroxylase ($R = 0.7351$, $p < 0.01$). Closed circles, LTP; open circles, BMT; circles with dots, control.

One of the conclusions in Paper II was that pulmonary vessels in patients with OB after lung transplantation are more remodelled than after bone marrow transplantation; this could be explained by the lung transplantation *per se*. For technical reasons, the vessels of the bronchial circulation are not restored during the lung transplantation (153), while patients with OB after bone marrow transplantation have an intact bronchial circulation. A permanent extension of the microvasculature of the bronchial circulation starts directly after lung transplantation (154), and a comparison between lung transplantation with or without bronchial artery revascularization showed that revascularization delays the onset of BOS (14). In a murine orthotopic tracheal transplant model, it has been shown how a well-working vasculature in the bronchial tree reduces the risk of formation of fibrotic lesions (155).

Many research groups have investigated the amounts of circulating fibrocytes in blood. Between 0.1% and 0.5% of the nucleated cells in peripheral blood are defined as fibrocytes (79). Circulating cells in animals are often well-characterised, but in humans this type of study is associated with sources of errors such as undefined infections, latent virus infections, and hormonal changes as a result of the menstruation cycle or stress. Moreover, many studies have shown increased numbers of circulating fibrocytes in association with lung disease. The number of fibrocytes in the end-organ—in this case, the lung—does not have to change even if the pool of circulating fibrocytes is increased.

After the fibrocytes have entered the tissue, the fibrocytes can differentiate into other cell types and/or continue to migrate to the lumen of the airway. Asthmatic patients and IPF patients differ concerning the types of cells found in the BAL fluid. In asthmatic patients, a relatively high proportion of the fibroblast population expresses fibrocyte markers such as CD34, CD45RO, and α-SMA (74). In the cell population in BAL fluid from IPF patients, 1.0–3.4% of cells were of mesenchymal origin. It is possible that this cell population is of fibrocytic origin but has differentiated because of the local environment in the IPF lung, and for this reason does not express CXCR4 for example.

There is a connection between hypoxic milieus and increased expression of proteoglycans—at least in vessels, but probably also in lung tissue. Asplund *et al.* recently published a paper on hypoxic regulation of versican and perlecan, where they identified co-localization between versican and HIF-1α in atherosclerotic lesions, and an up-regulation of perlecan expression (128). HIF-1α has previously been reported to be an aggrecan and collagen II inducer in chondrocytes and HIF-2α has been shown to correlate with elevated expression of versican in stem cells (156;157).

In studies of fibroblasts obtained from lung-transplanted or asthmatic subjects, increased expression of versican has been identified both in centrally and distally derived cells (COPD patients show the same pattern (158)). Versican seems to be an important proteoglycan in fibrotic lung disorders and has many important functions in the fibrotic tissue, such as binding to cytokines and enzymes. The numerous negatively charged GAG chains attached to the protein core have a high capacity to bind water molecules. According to our results, versican appears to be associated with a reduced proliferation and migration rate in fibroblasts. In paper IV, two patients who had been lung transplanted were studied concerning proteoglycan production. One of largest changes was the production of versican, which was increased shortly after a viral infection.

In Papers III and IV, biopsies were obtained from both the patient groups and a control group. Primary fibroblast cultures were prepared from the biopsies. In work with primary cell cultures, many questions arise. What happens to cells when they are not in their local microenvironment in the tissue? The biopsies were put in a plastic flask in an artificial environment, where only the fibroblasts that were best suited would survive and proliferate. What conclusions can one draw? In our defence, all experiments were done at as low passage numbers as possible. The medium (DMEM containing 10% foetal clone III serum, gentamycin, and amphotericin B) had specific ingredients that favoured fibroblasts above other cell types. The passaging of the cell cultures eliminates macrophages, which are incapable of attaching to the flask after trypsinisation. Furthermore, working with primary cell lines makes it possible to analyse cells that are primed in a healthy or diseased microenvironment, which is not possible in work with cell lines or in animal studies.

From cell biological studies of asthmatic subjects, it has been concluded that there is strong involvement of remodelling of the distal part of the lung. A few years ago, the consensus was that only the bronchial part of the airways was affected in asthma. Furthermore, patients who have been lung transplanted have remodelling in distal parts of the lungs. The production of proteoglycans in the distal part of the lung is many times higher than the production in the central part in this patient group. These changes are recognised 6 months after transplantation, and occur independently of whether the patient develops BOS or not.

From the papers included in this thesis, we conclude that fibrocytes are located in the pulmonary tissue of patients with IPF and OB after lung or bone marrow transplantation. In IPF, there is a correlation between the numbers of fibrocytes in the tissue and the number of fibroblastic foci, and SDF-1/CXCL12 was elevated in plasma and BAL fluid and detectable in epithelial cells. In OB, there was a correlation between the number of fibrocytes and both vessel remodelling and thickening of alveolar parenchyma. The fibrocytes can differentiate into

fibroblasts and produce ECM molecules. In asthmatic patients and patients who have undergone lung transplantation, fibroblasts produce elevated levels of versican. Furthermore, the fibroblasts proliferate at a lower rate than fibroblasts from healthy controls. Both the central part and the distal part of the lung are involved in tissue repair in asthma and after lung transplantation.

Future Perspectives

Even though the results concerning fibrocytes and fibroblasts have answered some questions regarding IPF, OB, lung transplantation, and asthma, the insights obtained raise new questions. As mentioned in the discussion on local hypoxia, this phenomenon could either be a result of increased ECM or could be an inducer of production of more ECM. To investigate the problem, fibroblasts could be cultured under hypoxic conditions w/w matrix-coated cell culture flasks. Further investigations concerning the expression of VEGF/VEGFR and TGF-β could help us to understand the mechanism behind fibrotic disorders. Hypoxia could perhaps also affect the migratory and proliferative properties of fibroblasts.

A combination of analysis of tissue-specific fibroblasts and circulating fibrocytes at the same time point would open up the possibility of analysis of which pool of fibrocytes enters the tissue at different stages of disease. Fibrocytes in cell cultures could also be analysed concerning phenotype and how ECM molecules can stimulate differentiation. The mechanism of movement of the fibrocyte from the bloodstream to the tissue is hypothesised to be the same as for leucocytes: rolling. Such mechanisms could be a potential target for future therapeutic intervention.

Today, standard pharmacological therapy is the pharmaceuticals used against rejection after transplantation is cyclosporine A and azathioprin. In a study by Eickelberg *et al.*, cyclosporine A was shown to inhibit the effect of TGF- β -induced fibrosis in human lung fibroblasts (159). The effect of azathioprin is still not known, and the effects of both pharmaceuticals on primary lung fibroblasts from transplanted patients have not been studied. How these pharmaceuticals interfere with other pathophysiological features such as fibroblast differentiation and tissue recruitment is not known.

A possible stem cell origin of different cell types is debatable. Sex mismatch transplantation makes it conceivable to determine the origin of fibrocytes, epithelial cells, endothelial cells, and mast cells. The epithelial cells surrounding the neo-lumen in Figure 2 would have been interesting to analyse concerning origin; likewise, the endothelial cells in tissues with enlarged endothelial layers.

Summary in Swedish

I denna avhandling har sjukdomar såsom astma, idiopatisk fibroserande alveolit (IFA), obliterativ bronkiolit (OB) och förändringar efter lungtransplantation studerats beträffande förändringar i lungan. Gemensamt för dessa tillstånd är att de är kroniska. Astma hör till en av våra folksjukdomar medan IFA och OB är mindre vanligt förekommande. Lungtransplantation är en behandling av de senare stadierna av kronisk obstruktiv lungsjukdom, cystisk fibros, emfysem och IFA. Tyvärr drabbas hälften av de som lungtransplanterats av OB som är en kronisk avstötning.

En celltyp som i högsta grad är involverad i dessa tillstånd är fibroblasten. Denna celltyp har egenskaper såsom att dela sig och på så vis öka i antal och att förflytta sig, utan att använda blodkärl. En annan egenskap som är väldigt viktig i sjukdomarna som studerats i denna avhandling är att fibroblaster kan producera ett nätverk av trådar och flaskborstliknande strukturer. Dessa nätverk sitter delvis fast i fibroblasten, men fäster också till andra typer av celler. Nätverket ger stabilitet av exakt rätt grad för att vävnaden ska ha de specifika egenskaper som krävs för dess funktion (figur 6. sida 29). Vid en skada i vävnaden förändras nätverket så att skadan läks och sedan efter tid återgår till det normala. Vid vissa sjukdomar bildas det fel typ av nätverk vid vävnadsskadan. Detta leder ofta till att nätverket blir stelare och tappar sin funktion. Då detta sker i lungorna försämras således lungfunktionen. I avhandlingen har astmatiker och patienter som genomgått lungtransplantation studerats med avseende på vilka flaskborstliknande strukturer som fibroblasterna producerar. Vi har sett att både de övre delarna av lungan, bronkerna och de nedre delarna, lungblåsorna har förändrade egenskaper.

Som nämnts kan fibroblasterna dela sig för att öka i antal. Ett annat sätt att öka i antal är att rekrytera celler från blodet, fibrocyter som kan utvecklas till fibroblaster då de når vävnaden. I avhandlingen har fibrocyter som ännu inte har utvecklats till fibroblaster, identifierats i vävnaden vid IFA och OB. Vid IFA har vi även upptäckt hur fibrocyterna hittar till den skadade vävnaden och hur antalet fibrocyter i vävnaden stämmer överens med antalet område av extra tät nätverksstruktur. Vid OB har antalet fibrocyter i vävnaden visat sig stämma överens med att kärlen har blivit större och att vävnaden har blivit tätare.

Med forskningen kring dessa sjukdomar hoppas vi på att kunna förstå förändringarna i lungorna och på så vis kunna förutse dessa förändringar och i ett senare skeende också kunna bota desamma.

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Pappa & Lone
Min kära syster Lotta
Monica & Lasse

Alla släktingar och vänner!

Magnus, Hannes och Kajsa.
Ni är mitt allt!

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