

Article Type: Invited Reviews

Involvement of fibrocytes in asthma and clinical implications

Sabrina Mattoli

*Avail Biomedical Research Institute, Scientific Direction and Project Management Centre, Basel,
Switzerland*

Condensed title: Fibrocytes in asthma

Correspondence: Sabrina Mattoli, MD, PhD, Scientific Direction and Project Management Centre,
Avail Biomedical Research Institute, Elisabethen, Postfach 102, CH-4010 Basel, Switzerland.

Telephone: +41 61 262 3564; Fax: +41 61 544 7374; E-mail: smattoli@avail-research.com

Summary

Blood-borne fibrocytes are bone marrow-derived cells that participate in immune responses and exhibit pro-inflammatory and matrix remodelling properties. In asthmatic patients receiving an adequate treatment, the blood fibrocyte count is very low and comparable to that obtained in healthy individuals. In these patients, a transient increase in fibrocyte numbers in the peripheral blood and in the airways occurs in concomitance with increased bronchial inflammation, and reflects disease worsening and the need for more intensive treatment. Persistently elevated numbers of fibrocytes in the peripheral blood and in the bronchial mucosa are observed in chronically undertreated or corticosteroid-resistant asthma, and are associated with persistent airway

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cea.12525

This article is protected by copyright. All rights reserved.

Accepted Article

inflammation and ongoing remodelling of the bronchial wall. The asthmatic bronchial epithelium is the main source of fibrocyte chemoattractants in asthma, and contributes with T helper type 2 lymphocytes and eosinophils to promote the proliferation and pro-remodelling function of recruited fibrocytes. The presence of elevated numbers of fibrocytes in the bronchial mucosa of allergic patients with undertreated or treatment-resistant asthma may also increase the risk of acute exacerbations because these cells can amplify T helper type 2 lymphocyte-driven inflammation on every exposure to the clinically relevant allergen, and can promote further inflammation on rhinovirus infections by allowing viral replication and releasing additional pro-inflammatory factors. Improved methods for the isolation and functional analysis of pure populations of viable circulating fibrocytes have allowed a better understanding of the effector role of these cells. A reliable and clinically applicable assay has been developed to measure blood fibrocyte counts as outcome measure in future clinical trials. New therapeutic agents are needed to block both persistent inflammation and fibrocytosis in corticosteroid-resistant asthma.

Key words: Allergy; Asthma; Fibrocyte; Outcome measure; Rhinovirus.

Background information on fibrocytes and objectives of this review

Blood-borne fibrocytes were initially identified in murine wound chambers and in human wounded skin as CD34-positive cells with mixed features of fibroblasts and leukocytes [1]. Similar cells displayed an elongated or spindle-shaped morphology in long-term cultures of peripheral blood mononuclear cells (PBMCs), where they could be distinguished from the other cells because of the co-expression of CD34, CD45, CD11b, and CD13 on the cell surface, intracellular collagen type I (COL1) and COL3, and ability to acquire a myofibroblast-like contractile phenotype [1-3]. Analysis of the leukocyte infiltrating wound chambers over 5 days showed that COL1 was specifically expressed

at the mRNA level in CD34⁺ cells and was absent in CD14⁺ monocytes and in T lymphocytes [2].

Tracking studies in mice [4,5] have subsequently demonstrated that the fibrocytes originally identified in the wounds originate in the bone marrow and participate in the repair process at an early stage. Human studies in the settings of forensic pathology (determination of wound age) [6] and pulmonary intensive care (acute respiratory distress syndrome) [7] have confirmed the involvement of fibrocytes in the normal response to acute tissue injury.

Blood-borne fibrocytes exhibit multiple and complex functional properties that justify their early involvement in the normal repair processes and their potential contributions to the pathogenesis of disorders associated with persistent injury or defective tissue repair. They can exert both pro-fibrotic and pro-inflammatory activities [2,8-11], promote angiogenesis [12] and participate in innate and adaptive immune responses [10,11,13,14]. Similarly to some fibroblasts and to myofibroblasts [15-21], they produce collagenous and non-collagenous extracellular matrix (ECM) molecules and also contribute to collagen degradation through the Endo180-mediated endocytosis pathway [6]. Since the discovery of their role in wound healing, these cells have been implicated in the pathogenesis of several fibrotic disorders [22-25] and in the progression of diverse chronic inflammatory diseases, where persistent inflammation is driven by toll-like receptor danger signals [10] or dysregulated T cell activation and autoimmunity [22,23,26-28] and is associated with angiogenesis and excessive deposition of ECM molecules at the tissue sites [22-28]. Many studies from diverse laboratories and clinical centres have demonstrated the potential contribution of fibrocytes to the pathogenesis of airway remodelling in asthma [9,29-34] and their major role in the acute exacerbations caused by allergen exposure and viral infections [11,29,35]. My primary objective is to critically review the findings of these investigations and discuss their clinical implications.

The evaluation of the potential role and clinical relevance of fibrocytes in asthma and other diverse chronic inflammatory and fibrotic disorders has been a challenging area of investigation because of the rarity of circulating and tissue fibrocytes in normal individuals [9,22,23], the absence of a single

specific marker [22,23,26], and the lack of appropriate methods for the isolation of pure populations of viable fibrocytes from the peripheral blood and inflamed or fibrotic tissues [9,27]. In light of recent observations [11], the simple method commonly employed to isolate fibrocytes from cultures of PBMCs on the basis of their spindled shape and co-expression of CD45 and COL1 upon immunohistochemical or immunofluorescence analyses alone has some limitations. Circulating monocytes express the pan-leukocyte marker CD45, and those co-expressing CD163 and other scavenger receptors [36-39] acquire a spindle-shaped morphology in culture [40-43] similar to that of the originally identified fibrocytes [1]. Dendritic cells (DCs) are also present in the CD45⁺ PBMC fraction, and some of them express various scavenger receptors [39]. Neither monocytes nor DCs express the specific mRNA for COL1 [11], but can show COL1 immunoreactivity, particularly with the commonly used anti-COL1 polyclonal antibodies [11], because the scavenger receptors present on the cell membrane and inside the cytoplasm contain a collagenous domain analogous to the triple helical domain of fibrillar collagens [44-47]. The risk of non-specific staining may increase if the blood samples come from patients with fibrotic disorders or treated with systemically administered synthetic corticosteroids (from here onwards referred to as corticosteroids) for other conditions (also including severe asthma) because the frequency of monocytes expressing CD163 and other scavenger receptors is higher in the peripheral blood of these individuals than in normal subjects [48-54]. Even more challenging is the identification of fibrocytes in chronically inflamed or fibrotic tissues because of the presence of CD45⁺CD163⁺ monocytes and DCs [48-50,55] and the involvement of CD45⁺ monocyte-derived macrophages in collagen uptake and degradation [56]. In addition, fibrocytes recently recruited from the peripheral blood frequently acquire an elongated shape or dendriform morphology at the tissue site [4,29,30].

If appropriate procedures for the immune labelling of CD34⁺ cells (e.g. staining with phycoerythrin-conjugated class III monoclonal antibodies) are used [57], the identification of fibrocytes as cells co-expressing surface CD45 and CD34 in conjunction with intracellular COL1 [1,2] remains an accurate method because CD34 is not expressed on the surface of monocytes, macrophages, DCs or collagen-

producing cells different from fibrocytes (circulating or resident mesenchymal stem cells, their progenies and tissue fibroblasts) [58-60]. Most studies in asthma have employed this method for the enumeration of fibrocytes in the peripheral blood and in the airways [9,11,29-32,34,35,61], and those conducted in my laboratories [9,11,29,34,35,61] have often included an analysis of COL1 expression at the gene level to confirm cell identity [9,11,29,61]. In one of these studies [11], removal of CD34⁺ cells from the CD45⁺CD14⁺ PBMCs of asthmatic individuals completely removed the population of cells expressing *COL1A1* mRNA, as assessed by *in-situ* hybridisation with a validated probe [9,29,62,63].

The other molecules present on the fibrocyte surface or inside fibrocytes [1-3,8,9,11,30-35,61] are expressed by several cell types [59,60,64-76], but the co-expression of CD45/CD45RO, CD34, CD11b and CD13 at high level is a unique feature of fibrocytes, and the lack of CD115 is an additional characteristic that differentiates human [11] and mouse [77] fibrocytes from human and mouse monocytes [73]. Another objective of this review is to discuss the usefulness and clinical applicability of methods involving the identification and isolation of viable circulating fibrocytes on the basis of these phenotypic characteristics [9,11,34,35,61]. The readers can find a list of the markers attributed to fibrocytes and monocyte-derived fibrocyte-like cells in a recent review [26].

Fibrocyte trafficking and contribution to airway remodelling in asthma

Circulating fibrocytes

The analysis of the expression of *COL1A1* mRNA in circulating CD45⁺CD34⁺COL1⁺ cells has permitted to definitely ascertain that authentic fibrocytes are present as such in the whole blood of normal and asthmatic subjects [9], thereby confirming results obtained by flow cytometry analysis of CD45⁺CD34⁺COL1⁺ PBMCs [31,32]. However, the numbers of circulating fibrocytes in healthy subjects are very low, irrespective of the method used for their enumeration [9,31,32,35,61]. This observation

explains why sufficient numbers of normal fibrocytes for *in-vitro* testing are usually obtained after long-term cultures of PBMCs in media that selectively favour their proliferation and survival [1-3,8]. The same observation also excludes the possibility that all fibrocytes differentiate from monocytes or other cells present in the CD14⁺ PBMC fraction, as initially thought on the basis of the data available at that time [22,23]. Nonetheless, there are immature progenitor cells in the bone marrow and peripheral blood that co-express myeloid antigens and pluripotency/self-renewal markers and give rise to fibrocyte-like cells when cultured under conditions that favour the development and survival of such cells, as reviewed elsewhere [23].

The frequency of circulating CD45⁺CD34⁺COL1⁺/CD45⁺CD34⁺COL1A1 mRNA⁺ fibrocytes remains within the normal range in patients with treatment-responsive asthma of any severity, if their disease is adequately controlled by current therapy [31,34,61]. In contrast, there is a substantial increase in the number of peripheral blood fibrocytes in two specific groups of asthmatic subjects: patients with allergen-exacerbated asthma, whose disease is transiently uncontrolled and require a more intensive therapy to re-achieve adequate control [9,35,61]; patients with chronically treatment-refractory severe disease (also including those with “chronic obstructive asthma”), whose disease is persistently inadequately controlled despite an intensive treatment with high doses of inhaled corticosteroids and/or systemic corticosteroids [31,32-34,61]. The marked increase in the number of circulating fibrocytes demonstrated in my laboratories during acute exacerbations of asthma [9,35,61] has been recently confirmed by another group [78].

Airway fibrocytes

Studies involving comparative analyses of bronchial biopsy specimens from normal individuals and patients with various levels of asthma severity, have demonstrated the presence of an appreciable number of fibrocytes in the asthmatic airways and the virtual absence of similar cells in the normal airways [30,31]. These fibrocytes actively synthesise COL1, express α -SMA, localise to areas of ECM

Accepted Article

deposition beneath the epithelial basement membrane, in the *lamina propria* [30,31], and may also propagate toward the underlying smooth muscle bundle [31]. The density of fibrocytes in the subepithelial area correlates with the magnitude of subepithelial fibrosis, as estimated by measuring the thickness of the *lamina reticularis* [30]. Fibrocyte infiltration of the *lamina propria* also increases in patients requiring more intensive treatment to achieve disease control (e.g. those with treatment-responsive severe asthma), as demonstrated by the existence of a highly significant positive correlation between fibrocyte numbers in the *lamina propria* and asthma severity defined on the basis of the Global Initiative for Asthma treatment steps [31]. The highest densities of fibrocytes in the bronchial mucosa have been observed in patients labelled as mild asthmatics and not receiving an anti-inflammatory treatment (corticosteroid-naïve patients) [30], and in those who suffers from corticosteroid-resistant severe asthma [31]. The presence of numerous fibrocytes in the airways of the latter patients has been confirmed in a study involving asthmatics with chronic airflow obstruction despite the use of elevated doses of topical corticosteroids or oral corticosteroid treatment [79]. When the analysis of the bronchial biopsy specimens shows a prominent fibrocytes infiltration of the bronchial subepithelial area, it is possible to isolate CD34⁺COL1⁺α-SMA⁺ fibrocytes in cultures of BAL cells [30]. Moreover, an appreciable number of fibrocytes is also present in the induced sputum of patients with transiently uncontrolled asthma, as assessed by analysis of the cells co-expressing CD34 and *COL1A1* mRNA [9,27]. The fact that fibrocytes recently migrated from the peripheral blood toward the epithelium can be recovered by BAL and sputum induction is probably related to their high motility [30], which is a common feature of all cells bearing Endo180 on their surface [80].

In the bronchial mucosa of allergic patients with mild asthma, the density of CD34⁺ *COL1A1* mRNA⁺ fibrocytes is very low if the disease is adequately controlled by avoiding allergen exposure and using an appropriate treatment [29]. Following exposure to the clinically relevant allergen in laboratory,

the density of fibrocytes actively synthesising COL1 progressively increases in the *lamina propria* of these subjects between 4 and 24 hours post allergen exposure, and a substantial proportion of the CD34⁺COL1A1 mRNA⁺ cells also express the contractile protein α -SMA at 24 hours [29]. Taken together, these findings and the observations described above strongly suggest that fibrocytes may contribute to the development and progression of structural changes, such as subepithelial fibrosis and increased contractile mass, in patients with undertreated asthma and treatment-resistant disease, and that the structural alterations may further increase during the course of every acute exacerbation of asthma. The hypothesis that fibrocytes significantly contribute to the development and progression of airway remodelling in asthma is supported by the results of two studies employing a similar murine model of chronic allergic asthma [29,81], and by the evidence that CD45⁺CD34⁺CD11b⁺ mesenchymal stem cells (other potential sources of COL1⁺ α -SMA⁺ cells) do not have such an effect and even ameliorate established alterations [82]. Further support is provided by the observation that circulating fibrocytes from patients with transiently uncontrolled asthma and patients with treatment-resistant severe disease rapidly proliferate, produce collagenous and non-collagenous ECM components commonly increased in the asthmatic bronchial mucosa, and differentiate into α -SMA⁺ myofibroblast-like cells when stimulated with the soluble phase of autologous sputum [34] or the recombinant forms of cytokines present in the autologous sputum [9,27,35]. Although none of the studies mentioned above has demonstrated a significant correlation between the number of circulating fibrocytes and the level of airflow obstruction in asthma, a significant correlation exists between the number of circulating fibrocytes and the magnitude of the annual decrease in lung function over the previous 5 years in asthmatics with chronic airflow obstruction despite the intensive treatment [32].

A concomitant increase in the numbers of fibrocytes in the peripheral blood and in the airways of asthmatic individuals is observed in association with a transient exacerbation of airway inflammation during periods of disease worsening [9,35], and in the presence of ongoing eosinophilic inflammation, as in the case of patients with corticosteroid-resistant asthma [31,34]. The existence of an active recruitment of fibrocytes from the circulation into the inflamed airways of these patients is suggested by the observations that circulating fibrocytes from symptomatic asthmatics migrate in response to cytokines present in increased amounts in their sputum [34,35], and by the evidence of a positive correlation between the numbers of sputum fibrocytes and the sputum contents of some of these cytokines, such as IL-33 [27,35] (Fig. 1). Notably, more than 50% of the fibrocyte chemotactic activity present in the sputum fluid phase from patients with corticosteroid-resistant severe asthma has been attributed to a combination of known epithelium-derived eosinophil chemoattractants, the C-C motif chemokine ligand (CCL)5 (also known as regulated on activation normal T cell expressed and secreted or RANTES), CCL11 (also known as eotaxin) and CCL24 (also known as eotaxin 2 or myeloid progenitor inhibitor factor-2) [34]. Similar fibrocyte chemotactic activity cannot be detected in the sputum fluid phase from patients with treatment-responsive and well-controlled disease [34]. Such findings are in keeping with the observation that fibrocyte infiltration of the asthmatic airways and/or sputum fibrocytosis most frequently occur in association with bronchial and/or sputum eosinophilia, as consistently reported in atopic patients evaluated before and after allergen challenge [29,83], in atopic asthmatics tested during a period of spontaneous disease worsening [9], and in atopic and non-atopic patients with treatment-resistant severe asthma [31]. On the basis of studies that have evaluated the cytokines and chemokine receptors potentially involved in the enhanced release of CD34⁺ progenitor cells and fibrocytes from the bone marrow in asthma and experimental lung fibrosis [83-88], it is also reasonable to hypothesise that IL-33, CCL11 and the other CCR3/CCR5 ligands mentioned above may all contribute to increase the frequency of circulating fibrocytes in transiently uncontrolled asthma and in treatment-resistant severe disease through this mechanisms.

Some studies have provided evidence that the C-X-C motif chemokine ligand (CXCL)12 (also known as stromal cell-derived factor-1) may be another epithelium-derived factor potentially involved in the recruitment of circulating leukocytes [89,90] and fibrocytes [78] into the asthmatic airways and that it may contribute to angiogenesis and development of chronic airflow obstruction in asthma [89]. Moreover, this chemokine has been implicated in the tissue recruitment of fibrocytes in many diverse chronic inflammatory and fibrotic disorders [23,24,26]. CXCR4 is one of the receptors for CXCL12 and is expressed by normal fibrocytes [3], mesenchymal stem cells [68], all hematopoietic progenitor cells, most mature leukocytes, dendritic cells, macrophages, vascular endothelial cells, fibroblasts and many other tissue-resident cells [75,76]. Registered antagonists of CXCR4, such as AMD3100 (plerixafor), are used to induce the mobilisation of stem cells and progenitor cells from the bone marrow and cause leukocytosis [75,76]. During allergen-induced asthma exacerbations, an appreciable mobilisation of the population of CD45⁺CD34⁺CXCR4⁺CCR3⁺ cells occurs in concomitance with transient down-regulation of CXCR4 on these cells, reduced concentrations of CXCL12 in the bone marrow and increased levels of CCL11 in the peripheral blood [91]. The high concentrations of CXCL12 in the bronchial mucosa of these patients may then contribute to promote the airway recruitment of the mobilised cells, which also include fibrocytes. It is however worth noting that an ever increasing number of factors regulate CXCL12/CXCR4 signalling in a complex manner [75,76] and that this is a big limitation when trying to interpret existing studies addressed to analyse the mechanisms and functional consequences of the binding of CXCL12 to CXCR4 [75].

Activation of epithelial cell- and eosinophil-derived transforming growth factor- β (TGF- β) and increased release of endothelin (ET)-1 from the bronchial epithelium are major events implicated in the local expansion and pro-fibrotic function of fibrocytes in asthmatic airways. The effects of recombinant TGF- β 1 and ET-1 have been demonstrated in my laboratories by employing pure populations of normal and asthmatic peripheral blood fibrocytes exposed to these agents over 6 days

Accepted Article

in the absence of other stimuli [29] and by using freshly isolated circulating fibrocytes from normal and asthmatic individuals as well [61]. Similar effects, but with more marked *in-vitro* differences between asthmatic and normal fibrocytes, have been recently obtained by another group in long-term cultures of non-adherent, T cell-depleted PBMCs stimulated with 50 ng/ml stem cell factor in media highly supplemented with serum [32,79]. Although active signalling of TGF- β in the asthmatic bronchial mucosa may promotes the pro-fibrotic function of fibrocytes mainly because TGF- β increases the synthesis and release of collagenous proteins in these cells [3,29], additional mechanisms are suggested by the recent observation that TGF- β 1 markedly down-regulates the expression of Endo180 in lung fibroblast and fibroblast-like cells in the setting of experimental pulmonary fibrosis provoked by acute lung injury [19]. Thus, active signalling of TGF- β in the asthmatic bronchial mucosa may also promotes the pro-fibrotic function of fibrocytes by inhibiting their ability to internalise and degrade collagen fragments after protease cleavage at the tissue sites, with the consequent increase in cross-linking of extracellular collagen and enhanced stiffness of the ECM.

IL-4 and IL-13 are cytokines produced in the asthmatic airways by T helper type 2 (T_H2) lymphocytes and their concentrations increase in the sputum of atopic asthmatics during spontaneous disease exacerbations [9]. Both these cytokines enhance the production of collagenous and non-collagenous ECM components relevant to asthma (COL3, COL5, hyaluronan, versican, perlecan/heparan sulphate proteoglycan 2, tenascin-C) in autologous circulating fibrocytes [9], and induce the expression of α -SMA in these cells [9]. The T_H17 cell-derived cytokine IL-17A is also increased in the sputum of the same patients, but it mainly stimulates the proliferation of autologous circulating fibrocytes and their production of factors that promote neutrophil recruitment and airway hyperresponsiveness, such as CXCL1, CXCL8 and tumor necrosis factor- α (TNF- α) [9]. It does not affect the synthesis of collagens in these cells but promotes their expression of α -SMA, both directly and *via* autocrine effects

apparently mediated by the IL-17A induced up-regulation of CXCL8 release, without activating TGF- β signalling [9]. These findings from my laboratories are in keeping with other observations [92,93] that CXCL8 can induce α -SMA in fibroblast-like cells and hepatic stellate cells without increasing collagen production and in the absence of TGF- β activation. Moreover, a more recent study [94] has reported similar disjunctive effects of IL-17A on the expression of α -SMA and COL1 production in fibrocyte-like cells that were obtained from long-term cultures of normal PBMCs after stimulation with platelet-derived growth factor-BB to increase the yield of spindle-shaped cells.

Fig. 2 schematically summarise the main mechanisms through which factors released from the bronchial epithelium, T lymphocytes and other cells present in the inflammatory infiltrate can promote the recruitment, local proliferation and pro-fibrotic function of fibrocytes in asthmatic airways, on the basis of the data discussed above. It is important to clarify that although the expression of α -SMA in non-smooth muscle cells at the tissue sites is a feature of the contractile fibroblasts termed myofibroblasts [95], several cell types express this contractile protein, either constitutively or upon appropriate stimulation [65,96], also including amongst others follicular DCs [67] and subepithelial cells with antigen-presenting function in the colonic mucosa [66], which may not be easily distinguished from contractile fibroblasts. Only some of the cells that express α -SMA (myofibroblast and fibrocytes) also produce collagens, and the notion that myofibroblasts are the major pro-fibrotic players in the pathogenesis of organ fibrosis has been recently challenged by the evidence that deficiency of α -SMA or reduced expression of α -SMA in fibroblasts are associated with enhanced experimentally induced renal and pulmonary fibrosis [97,98] and with the development of cardiac fibrosis in aged mice [99]. In the asthmatic airways, the increased deposition of specific ECM components, the enhanced stiffness of this matrix, and the contractile force generated by α -SMA⁺ fibrocytes in the subepithelial zone may certainly concur to lead to chronic airflow obstruction, but may also serve to prevent further damage to the inflamed bronchial mucosa during the increased

mechanical stress generated by episodic bronchial smooth muscle contractions [27]. In keeping with this hypothesis are the results of a comparative analysis of the expression of collagens and proteoglycans in fibrocytes and lung fibroblasts [8], suggesting that fibrocytes have matrix remodelling properties consistent with a predominant tissue-stabilising function, similar to that originally attributed to myofibroblasts present in the granulation tissue [100,101], while most resident fibroblasts have a predominant matrix-building function [8].

Role of fibrocytes in the provocation of asthma exacerbations

The fibrocytes exhibit antigen-presenting properties *in vitro* [11,13] and *in vivo* [13]. Cultured normal fibrocytes [13] and fibrocytes freshly isolated from the peripheral blood of allergic asthmatic individuals [11] express HLA-DR, the co-stimulatory molecules CD80 and CD86, and all the adhesion molecules required for an adequate accessory function. Similarly to myeloid DCs (mDCs), they also express CCR7 [3] and the CCL21-CCR7 axis significantly contributes to regulate the allergen-induced fibrocyte trafficking in a murine model of allergic asthma [102]. Fibrocytes are however phenotypically and functionally different from freshly isolated circulating (CD3, CD14, CD16, CD19, CD20, CD56)⁻CD1c/BDCA-1⁺CD141/BDCA-3⁺CD11c⁺HLA-DR⁺ myeloid DCs (mDCs) [11]. Cultured CD45⁺CD34⁺COL1⁺COL1 mRNA⁺ fibrocytes [8] as well as cultured monocyte-derived fibrocyte-like cells [14,42] also show detectable levels of expression of CD206, a mannose receptor that has been implicated in the *in-vitro* uptake of some native allergens by monocytes, monocyte-derived DCs [103,104] and the monocyte-derived fibrocyte-like cells themselves [14].

Fibrocytes freshly isolated from the peripheral blood of allergic asthmatic patients induce the proliferation of autologous circulating CD4⁺ naive and memory T cells, and the predominant release of T_H2 cytokines from effector CD4⁺ memory T cells, when pulsed with the clinically relevant allergen

[11]. Their ability to increase the release of these lymphokines from CD4⁺ memory T cells is comparable to that observed with autologous allergen-pulsed circulating mDCs, and both fibrocytes and mDCs also induce an appreciable release of IL-17A from CD4⁺ memory T lymphocytes when co-cultured with these cells in the presence of the clinically relevant allergen [11]. Fibrocytes freshly isolated from the peripheral blood of allergic asthmatic patients are also susceptible to rhinovirus infections [11]. Viral replication and virus release are more intense and last longer in fibrocytes pulsed with the clinically relevant allergen than in unpulsed fibrocytes [11]. Moreover, allergen-pulsed and infected fibrocytes release much larger amounts of the pro-inflammatory cytokines IL-6, CXCL8 and TNF- α than unpulsed but similarly infected cells, and the magnitude of cytokine release depends on the intensity of virus replication [11]. These effects may be related to the allergen-induced increase in the expression of a major rhinovirus receptor on the fibrocyte surface, which may facilitate virus entry, and to the absence of a significant up-regulation of the production of anti-viral type I interferons in the allergen-pulsed and infected fibrocytes, which may favour virus replication [11].

These novel observations from my laboratories suggest that the presence of elevated numbers of fibrocytes in the airways of allergic patients with undertreated or treatment-resistant asthma may concur to increase the risk of acute exacerbations because fibrocytes can amplify T_H2 cell-driven inflammation on every exposure to the clinically relevant allergen, and can promote further inflammation on rhinovirus infections, by serving as viral reservoirs and by releasing additional pro-inflammatory factors in the inflamed bronchial mucosa (Fig. 3).

Interestingly, co-culturing allergen-pulsed fibrocytes from asthmatic individuals with autologous CD4⁺ memory T cells, in the continuing presence of the clinically relevant allergen, results in increased expression of α -SMA in fibrocytes [11]. According to the observations mentioned above [9,94], this

Accepted Article

effect can be explained with the release of T_H2 cytokines and IL-17A from the activated T lymphocytes. However, direct cell-to-cell interaction may be important, as suggested by the detection of clusters of activated T cells and immature α -SMA-positive cells with a fibrocyte-like morphology in the airways of patients with moderate or severe asthma [105]. Because effector CD4⁺ memory T cells are recruited from the circulation or derive from the activation of tissue resident CD4⁺ memory T lymphocytes as result of an allergen challenge [106,107], the occurrence of a sustained local interaction between fibrocytes and these cells may also explain why α -SMA⁺ fibrocytes are detectable in the bronchial mucosa of allergic asthmatic patients at 24 hours but not at 4 hours following allergen inhalation in laboratory [29]. Ultrastructural studies and analyses of cell secretomes and exosomes are under way in my laboratories to identify the mechanisms of communication between fibrocytes and T cell subpopulations in asthma.

Clinical implications

The recent development of methods for the isolation of pure populations of viable circulating fibrocytes has allowed a better understanding of the origin and effector function of these cells [9,11,34,35]. Fibrocytes are directly obtained from the peripheral blood and can be interrogated without requiring long-term cultures of mixed cell populations or manipulations that may alter their phenotype and functional properties.

By using a multi-parameter approach, an analytically valid flow cytometry assay for the enumeration of fibrocytes in 100 μ l of whole blood has been standardised and adapted for use in clinical trials [61]. This assay detects significant differences among asthmatic individuals on the basis of the current level of disease control, and reliably distinguishes these patients from normal subjects (Fig. 4a) [61]. Moreover, it provides reproducible results over one week in healthy individuals, in asthmatic patients with well-controlled asthma and in those with treatment-resistant severe disease (Fig. 4b) [61]. This assay would be useful for the identification and monitoring of patients at high risks

of adverse outcomes because abnormally high blood fibrocyte counts reflect ongoing airway inflammation and the progression of structural changes, on the basis of the studies reviewed above [31,32,34]. These alterations can be alternatively detected in a minimally invasive way only by combining the analysis of inflammatory cells in induced sputum with the evaluation of signs of airway remodelling by high-resolution computed tomography [108]. In comparison with other prognostic biomarkers [108], blood fibrocyte count may not be influenced by the atopic status alone (Fig. 3a) [61]. Moreover, the fibrocyte count is not influenced by the dose and route of administration of corticosteroids, because patients with treatment-resistant severe asthma by definition use high doses of inhaled corticosteroids and/or oral corticosteroids and have higher numbers of fibrocytes in the peripheral blood and in the airways than other patients adequately controlled by this anti-inflammatory therapy [31,33,34]. Thus, the enumeration of circulating fibrocytes by an optimised assay can be considered an emerging biomarker of asthma control and therapeutic responsiveness, with potential applicability in clinical trials. Studies in larger populations are however required for establishing the clinical validity and utility of the use of blood fibrocyte count as an outcome measure in asthma.

In terms of therapeutic approach, the most urgent need is to block persistent fibrocytosis in corticosteroid-resistant asthma. This goal may be probably achieved by reducing the persistent airway inflammation that drives fibrocyte accumulation, although a combined approach against inflammation and fibrocytosis would be desirable. Future studies could be addressed to evaluate the effects on circulating and airway fibrocytes of anti-inflammatory agents that are approved for the treatment of asthma poorly controlled with corticosteroids in allergic individuals, such as omalizumab (Xolair®) [109], and the effects of newer agents under clinical development that have demonstrated improvement in asthma control in corticosteroid-resistant asthma, such as drug candidates targeting IL-5, IL-4 and IL-13 [110]. Inhibition of IL-4 and IL-13 signalling would directly

reduce the pro-fibrotic effects of the endogenous proteins on airway fibrocytes [9], in addition to having potential beneficial effects on airway remodelling and inflammation through other, multiple mechanisms [110]. As discussed above, CXCL12 and the chemokine receptors CCR3 and CCR5 are involved in the airway recruitment of inflammatory cells and fibrocytes in asthma. Promising findings have been reported with a drug candidate in phase II trials for asthma, which targets CCR3 and the common β -chain of the receptors for IL-3, IL-5 and granulocyte-macrophage colony-stimulating factor [111,112]. Another interesting compound is an inhibitor of the binding of CXCL12 to CXCR4 and to its alternative receptor CXCR7, which has demonstrated broad anti-inflammatory activity in a murine model of asthma [113]. This agent is given by inhalation and therefore does not interfere with the function of the CXCL12-CXCR4 axis in the bone marrow and other organs and lacks the cardiovascular liability of systemically administered CXCR4 antagonists [75,76]. Compounds targeting CCR5 are currently under development for indications other than asthma, and compounds targeting IL-33 are not yet available [76]. A recombinant form of pentraxin-2/serum amyloid P component (SAP) attenuates experimental fibrosis by inhibiting the pro-fibrotic function of a sub-population of macrophages *in vivo* [36, 114] and reduces the generation of monocyte-derived fibrocyte-like cells in cultures of PBMCs from patients with idiopathic pulmonary fibrosis after stimulation for 48 hours with 1 μ g/ml lipopolysaccharide [115]. There are no data about the ability of pentraxin-2/SAP to reduce the number of fibrocytes actually present in the peripheral blood of these individuals. Moreover, the possibility of using a recombinant form of pentraxin-2/SAP (e.g. the drug candidate PRM-151) for the treatment of asthma, if developed for this indication in the future, is greatly limited by its pro-inflammatory properties[116] and the proven toxicity of abnormally high levels of the endogenous protein in the circulation and at tissue sites [117-120].

Conflicts of interest

I am founding shareholder and board member of AVAIL GmbH.

References

- [1] Bucala R, Spiegel LA, Chesney J, Hogan M, Cerami A. Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. *Mol Med* 1994; **1**:71-81.
- [2] Chesney J, Metz C, Stavitsky AB, Bacher M, Bucala R. Regulated production of type I collagen and inflammatory cytokines by peripheral blood fibrocytes. *J Immunol* 1998; **160**:419-25.
- [3] Abe R, Donnelly SC, Peng T, Bucala R, Metz CN. Peripheral blood fibrocytes: Differentiation pathway and migration to wound sites. *J Immunol* 2001; **166**:7556-62.
- [4] Mori L, Bellini A, Stacey MA, Schmidt M, Mattoli S. Fibrocytes contribute to the myofibroblast population in the wounded skin and originate from the bone marrow. *Exp Cell Res* 2005; **304**:81-90.
- [5] Suga H, Rennert RC, Rodrigues M *et al.* Tracking the elusive fibrocyte: identification and characterization of collagen-producing hematopoietic lineage cells during murine wound healing. *Stem Cells* 2014; **32**:1347-60.
- [6] Ishida Y, Kimura A, Takayasu T, Eisenmenger W, Kondo T. Detection of fibrocytes in human skin wounds and its application for wound age determination. *Int J Legal Med* 2009; **123**:299-304.
- [7] Quesnel C, Piednoir P, Gelly J *et al.* Alveolar fibrocyte percentage is an independent predictor of poor outcome in patients with acute lung injury. *Crit Care Med* 2012; **40**:21-8.
- [8] Bianchetti L, Barczyk M, Cardoso J, Schmidt M, Bellini A, Mattoli S. Extracellular matrix remodelling properties of human fibrocytes. *J Cell Mol Med* 2012; **16**:483-95.

- Accepted Article
- [9] Bellini A, Marini MA, Bianchetti L, Barczyk M, Schmidt M, Mattoli S. Interleukin (IL)-4, IL-13, and IL-17A differentially affect the profibrotic and proinflammatory functions of fibrocytes from asthmatic patients. *Mucosal Immunol* 2012; **5**:140-9.
- [10] Sazuka S, Katsumo T, Nakagawa T *et al.* Fibrocytes are involved in inflammation as well as fibrosis in the pathogenesis of Crohn's disease. *Dig Dis Sci* 2014; **59**:760-8.
- [11] Isgrò M, Bianchetti L, Marini MA, Mattoli S. Involvement of fibrocytes in allergen-induced T cell responses and rhinovirus infections in asthma. *Biochem Biophys Res Commun* 2013; **437**:446-51.
- [12] Hartlapp I, Abe R, Saeed W *et al.* Fibrocytes induce an angiogenic phenotype in cultured endothelial cells and promote angiogenesis in vivo. *FASEB J* 2001; **15**:2215-24.
- [13] Chesney J, Bacher M, Bender A, Bucala R. The peripheral blood fibrocyte is a potent antigen-presenting cell capable of priming naive T cells in situ. *Proc Natl Acad Sci USA* 1997; **94**:6307-12.
- [14] Tsai Y-M, Hsu S-C, Zhang J *et al.* Functional interaction of cockroach allergens and mannose receptor (CD206) in human circulating fibrocytes. *PLoS ONE* 2013; **8**:e64105.
- [15] East L, McCarthy A, Wienke D, Sturge J, Ashworth A, Isacke CM. A targeted deletion in the endocytic receptor gene Endo180 results in a defect in collagen uptake. *EMBO Rep* 2003; **4**:710-16.
- [16] Engelholm LH, List K, Netzel-Arnett S *et al.* uPARAP/endo180 is essential for cellular uptake of collagen and promotes fibroblast collagen adhesion. *J Cell Biol* 2003; **160**: 1009-15.
- [17] Madsen DH, Engelholm LH, Ingvarsen S *et al.* Extracellular collagenases and the endocytic receptor, urokinase plasminogen activator receptor-associated protein/Endo180, cooperate in fibroblast-mediated collagen degradation. *J Biol Chem* 2007; **282**:27037-45.

- Accepted Article
- [18] Behrendt N. The urokinase receptor (uPAR) and the uPAR-associated protein (uPARAP/Endo180): membrane proteins engaged in matrix turnover during tissue remodeling. *Biol Chem* 2004; **385**:103-36.
- [19] Bundesmann MM, Wagner TE, Chow YH, Altemeier WA, Steinbach T, Schnapp LM. Role of urokinase plasminogen activator receptor-associated protein in mouse lung. *Am J Respir Cell Mol Biol* 2012; **46**:233-9.
- [20] Mousavi SA, Fønhus MS, Berg T. Up-regulation of uPARAP/Endo180 during culture activation of rat hepatic stellate cells and its presence in hepatic stellate cell lines from different species. *BMC Cell Biology* 2009; **10**:39.
- [21] Madsen DH, Jürgensen HJ, Ingvarsen S *et al.* Endocytic collagen degradation: a novel mechanisms involved in protection against liver fibrosis. *J Pathol* 2012; **227**:95-105.
- [22] Bellini A, Mattoli S. The role of the fibrocyte, a bone marrow-derived mesenchymal progenitor, in reactive and reparative fibroses. *Lab Invest* 2007; **87**:858-70.
- [23] Mattoli S, Bellini A, Schmidt M. The role of a human hematopoietic mesenchymal progenitor in wound healing and fibrotic diseases and implications for therapy. *Curr Stem Cell Res Ther* 2009; **4**:266-80.
- [24] Keeley EC, Mehrad B, Strieter RM. The role of fibrocytes in fibrotic diseases of the lungs and heart. *Fibrogenesis Tissue Repair* 2011; **4**:2.
- [25] Gilpin SE, Lung K, de Couto GT *et al.* Bone marrow-derived progenitor cells in end-stage lung disease patients. *BMC Pulmonary Medicine* 2013; **13**:48.
- [26] Peng H, Herzog EL. Fibrocytes: emerging effector cells in chronic inflammation. *Curr Opin Pharmacol* 2012; **12**:491-6.

- [27] Bellini A, Schmidt M, Mattoli S. Interactions between the bronchial epithelium and fibrocytes in the pathogenesis of airway remodeling in asthma. *J Epithelial Biol Pharmacol* 2013; **6**:1-10. <http://dx.doi.org/10.2174/1875044301306010001>.
- [28] Galligan CL, Fish EN. The role of circulating fibrocytes in inflammation and autoimmunity. *J Leukoc Biol* 2013; **93**:45-50.
- [29] Schmidt M, Sun G, Stacey MA, Mori L, Mattoli S. Identification of circulating fibrocytes as precursors of bronchial myofibroblasts in asthma. *J Immunol* 2003; **171**:380-9.
- [30] Nihlberg K, Larsen K, Hultgårdh-Nilsson A, Malmström A, Bjermer L, Westergren-Thorsson G. Tissue fibrocytes in patients with mild asthma: a possible link to thickness of reticular basement membrane? *Respir Res* 2006; **7**:50.
- [31] Saunders R, Siddiqui S, Kaur D, *et al*. Fibrocyte localization to the airway smooth muscle is a feature of asthma. *J Allergy Clin Immunol* 2009; **123**:376-84.
- [32] Wang CH, Huang CD, Lin HC, *et al*. Increased circulating fibrocytes in asthma with chronic airflow obstruction. *Am J Respir Crit Care Med* 2008; **178**:583-91.
- [33] Lo CY, Michaeloudes C, Bhavsar PK *et al*. Increased phenotypic differentiation and reduced corticosteroid sensitivity of fibrocytes in severe asthma. *J Allergy Clin Immunol* 2014 Dec 6. pii: S0091-6749(14)01573-5. <http://dx.doi.org/10.1016/j.jaci.2014.10.031>. [Epub ahead of print]
- [34] Isgrò M, Bianchetti L, Marini MA, Bellini A, Schmidt M, Mattoli S. The C-C motif chemokine ligands CCL5, CCL11, and CCL24 induce the migration of circulating fibrocytes from patients with severe asthma. *Mucosal Immunol* 2013; **6**:718-27.
- [35] Bianchetti L, Marini MA, Isgrò M, Bellini A, Schmidt M, Mattoli S. IL-33 promotes the migration and proliferation of circulating fibrocytes from allergen-exacerbated asthma. *Biochem Biophys Res Commun* 2012; **426**:116-21.

- [36] Murray LA, Rosada R, Moreira AP *et al.* Serum amyloid P therapeutically attenuates murine bleomycin-induced pulmonary fibrosis via its effects on macrophages. *PLoS ONE* 2010 ; **5**:e9683.
- [37] Tippet E, Cheng W-J, Westhorpe C *et al.* Differential expression of CD163 on monocyte subsets in healthy and HIV-1 infected individuals. *PLoS ONE* 2011; **6**:e19968.
- [38] Gibbons MS, MacKinnon AC, Ramachandran P *et al.* Ly6C^{hi} monocytes direct alternatively activated profibrotic macrophages regulation of lung fibrosis. *Am J Respir Crit Care Med* 2011; **184**: 569-81.
- [39] Maniecki MB, Møller HJ, Moestrup SK, Møller BK. CD163 positive subsets of blood dendritic cells: the scavenging macrophage receptors CD163 and CD91 are coexpressed on human dendritic cells and monocytes. *Immunobiology* 2006; **211**:407-17.
- [40] Fraser I, Hughes D, Gordon S. Divalent cation-independent macrophage adhesion inhibited by monoclonal antibody to murine scavenger receptor. *Nature* 1993; **364**:343-6.
- [41] Robbins AK, Horlick RA. Macrophage scavenger receptor confers an adherent phenotype to cells in culture. *Biotechniques* 1998; **25**:240-4.
- [42] Pilling D, Fan T, Huang D, Kaul B, Gomer RH. Identification of markers that distinguish monocyte-derived fibrocytes from monocytes, macrophages, and fibroblasts. *PLoS ONE* 2009; **4**:e7475.
- [43] Rey-Giraud F, Hafner M, Ries CH. In vitro generation of monocyte-derived macrophages under serum-free conditions improves their tumor promoting functions. *PLoS ONE* 2012; **7**:e42656.
- [44] Shirai H, Murakami T, Yamada Y, Doi T, Hamakubo T, Kodama T. Structure and function of type I and II macrophage scavenger receptors. *Mech Age Dev* 1999; **111**:107-21.

- [45] Franzke C-W, Tasanen K, Schumann H *et al.* Collagenous transmembrane proteins: collagen XVII as a prototype. *Matrix Biol* 2003; **22**:299-309.
- [46] Myllyharju J, Kivirikko KI. Collagens, modifying enzymes and their mutations in humans, flies and worms. *Trends Genet* 2004; **20**: 33-43.
- [47] Bowdish DM, Gordon S. Conserved domains of the class A scavenger receptors: evolution and function. *Immunol Rev* 2009; **227**:19-31.
- [48] Prasse A, Pechkovsky DV, Toews GB *et al.* A vicious circle of alveolar macrophages and fibroblasts perpetuates pulmonary fibrosis via CCL18. *Am J Respir Crit Care Med* 2006; **173**:781-92.
- [49] Prasse A, Pechkovsky DV, Toews GB *et al.* CCL18 as an indicator of pulmonary fibrotic activity in idiopathic interstitial pneumonias and systemic sclerosis. *Arthritis Rheum* 2007; **56**:1685-93.
- [50] Higashi-Kuwata N, Jinnin M, Makino T *et al.* Characterization of monocyte/ macrophage subsets in the skin and peripheral blood derived from patients with systemic sclerosis. *Arthritis Res Ther* 2010; **12**:R128.
- [51] Mathai SK, Gulati M, Peng X *et al.* Circulating monocytes from systemic sclerosis patients with interstitial lung disease show an enhanced profibrotic phenotype. *Lab Invest* 2010; **90**: 812-23.
- [52] Zwadlo-Klarwasser G, Bent S, Haubeck HD, Sorg C, Schmutzler W. Glucocorticoid-induced appearance of the macrophage subtype RM 3/1 in peripheral blood of man. *Int Arch Allergy Appl Immunol* 1990; **91**:175-80.
- [53] Yamazaki H, Ohta K, Tsukiji H *et al.* Corticosteroid enhances heme oxygenase-1 production by circulating monocytes by up-regulating haemoglobin scavenger receptor and amplifying the receptor-mediated uptake of haemoglobin-haptoglobin complex. *Biochem Biophys Res Commun* 2007; **358**:506-12.

- [54] Moniuszko M, Bodzenta-Lukaszyk A, Kowal K, Lenczewska D, Dabrowska M. Enhanced frequencies of CD14⁺⁺CD16⁺, but not CD14⁺CD16⁺, peripheral blood monocytes in severe asthmatic patients. *Clin Immunol* 2009; **130**:338-46.
- [55] Beitnes ACR, Råki M, Lundin KEA, Jahnsen J, Sollid LM, Jahnsen FL. Density of CD163⁺CD11c⁺ dendritic cells increases and CD103⁺ dendritic cells decreases in the coeliac lesion. *Scand J Immunol* 2011; **74**:186-94.
- [56] Atabai K, Jame S, Azhar N *et al.* Mfge8 diminishes the severity of tissue fibrosis in mice by binding and targeting collagen for uptake by macrophages. *J Clin Invest* 2009; **119**:3713-22.
- [57] Barnett D, Janossy G, Lubenko A, Matutes E, Newland A, Reilly JT. Guideline for the flow cytometric enumeration of CD34⁺ haematopoietic stem cells. Prepared by the CD34⁺ Haematopoietic Stem Cell Working Party. *Clin Lab Haem* 1999; **21**: 301-8.
- [58] Oertel J, Oertel B, Schleicher J, Huhn D. Detection of small numbers of immature cells in the blood of healthy subjects. *J Clin Pathol* 1998; **51**:886-90.
- [59] Dzionek A, Fuchs A, Schmidt P *et al.* BDCA-2, BDCA-3, and BDC-4: three markers for distinct subsets of dendritic cells in human peripheral blood. *J Immunol* 2000; **165**: 6037-46.
- [60] Murphy MB, Moncivais K, Caplan AI. Mesenchymal stem cells: environmental responsive therapeutics for regenerative medicine. *Exp Mol Med* 2013; **45**:e54.
- [61] Bianchetti L, Isgrò M, Marini MA, Bellini A, Schmidt M, Mattoli S. Enumeration of circulating fibrocytes for clinical use in asthma by an optimized single-platform flow cytometry assay. *BBA Clinical* 2014; **1**:52-8.
- [62] D'Alessio M, Bernard M, Benson-Chanda V *et al.* Complete nucleotide sequence of the region encompassing the first twenty-five exons of the human pro- α 1(I) collagen gene (COL1A1). *Gene* 1990; **67**:105-15.

- [63] Hamada K, Okawara Y, Fryer JN, Tomonaga A, Fukuda H. The detection of the mRNAs of procollagen types I, II and III in human fetal fingers by in situ hybridization using digoxigenin-labelled oligonucleotide probes. *Histochem J* 1995; **27**:309-17
- [64] Brewster CEP, Howarth PH, Djukanovic R, Wilson J, Holgate ST, Roche WR. Myofibroblasts and subepithelial fibrosis in bronchial asthma. *Am J Respir Cell Mol Biol* 1990; **3**:507-11.
- [65] Heyden B. Smooth-muscle-type myofilaments and actin in reactive and neoplastic nonmuscle cells. Reader's Forum. *Ultrastruct Pathol* 2000; **24**:347-52.
- [66] Saada JI, Pinchuk IV, Barrera CA *et al.* Subepithelial myofibroblasts are novel nonprofessional APCs in the human colonic mucosa. *J Immunol* 2006; **177**:5968-79.
- [67] Muñoz-Fernández R, Blanco FJ, Frecha C *et al.* Follicular dendritic cells are related to bone marrow stromal cell progenitors and to myofibroblasts. *J Immunol* 2006; **177**: 80-9.
- [68] Son BR, Marquez-Curtis LA, Kucia M *et al.* Migration of bone marrow and cord blood mesenchymal stem cells in vitro is regulated by stromal-derived factor-1-CXCR4 and hepatocyte growth factor-c-met axes and involves matrix metalloproteinases. *Stem Cell* 2006; **24**:1254-64.
- [69] Faucher JL, Lacronique-Gazaille C, Frébet E *et al.* "6 markers/5 colors" extended white blood cell differential by flow cytometry. *Cytometry* 2007; **A71**:934-44.
- [70] Han X, Jorgensen JL, Brahmandam A *et al.* Immunophenotypic study of basophils by multiparameter flow cytometry. *Arch Pathol Lab Med* 2008; **132**:813-9.
- [71] Beare A, Stockinger H, Zola H, Nicholson I. The CD system of leukocyte surface molecules. *Curr Protoc Immunol* 2008; **80**:A.4A.1-73.
- [72] Crisan M, Yap S, Casteilla L *et al.* A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell* 2008; **3**:301-13.

- [73] Ingersoll MA, Spanbroek R, Lottaz C *et al.* Comparison of gene expression profile between human and mouse monocyte subsets. *Blood* 2010; **115**:e10-9.
- [74] To WS, Midwood KS. Plasma and cellular fibronectin: distinct and independent functions during tissue repair. *Fibrogenesis Tissue Repair* 2011; **4**:21.
- [75] Döring Y, Pawig L, Weber C, Noels H. The CXCL12/CXCR4 chemokine ligand/receptor axis in cardiovascular disease. *Front Physiol* 2014; **5**:212.
- [76] Bachelier F, Ben-Baruch A, Burkhardt AM *et al.* International Union of Pharmacology. LXXXIX. Update on the extended family of chemokine receptors and introducing a new nomenclature for atypical chemokine receptors. *Pharmacol Rev* 2014; **66**:1-79.
- [77] Reich B, Schmidbauer K, Gomez MR *et al.* Fibrocytes develop outside the kidney but contribute to renal fibrosis in a mouse model. *Kidney Int* 2013; **84**:78-89.
- [78] Wang CH, Punde TH, Huang CD *et al.* Fibrocyte trafficking in patients with chronic obstructive asthma and during an acute asthma exacerbation. *J Allergy Clin Immunol* 2014 Oct 24. pii: S0091-6749(14)01321-9. <http://dx.doi.org/10.1016/j.jaci.2014.09.011>. [Epub ahead of print]
- [79] Weng CM, Chen BC, Wang CH *et al.* The endothelin A receptor mediates fibrocyte differentiation in chronic obstructive asthma. The involvement of connective tissue growth factor. *Am J Respir Crit Care Med* 2013; **188**:298-308.
- [80] Sturge J, Wienke D, Isacke CM. Endosomes generate localized Rho-ROCK-MLC2-based contractile signals via Endo180 to promote adhesion disassembly. *J Cell Biol* 2006; **175**:337-47.
- [81] Ou-Yang H-F, Han X-P, Zhao F, Ti X-Y, Wu C-G. The role of bone marrow-derived adult stem cells in a transgenic mouse model of allergic asthma. *Respiration* 2012; **83**:74-80.
- [82] Mariñas-Pardo L, Mirones I, Amor-Carro Ó *et al.* Mesenchymal stem cells regulate airway contractile tissue remodeling in murine experimental asthma. *Allergy* 2014; **69**:730-40.

- [83] Brown JR, Kleimberg J, Marini M, Sun G, Bellini A, Mattoli S. Kinetics of eotaxin expression and its relationship to eosinophil accumulation and activation in bronchial biopsies and bronchoalveolar lavage of asthmatic patients after allergen inhalation. *Clin Exp Immunol* 1998; **114**:137-46.
- [84] Kim J, Kim W, Le HT *et al.* IL-33-induced hematopoietic stem and progenitor cell mobilization depends upon CCR2. *J Immunol* 2014; **193**:3792-802.
- [85] Le H, Kim W, Kim J, Cho HR, Kwon B. Interleukin-33: a mediator of inflammation targeting hematopoietic stem and progenitor cells and their progenies. *Front Immunol* 2013; **4**:104.
- [86] Peled A, Gonzalo JA, Lloyd C, Gutierrez-Ramos J-C. The chemotactic cytokine eotaxin acts as a granulocyte-macrophage colony stimulating factor during lung inflammation. *Blood* 1998; **91**:1909-16.
- [87] Sehmi R, Dorman S, Baatjes A *et al.* Allergen-induced fluctuation in CC chemokine receptor 3 expression on bone marrow CD34+ cells from asthmatic subjects: significance for mobilization of haemopoietic progenitor cells in allergic inflammation. *Immunology* 2003; **109**:536-46.
- [88] Ishida Y, Kimura A, Kondo T *et al.* Essential roles of the CC chemokine ligand 3-CC chemokine receptor 5 axis in bleomycin-induced pulmonary fibrosis through regulation of macrophages and fibrocyte infiltration. *Am J Pathol* 2007; **170**:843-54.
- [89] Hoshino M, Aoike N, Takahashi M, Nakamura Y, Nakagawa T. Increased immunoreactivity of stromal cell-derived factor-1 and angiogenesis in asthma. *Eur Respir J* 2003; **21**:804-9.
- [90] Negrete-García MC, Velazquez JR, Popoca-Coyotl A, Montes-Vizuet R, Juárez-Carvajal E, Teran LM. Chemokine (C-X-C motif) ligand 12/stromal cell-derived factor-1 is associated with leukocyte recruitment in asthma. *Chest* 2010; **138**:100-6.

- [91] Dorman SC, Babirad I, Post J *et al.* Progenitor egress from the bone marrow after allergen challenge: role of stromal cell-derived factor 1 α and eotaxin. *J Allergy Clin Immunol* 2005; 115:501-7.
- [92] Schauer IG, Ressler SJ, Tuxhorn JA, Dang TD, Rowley DR. Elevated epithelial expression of interleukin-8 correlates with myofibroblast reactive stroma in benign prostatic hyperplasia. *Urology* 2008; **72**:205-13.
- [93] Clément S, Pascarella S, Conzelmann S, Gonelle-Gispert C, Guillox K, Negro F. The hepatitis C virus core protein indirectly induces alpha-smooth muscle actin expression in hepatic stellate cells via interleukin-8. *J Hepatol* 2010; **52**:635-43.
- [94] Hayashi H, Kawakita A, Okazaki S, Yasutomi M, Murai H, Ohshima Y. IL-17A/F modulates fibrocyte functions in cooperation with CD40-mediated signalling. *Inflammation* 2013; **36**:830-8.
- [95] Schmitt-Gräff A, Desmoulière A, Gabbiani G. Heterogeneity of myofibroblast phenotypic features: an example of fibroblastic cell plasticity. *Virchows Arch* 1994; **425**: 3-24.
- [96] Hinz B. The myofibroblast: Paradigm for a mechanically active cell. *J Biomech* 2010; **43**:146-55.
- [97] Takeji M, Moriyama T, Oseto S *et al.* Smooth muscle alpha-actin deficiency in myofibroblasts leads to enhanced renal tissue fibrosis. *J Biol Chem* 2006; **281**:40193-200.
- [98] Liu T, Warburton RR, Guevara OE *et al.* Lack of MK2 inhibits myofibroblast formation and exacerbates pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2007; **37**:507-17.
- [99] Cieslik KA, Trial J, Carlson S, Taffet GE, Entman ML. Aberrant differentiation of fibroblast progenitors contribute to fibrosis in the aged murine heart: role of elevated circulating insulin levels. *FASEB J* 2013; **27**:1761-71.

- [100] Gabbiani G, Ryan GB, Majno G. Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. *Experientia* 1971; **27**:549-50.
- [101] Darby I, Skalli O, Gabbiani G. α -smooth muscle actin is transiently expressed by myofibroblasts during experimental wound healing. *Lab Invest* 1990; **63**:21-9.
- [102] Mattoli S. Tissue repair in asthma: The origin of subepithelial fibroblasts and myofibroblasts. In: Chaponnier C, Desmoulière A, Gabbiani G, eds. Tissue Repair, Contraction and the Myofibroblasts. Georgetown (TX): Landes Bioscience and Springer Science + Business Media. 2006:40-6.
- [103] Deslee G, Charbonnier AS, Hammad H *et al*. Involvement of the mannose receptor in the uptake of Der p 1, a major mite allergen, by human dendritic cells. *J Allergy Clin Immunol* 2002; **110**:763-70.
- [104] Royer PJ, Emara M, Yang C *et al*. The mannose receptor mediates the uptake of diverse native allergens by dendritic cells and determines allergen-induced T cell polarization through modulation of IDO activity. *J Immunol* 2010; **185**:1522-31.
- [105] Ramos-Barbón D, Fraga-Iriso R, Brienza NS *et al*. T cells localize with proliferating smooth muscle α -actin⁺ cell compartments in asthma. *Am J Respir Crit Care Med* 2010; **182**:317-24.
- [106] Lloyd CM, Hessel EM. Functions of T cells in asthma. More than just T(H)2 cells. *Nat Rev Immunol* 2010; **10**:838-48.
- [107] Turner DL, Farber DL. Mucosal resident memory CD4 T cells in protection and immunopathology. *Front Immunol* 2014; **5**:331.
- [108] Szeftler SJ, Wenzel S, Brown R *et al*. Asthma outcomes: biomarkers. *J Allergy Clin Immunol* 2012; **129**:S9-S23.

- [109] Normansell R, Walker S, Milan SJ, Walters EH, Nair P. Omalizumab for asthma in adults and children. *Cochrane Database Syst Rev* 2014; **1**:CD003559.
<http://dx.doi.org/10.1002/14651858.CD003559.pub4>.
- [110] Olin JT, Wechsler ME. Asthma: pathogenesis and novel drugs for treatment. *BMJ* 2014; **349**:g5517.
- [111] Gauvreau GM, Boulet LP, Cockcroft DW *et al*. Antisense therapy against CCR3 and the common beta chain attenuates allergen-induced eosinophilic responses. *Am J Respir Crit Care Med* 2008; **177**:952-8.
- [112] Imaoka H, Campbell H, Babirad I *et al*. TPI ASM8 reduces eosinophil progenitors in sputum after allergen challenge. *Clin Exp Allergy* 2011; **41**: 1740-6.
- [113] Daubeuf F, Hachet-Haas M, Gizzi P *et al*. An antedrug of the CXCL12 neutraligand blocks experimental allergic asthma without systemic effect in mice. *J Biol Chem* 2013; 288:11865-76.
- [114] Castaño AP, Lin SL, Surowy T *et al*. Serum amyloid P inhibits fibrosis through FcγR-dependent monocyte-macrophage regulation in vivo. *Sci Transl Med* 2009; **1**:5a13.
- [115] Dillingh MR, van den Blink B, Moerland M *et al*. Recombinant human serum amyloid P in healthy volunteers and patients with pulmonary fibrosis. *Pulm Pharmacol Ther* 2013; 26:672-6.
- [116] Lu J, Marjon KD, Mold C, Du Clos TW, Sun PD. Pentraxins and Fc receptors. *Immunol Rev* 2012; **250**:230-8.
- [117] Tennent GA, Lovat LB, Pepys MB. Serum amyloid P component prevents proteolysis of the amyloid fibrils of Alzheimer disease and systemic amyloidosis. *Proc Natl Acad Sci USA* 1995; 92:4299-303.
- [118] Pepys MB, Herbert J, Hutchinson WL *et al*. Targeted pharmacological depletion of serum amyloid P component for treatment of human amyloidosis. *Nature* 2002; 417:254-9.

[119] Kolstoe SE, Ridha BH, Bellotti V *et al.* Molecular dissection of Alzheimer's disease neuropathology by depletion of serum amyloid P component. *Proc Natl Acad Sci USA* 2009; 106:7619-23.

[120] Gillmore D, Tennent GA, Hutchinson WL *et al.* Sustained pharmacological depletion of serum amyloid P component in patients with systemic amyloidosis. *Br J Haematol* 2010; 148:760-7.

Legends to figures

Fig. 1. Involvement of interleukin-33 (IL-33) in the airway recruitment and local proliferation of fibrocytes in the acute exacerbations of allergic asthma: **(a)** increased expression of the specific component of the IL-33 receptor, ST2L, on the surface of freshly isolated circulating CD34⁺CD45RO⁺CD11b⁺CD13⁺COL1⁺ fibrocytes from patients with allergen-exacerbated asthma (PwAA) in comparison with the cells from non-asthmatic individuals (NAI); effects of the recombinant form of IL-33 (rIL-33) on the migration **(b)** and proliferation **(c)** of circulating fibrocytes from PwAA and NAI (***p* < 0.01 and **p* < 0.05 compared with 0 ng/ml rIL-33); **(d)** detection of fibrocytes by a double immunohistochemical and *in-situ* hybridisation assay [9,27,29] in a representative cytospin of sputum leukocytes from a PwAA (the white arrow indicates a CD34⁺COL1A1 mRNA⁺ fibrocyte, the arrowhead indicates a CD34⁺COL1A1 mRNA⁻ cell and the empty arrow indicates a CD34⁻COL1A1 mRNA⁻ cell); **(e)** significant correlation between the concentrations of immunoreactive IL-33 and the number of fibrocytes in the sputum of PwAA; **(f)** significant correlation between the concentrations of immunoreactive IL-33 in the sputum of PwAA and the frequency of symptoms requiring reliever use. Reproduced with permission of the publisher from **(a, b and c)** Isgrò M. *et al.* [35], copyright© 2012 Elsevier Inc., and under the CC BY-NC-ND license from **(e and f)** Bellini A. *et al.* [27], copyright© 2013 The Authors.

Fig. 2. Schematic illustration of the main potential effects of locally released factors on the accumulation and pro-remodelling properties of fibrocytes in the inflamed bronchial mucosa of patients with transiently uncontrolled or treatment-resistant severe asthma, on the basis of published studies involving the stimulation of normal and asthmatic circulating fibrocytes with recombinant agonists of receptors expressed by fibrocytes and/or the fluid phase of autologous sputum [3,8,9,12,27,29,31,34,35], and information about the modulation of Endo180 on fibroblasts and fibroblast-like cells in the setting of experimentally induced lung fibrosis [19]. α -SMA = α -smooth muscle actin; CCL = chemokine C-C motif ligand; COL = collagen type; CXCL = chemokine C-X-C motif ligand; ECM = extracellular matrix; ET= endothelin; IL = interleukin; TGF- β = transforming growth factor- β ; T_H = T helper type cell.

Designed by using the ScienceSlides software (VisiScience Corporation, Chapel Hill, NC, USA).

Fig. 3. Schematic illustration of the potential contribution of bronchial fibrocytes to the provocation of acute exacerbations in allergic asthmatic subjects during exposure to the clinically relevant allergens or rhinovirus infections, on the basis of data obtained with allergen-pulsed and/or rhinovirus infected circulating fibrocytes from allergic patients with moderate or severe asthma and a history of allergen- and virally-induced acute exacerbations [11]. CCL = chemokine C-C motif ligand; CXCL = chemokine C-X-C motif ligand; DC = dendritic cell; IL = interleukin; INF = interferon; T_H = T helper type cell; TNF- α = tumor necrosis factor- α . Designed by using the ScienceSlides software (VisiScience Corporation, Chapel Hill, NC, USA).

Fig. 4. Comparison **(a)** and repeatability over one week **(b)** of fibrocyte counts in the whole blood of healthy individuals and patients with well-controlled asthma, disease transiently uncontrolled by current treatment, or treatment-resistant severe asthma. The graph in **(a)** shows individual values and the median value (horizontal line) for each group. Closed symbols represent atopic individuals. The dotted line indicates the upper limit of the 95% confidence interval in healthy subjects (12.8 fibrocytes per μ l). The Bland-Altman plot in **(b)** displays the differences in fibrocyte counts between

Day 1 and Day 2 as a percentage of the average counts *versus* the average values, with individual % differences, the mean value, and the limits of agreement. ICC = intraclass correlation coefficient; SD = standard deviation; SS = sum of squares (from analysis of variance, one way random model).

Fibrocytes were identified as viable CD45⁺[CD3/CD19/ CD20/CRTH2]⁻CD16⁻CD115⁻CD11b⁺CD34⁺ cells in the whole blood by flow cytometry, and the analytical validity of the assay was demonstrated in sorted cells by evaluation of the expression of *COL1A1* mRNA and expression of α -smooth muscle actin on stimulation with endothelin-1, as previously reported [29]. Reproduced under the CC BY-NC-ND license from Bianchetti L. *et al.* [61], copyright© 2014 The Authors.





