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Review Article

# The Monocyte/Macrophage Lineage as a Therapeutic Target in Idiopathic Pulmonary Fibrosis

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## Abstract

Idiopathic pulmonary fibrosis (IPF), is a rare but increasingly prevalent lung disease, affecting approximately three million people worldwide. With a median survival post diagnosis of two to four years, IPF is as devastating as some of the most aggressive cancers. Despite the recent approval by the FDA of two drugs able to slow down the disease progression, there is still no drug able to cure IPF, and the mortality remains high in the populations affected. Due to the ineffectiveness of anti-inflammatory treatments (i.e., corticosteroids and azathioprine) in IPF, the role of inflammation and immune cells in the disease pathogeny has become a controversial subject. Here we are reviewing data highlighting a role for the monocyte/macrophage lineage in IPF and experimental models of lung fibrosis. We will also examine recent publications shedding new lights on the mechanisms of alveolar macrophages resistance to corticosteroids and the effect of current and prospective IPF treatments on lung macrophage biology.

***Keywords:*** *Idiopathic Pulmonary Fibrosis; Macrophages; Monocytes; Therapy*

## Abbreviations

AAM: Alternatively Activated Macrophages; AE-IPF: Acute Exacerbation-Idiopathic Pulmonary Fibrosis; AM: Alveolar Macrophages; BALF: Bronchoalveolar Lavage Fluid; C/EBPβ: CCAAT/enhancer binding protein β; CCRX: C-C chemokine receptor type X; CD: Cluster of Differentiation; CEACAMX: Carcinoembryonic antigen-related cell adhesion molecule X; Clec10a: C-Type Lectin Domain Containing 10A; CXCL: C-X-C Motif Chemokine Ligand; CXCRX: C-X-C chemokine receptor type X; DLCO%: Diffusing capacity of the lung for carbon monoxide; ELMOD2: ELMO Domain Containing 2; EMR1: EGF-like module-containing mucin-like hormone receptor-like 1; EMT: Epithelial to Mesenchymal Transition; ER stress: Endoplasmic Reticulum stress; FCGR2A: Fc Gamma Receptor IIa; FF: Fibroblastic Focus; FGFR-X: Fibroblast Growth Factor Receptor-X; FVC%: Forced Percentual Vital Capacity; GM-CSF: Granulocytes Macrophages-Colony stimulating Factor; GM-CSFR: Granulocytes Macrophages-Colony stimulating Factor Receptor; HC: Healthy Controls; HP: Hypersensitivity Pneumonitis; hTR: human Telomerase RNA; IFN-γ: Interferon-γ; IL-17RB: Interleukin 17 Receptor Beta; IL-1Ra: Interleukin Receptor antagonist; IL-23A: Interleukin-23 subunit alpha; IL-2RA: nterleukin-2 receptor alpha chain; ILD: Interstitial Lung Diseases; IL-x: Interleukin-x; INF-γ: Interferon-γ ; IPF: Idiopathic Pulmonary Fibrosis; IRAK-M: Interleukin Receptor-Associated Kinase-M; LCN2: Lipocalin-2; LPS: Lipopolysaccharides; LTF: Lactotransferrin; Ly6C: Lymphocyte antigen 6 complex, locus C; MAP3K19: Mitogen-Activated Protein Kinase Kinase Kinase 19; MCP-1: Monocyte Chemoattractant Protein-1; M-CSF: Macrophage-Colony stimulating Factor; MDM: Monocyte-Derived Macrophages; MIF: Macrophage migration inhibitory factor; mL: millilitre; MMP: Matrix metalloproteinases; M-MDSCs: Monocytic Myeloid-Derived Suppressor Cells; mTOR: mechanistic Target Of Rapamycin; MUC5B: Mucin 5B; MYD88: Myeloid differentiation primary response 88; NTHi: Non-typeable Haemophilus influenza; OPN: Osteopontin; PaO2: Partial pressure of $O_2$; PARC: Pulmonary and activation-regulated chemokine; PCEC: Pulmonary Capillary Endothelial Cells; PDGFA: Platelet-derived growth factor subunit A; PDGFRα: Platelet Derived Growth Factor Receptor alpha; PGD2: Prostaglandins D2; PGE2: Prostaglandins E2; PI3K: Phosphatidylinositol-4,5-bisphosphate 3-kinase; PIR-B: Paired Immunoglobulin-like Receptor B; S100A9: S100 Calcium Binding Protein A9; SatM: segregated-nucleus-containing atypical monocytes; SMA: Smooth Muscle Actin; TGF-β: Transforming Growth Factor-β; Th1: T helper 1; Th2: T helper 2; TLR3: Toll-like receptor 3; Tollip: Toll interacting protein; TRM: Tissue-resident macrophages; TNFα: Tumour Necrosis Factor alpha; UIP: Usual Interstitial Pneumonia; UPAR: uPA receptor; VEGF: Vascular endothelial growth factor; VEGFR-X: Vascular endothelial growth factor receptor-X.

## Introduction

Idiopathic pulmonary fibrosis (IPF) is a rare and progressive parenchymal/interstitial lung disease of unknown origin, whose incidence in the western world has risen in the past decades to reach 2.8 to 18 cases per 100 000 people per year [1]. Patients with IPF usually present with slowly progressive dyspnoea on exertion and dry cough. The disease mainly affects men, over 50-60 years of age, and for lack of effective treatments other than lung transplantation, the median survival time from diagnosis range between two and four years [1]. Although a progressive disease, 5 to 15% of IPF patients a year, can experience episodes of acute exacerbations of the disease (AE-IPF), characterised by a rapid deterioration of their lung function outside any known cause (e.g. infections) [2]. Despite treatments with high doses of corticosteroids and antibiotics, AE-IPF is fatal in approximately 50% of patients [2].

IPF is characterised by a histological pattern of usual interstitial pneumonia (UIP), which distinguish it from other interstitial lung diseases (ILD), such as, chronic hypersensitivity pneumonitis (HP), which is characterised by an airway-centered pattern of fibrosis [3]. The UIP pattern presents three main features: (1) A patchy pattern of fibrosis mainly in the subpleural/paraseptal areas which alternate abruptly with histologically normal alveolar areas. (2) Enlarged alveolar spaces, lined by bronchiolar metaplastic epithelium, filled with mucus and inflammatory cells, called Honeycomb. (3) The presence of dome-shaped areas, formed by actively proliferating fibroblasts/myofibroblasts, embedded in a pale myxoid matrix, and covered by hyperplastic pneumocytes called fibroblastic focus (FF) [3].

Although of unknown aetiology, many external and genetic risk factors have been associated with the development of IPF (Fig 1). Thus epidemiologic studies have highlighted a history of cigarette smoking in 73% of IPF patients, whereas other studies have incriminated exposure to metal, wood, stone and silica dust, often in a professional environment [4]. Apart from inhaled particles, gastroesophageal reflux is also believed to contribute to the external aggression of the lung and is found in as much as 90% of patients suffering from IPF [5]. Finally, bacterial and viral infections, especially herpes viruses, Epstein-bar and hepatitis C virus infections have been observed at a higher rate in IPF patients and could participate in disease initiation and progression [6]. In addition to these external factors, the recent advances in genomics have led to numerous genome-wide association studies in patients with sporadic as well as familial forms of IPF. These studies led to the identification of genetic variants positively associated with IPF, in genes related to lung epithelial cell functions (e.g., MUC5B, SFTPC, SFTPA2), telomere size maintenance (e.g., TERT, hTR) and innate immunity (e.g., TLR3, Tollip, ELMOD2) (Fig 1) [7]. All these data suggest that IPF emerge from a lifetime chronic aggression of the lung by various external factors in individuals whose genetic predispose to premature ageing, Endoplasmic Reticulum stress (ER stress) and abnormal innate immune response.

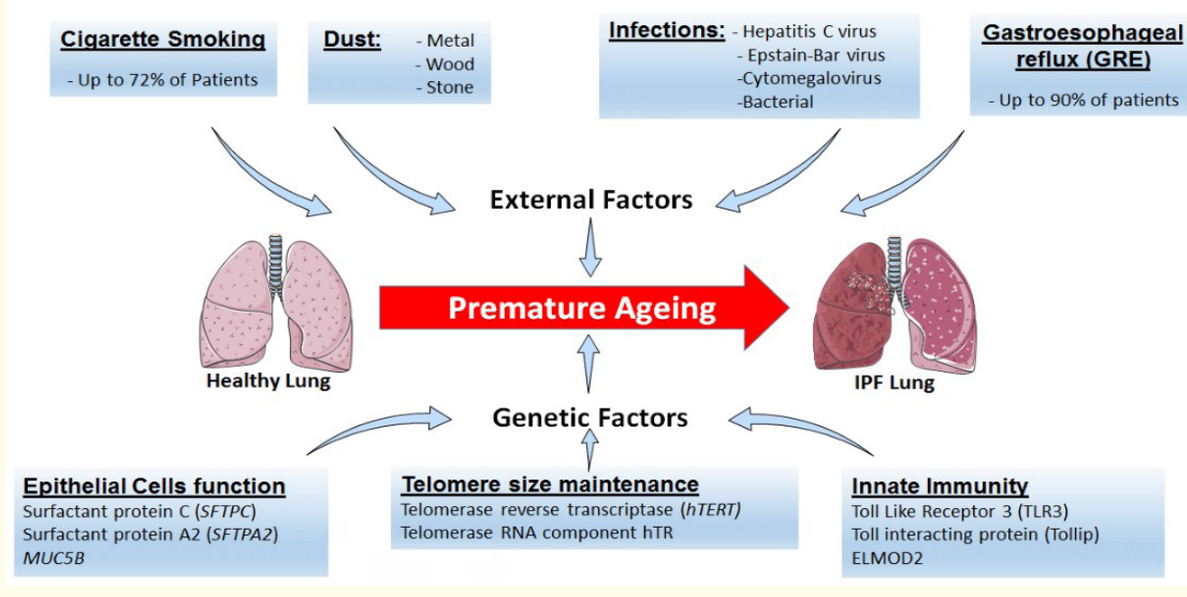


***Figure 1:*** *Summary of the main external and genetic risk factors associated with IPF development.*

From a mechanistic perspective, IPF has long been perceived as a fibrotic lung disease driven by an underlying chronic inflammatory response of the lung parenchyma, in response to repeated micro injuries of unknown origin. However, the inefficiency of anti-inflammatory treatments (e.g., corticosteroids) together with the mild inflammatory findings in histological specimens compared to others lung interstitial diseases, prompted the scientific community to reassess its view of IPF pathogeny [1]. The current predominant concept involves repetitive micro-injury of the lung epithelium leading to aberrant epithelium/mesenchyme communication and fibrosis development [1]. Several key cellular and molecular processes involved in epithelium/mesenchyme interactions, such as epithelial to mesenchymal transition (EMT), ER stress, TGF-β pathway activation, have been shown to be active in IPF [8]. However, changes in, inflammatory cells proportion, cytokines and chemokines concentration, in the blood, lung and Bronchoalveolar Lavage Fluid (BALF) of IPF patients also support a role for the immune system and in particular the monocyte/macrophage lineage in this disease. Here we are presenting a review of data supporting the involvement of the monocyte/macrophage lineage in IPF and experimental lung fibrosis while highlighting the effect of past, current and prospective IPF treatments on their function.

## Origin, diversity, and function of monocytes and pulmonary macrophages

Macrophages are leucocytes, first described in 1883 by Ilya Metchnikoff for their phagocytic abilities [9]. They are present in virtually all the tissues of our organism, where their phagocytosis of apoptotic cells and other debris, make them active participants in tissue homeostasis and defence against pathogens. The latter function explains, in particular, their presence in different organs during the early steps of embryogenesis which is associated with intense proliferation, apoptosis and morphogenesis [9]. Whereas most tissue-resident leucocytes arise from haematopoietic stem cells found in the bone marrow, macrophages from many mammalian organs such as the brain, the lung and the liver find their origin in the yolk sac and the foetal liver. Consequently, these cells are referred to as tissue-resident macrophages (TRM) in adult tissues [10]. On the other hand, the intestine is mainly populated by macrophages derived from the bone marrow haematopoietic stem cell niche with the monocytes as immediate precursors. Therefore, these cells are referred to as monocyte-derived macrophages (MDM) [11]. TRM have been shown to be, long living and self-renewing, thereby maintaining themselves in adult tissues throughout life [10]. In most adult tissues, there is a co-existence, between both macrophage populations, with a ratio, depending on the tissue and the age of the organism. However, during episodes of infections, and other tissue damages, MDM are massively recruited to most organs and actively contribute with TRM to the immune response [12].

Unlike macrophages, monocytes are found primarily in the blood, and secondary lymphoid organs such as the spleen and are produced in the bone marrow from the differentiation of hematopoietic stem cells [13; 14]. Although tissue-resident monocytes, have been described in some tissues such as the lung, the scientific consensus is that after migration into the tissues, monocytes tend to differentiate into macrophages, dendritic cells and fibrocytes [15]. Three different populations of monocytes have been identified in human blood, according to their expression of the cluster of differentiation (CD) 14 and 16. Thus the classical monocytes are described as CD14++/CD16-, the intermediate monocytes as CD14++/CD16+ and the non-classical monocytes as CD14+/CD16++ [15; 16]. Recently taking advantage of the accuracy and sensitivity of single-cell RNA-sequencing Villani et al., identified four human monocyte subsets, adding thereby more depth to our initial understanding of monocytes diversity [17].

In the lung, there is three main types of macrophages named according to their location. Most pulmonary macrophages are found in the bronchiolar and alveolar space and are called respectively bronchiolar and alveolar macrophages (AM), whereas those present within the pulmonary interstitium are referred to as interstitial macrophages [18; 19]. AM play a critical role in pulmonary homeostasis by preventing the accumulation of surfactant proteins, mucus and dust particles that could impair lung function and damage the epithelium [19]. They also contribute to a tolerogenic response to innocuous inhaled particles to avoid excessive inflammation and tissue damages [20]. However, despite sharing common origins, TRM are undertaking very different, tissue-specific functions, (e.g. clearance of amyloid beta plaques in the brain, recycling of iron in the spleen and liver), that require the expression of particular sets of proteins [21]. A recent article from Lavin et al., has highlighted the marked plasticity of macrophages, and the critical role of environmental factors in shaping

their transcriptional landscape through epigenetic remodelling of their chromatin [22]. This capacity to adapt the set of protein they express to the environmental context defined different phenotype/polarisation state [23]. One of the specificities of pulmonary macrophages compared to other populations is their dependency upon the Granulocytes Macrophages-Colony stimulating Factor (GM-CSF), whereas most macrophage populations are rather dependent upon Macrophage-Colony Stimulating Factor (M-CSF) [24; 25]. Thereby patients with anti-GM-CSF autoantibodies or genetic mutations affecting the GM-CSF/GM-CSF receptor pathway present impaired pulmonary macrophage function, leading to surfactant protein accumulation in the alveolar space and subsequently to a condition named pulmonary alveolar proteinosis [26].

## The M1/M2 paradigm: Implication for the monocyte/macrophage lineage in IPF

Advances in the field of lymphocyte biology have led to the characterisation of different inflammatory responses to intracellular and extracellular pathogens [27]. Thus intracellular pathogens are classically associated with the release of interferon-gamma (IFN-γ) by T helper lymphocytes and define a Th1 polarisation, whereas parasitic pathogens classically trigger the release of Interleukin-4 (IL-4) determining a Th2 response [27]. Because macrophages are active participant in these responses, in vitro experiments have been carried out using stimuli triggering or released during Th1 and Th2 responses, to determine their effects on macrophage phenotype. These experiments led to the establishment of the classically activated macrophage or M1 macrophage phenotype and the alternatively activated macrophage (AAM) or M2 macrophage phenotype [28]. The M1 phenotype is induced by stimuli such as GM-CSF, IFN-γ, lipopolysaccharides (LPS) and Tumour Necrosis Factor alpha (TNFα) whereas, the M2 phenotype is induced by stimuli such as M-CSF, Interleukin-4 (IL-4), -13 (IL-13), -10 (IL-10) and TGF-β. Further analysis of the M2 phenotype led to the establishment of subtypes (M2a-c) depending on the mediators used for their generation and the profiles of mediators they secrete [28]. In vitro experiments suggest that these phenotypes are not exclusive of one another and are reversible. Indeed, Smith et al., recently demonstrated that M1 macrophages could switch to an M2 phenotype, when stimulated for an extended period with M2 inducers. Moreover, co-exposition of macrophages to M1 and M2 inducers (i.e., IFN-γ, LPS, IL-4 and IL-13) lead to a mixed phenotype with co-expression of M1 and M2 markers [29]. Interestingly the authors showed that M1 polarisation is associated with an enhanced response to M2 inducers whereas, M2 polarisation tends to inhibit the sensitivity to M1 inducers [29]. *In vivo*, macrophage phenotype/polarisation results from the integration of multiple and sometimes conflicting stimulations based on the M1/M2 nomenclature. Thereby, the *in vivo* phenotype of macrophages in IPF and other diseases is undoubtedly more complex than suggested by the M1/M2 classification.

## The Monocyte/Macrophage lineage in Idiopathic pulmonary fibrosis

Unlike sarcoidosis and other connective tissue diseases that trigger lung fibrosis by changing the concentration of systemic factors (e.g., Inflammatory and growth factors), IPF is a lung centered disease most likely driven by inhaled particles and with discrete systemic repercussion [3]. Throughout the years' several reports have pointed at changes in term of inflammatory cells proportion, cytokines, and chemokines levels in the blood, and BALF from IPF patients with some reports highlighting changes in monocyte or macrophage function.

## Alveolar Macrophages in the Bronchoalveolar lavage fluid of IPF patients

Bronchoalveolar lavage is a medical procedure often used in the diagnosis of lung diseases, and consisting in injecting and recovering a saline solution in one lobe of the lung using a bronchoscope. The collected BALF allows the analysis of the inflammatory state of the lung. Thus, many clinical studies conducted on patients suffering from ILD have investigated the changes in inflammatory cell and mediators content in their BALF. However, one of the limitations of BALF analysis is due to the fact that it doesn't allow an appreciation of the initial location of the harvested cells. In diseases with a uniform pattern of fibrosis such as connective tissue disease, this is not a hurdle to the interpretation of the results. However, due to the irregular pattern of fibrosis in IPF, the results from BALF cellularity might mask essential disparities between the number and phenotype of cells in active and healthy areas of the lung.

Keeping this in mind, Table 1 presents the summary of the BALF cellularity from six independent studies [30-35]. The results show that the BALF of normal individuals contain in average 158*103 cells/mL with AM representing around 90% of these cells, followed by the lymphocytes 8%, the neutrophils 1.43%, and the eosinophils 0.3%. In IPF patients, on the other hand, the BALF contain in average 288*103 cells/mL representing an 80% increase in cellularity mainly due to the recruitment and/or proliferation of leucocytes. Thus, in IPF the BALF contain on average 80.4% AM, 7.6% lymphocytes, 8.6% neutrophils, and 3.6% eosinophils (See Table 1). Whereas the absolute number of all these populations are increased in the BALF of IPF patients the number of neutrophils and eosinophils is increased by 11 and 26 fold respectively, the neutrophils even outnumbering the lymphocytes (26.5 vs 19.6*103 cells/mL) on average. Interestingly, some studies comparing these trends with the changes in other ILD showed that sarcoidosis and HP have a different pattern of inflammation. Indeed, in sarcoidosis, there is a sharp increase in the proportion of lymphocytes and minor changes in neutrophils and eosinophils counts [32; 33], whereas, in HP, both lymphocytes and granulocytes were significantly increased [33; 35]. These differences indicate that unlike other ILD the BALF inflammatory signature of IPF is marked by the activation of the innate arm of the immune system. Despite a slight increase in the absolute number of lymphocytes in the BALF of IPF patients compared to the healthy controls (HC) (20 vs 11*103 cells/mL), the CD4/CD8 ratio remains similar to that of the HC. In the BALF of sarcoidosis patients on the other hand, the CD4/CD8 ratio increases by 2.77 fold [32; 36], signing the activation of a T helper response. Despite having a CD4/CD8 ratio similar to the HC, IPF lymphocytes present a decreased expression of the CCR5 receptor. Since CCR5 is a marker of Th1 lymphocytes, this suggests a possible Th2 polarisation of lymphocytes in IPF [30].

| | Average HC | Average IPF | Delta IPF-HC | FC IPF vs HC |
|---|---|---|---|---|
| Cells. $mL^{-1}$ x $10^3$ | 158 (91 - 240) | 288 (120 - 400) | 130.00 | 1.82 |
| Macrophages $mL^{-1}$ x $10^3$ | 143.6 (82 - 230) | 231.7 (89 - 363) | 88.07 | 1.61 |
| Lymphocytes $mL^{-1}$ x $10^3$ | 11.0 (6.3 - 17.3) | 19.6 (9.6 - 36.4) | 8.67 | 1.79 |
| Neutrophils $mL^{-1}$ x $10^3$ | 2.3 (0.73 - 6.5) | 26.5 (6.2 - 61.6) | 24.16 | 11.52 |
| Eosinophils $mL^{-1}$ x $1^03$ | 0.41 (0 - 1.2) | 10.6 (3.1 - 24) | 10.21 | 25.67 |
| Macrophages % | 90 (79 - 96) | 80.4 (74 - 84) | | |
| Lymphocytes % | 8 (2.9 - 16.2) | 7.6 (3.6 - 11.6) | | |
| Neutrophils % | 1.43 (0.6 - 2.97) | 8.6 (3.0 - 13) | | |
| Eosinophils % | 0.3 (0 - 0.55) | 3.6 (1.8 - 5.0) | | |

***Table 1:*** *Summary of BALF cell counts in Healthy Control (HC) and IPF patients across six studies.*

*Definition of abbreviations: HC: Healthy Controls; IPF: Idiopathic Pulmonary Fibrosis; FC: Fold Change.*

It is not always clear from those studies whether the patients were undergoing treatments before the BALF sampling. Therefore, the question of the impact of those treatments on the cell count can be raised. In a 2013 article, Abdel Khalik et al., analysed the effects of some conventional IPF treatments (i.e., Corticosteroids, Azathioprine, Acetylcysteine) on BALF cellularity. The authors found that BALF neutrophils count was positively correlated with disease severity (e.g. FVC%, PaO2) and that none of the treatments significantly influenced BALF composition [37]. These results suggest that the inflammatory response in IPF is resistant to standard immunosuppressant, and anti-inflammatory drugs, a topic that we will later develop in this review.

One of the primary functions of AM is to prevent unnecessary inflammation by phagocytising dying/dead cells in a process called efferocytosis. The analysis of AM efferocytosis in patients suffering from different ILD and HC showed a marked decrease of AM efferocytosis in IPF patients compared to other ILD and HC. Thereby, defective efferocytosis may be a hallmark of AM dysfunction in IPF and offer potential insight into their contribution to IPF pathogeny [38]. Interestingly enough to be mentioned, the phagocytosis of apoptotic cells by macrophages play a crucial role in their production and secretion of prostaglandins (i.e., PGE2, PGD2) and hepatocytes growth factor, which have been proven essential to the prevention by macrophages of EMT a process favouring fibrosis and observed in IPF [39].

AM from IPF patients have also been shown to be resistant to apoptosis [40], and present an exaggerated expression of TNFα and IL-10 in response to LPS stimulation [41]. This last point is of particular interest. Indeed, as monocytes transit from the blood to the tissues and differentiates into macrophages their expression of CD14 which play a crucial role in LPS response and TLR signalling is decreased [42]. The rationale for such a change is the fact that as the cell is leaving the bloodstream for mucosal/peripheral areas the level of tolerance toward pathogens must increase to prevent unnecessary inflammation. However, several studies have highlighted a more monocytic-like phenotype of AM in IPF reflecting either an alteration of the monocyte to macrophage differentiation process or the active and rapid recruitment of MDM. Thus Kiemle-Kallee *et al*., reported a monocytic-like phenotype of AM in IPF characterised by the retention of monocytes markers and an increase in the release of free oxygen radicals [43]. In another study evaluating AM phenotype in ILD, the monocytic-like phenotype of AM in IPF was confirmed and demonstrated as well, in other ILD such as sarcoidosis and HP [44]. More recently Yu et al., reported in the BALF of IPF patients the presence of a population of AM with high expression of the monocyte marker CD14 ($CD206^+$/$CD169^+$/$CD14^+$ cells) that was absent in HC individuals whether smoker or not [45]. Overall, these data are supportive of an alteration of AM phenotype and function in IPF patients that may contribute to disease progression.

## Peripheral blood monocytes phenotype in IPF

In addition to changes in BALF composition, changes in peripheral blood leucocytes of IPF patients have also been observed, although less drastic than in other ILD. Indeed, analysis of blood leucocytes from, 35 IPF patients showed that increases in the circulating CD14hi/CD16hi subset of monocytes (i.e., > 0.5% of total blood mononuclear cells) measured at baseline correlated with disease progression and poorer outcome. However, longitudinal monitoring of these patients showed considerable variation in the level of circulating monocytes over time, suggesting a disease progression made up of, short inflammatory episodes [46]. These results indicate that a shift in the proportions of the different monocyte subsets in IPF could favour disease progression. In a 2014 article, Fahim et al., observed an increase in platelet binding to monocyte from IPF patients compared to other ILD and HC, suggesting an increase platelet activation in these patients [47]. Indeed, an earlier publication has shown that platelet from IPF patients had increased reactivity due to an unidentified plasma factor [48]. Interestingly, it has been demonstrated that platelet aggregation to monocytes trigger the acquisition of a pro-inflammatory phenotype by these cells that may favour IPF development [49].

In another study involving the phenotyping of blood leucocytes from 18 IPF patients and 20 HC, the authors observed a shift in the expression of chemokines receptors by monocytes in IPF with an increase and a decrease in the number and percentage of IL-17RB and CXCR4 positive monocytes respectively [50]. Further analysis of the gene expression profile of the peripheral blood mononuclear cells from these patients revealed the differential expression of 18 genes involved in inflammation and signalling. Of these, EMR1, CCR3, UPAR, FCGR2A, OPN, CEACAM3, CD16a, CD18, CD11b, LTF, and LCN2 were up-regulated, whereas IL-17RB, IL-10, PDGFA, CD301/Clec10a, CD25/IL-2RA, IL-23A, and IL-15 were down-regulated in IPF [50]. Interestingly, some of the up-regulated genes such as osteopontin (OPN) have been shown to strongly regulate monocyte/macrophage development and function [51]. Moreover, OPN has been found to be highly expressed by AM located in fibrotic areas in the bleomycin model of lung fibrosis and was found to promote the migration, proliferation, and adhesion of fibroblasts, all participating in the development of lung fibrosis [52].

Lastly, another study conducted on 13 IPF patients and 12 age-matched HC showed an increase in the number of monocytes expressing the M2 marker CD163 [53]. Furthermore, the number of CD163 expressing monocytes was significantly increased in patients with progressive IPF compare to the stable ones [53]. This skewed M2 phenotype of monocytes in IPF may favour the recruitment to the lung of AAM with a "repair" rather than "anti-inflammatory" phenotype that could contribute to the disease progression. To get more clues in the molecular mechanisms driving these changes in IPF, researchers also measured the level of cytokines and chemokines in the BALF and serum from IPF patients.

## Monocyte/Macrophage related mediators in the BALF and serum of IPF patients

Cytokines and chemokines are inflammatory mediators released by immune and non-immune cells and playing an essential role in the polarisation of the immune response. The monocyte/macrophage lineage is all at once, a great source of many chemokines and cytokines, as well as a target of many of them. In Table 2, we summarised the changes of mediators relevant to the monocyte/macrophage lineage, observed in the BALF and serum from IPF patients. Since the recruitment of neutrophils is one of the leading discovery of IPF patients BALF analysis, we will focus in this section on the mediators likely to be involved in this process.

In a study investigating the level of the key inflammatory cytokines, IL-1β and TNFα in the BALF of IPF patients, Losa-Garcia et al., observed a modest but significant increase in their concentration [33]. To distinguish the respective contribution of BALF cells and epithelial cells to the cytokine and the chemokine content of the BALF, Schupp et al., measured their level in vitro in the supernatant of BAL cells from IPF patients and HC. They found that BAL cells from IPF patients secrete higher levels of, IL-1β and its antagonist IL-1Ra, but no change in TNFα production was observed [31]. However, another study reported higher production of both IL-1β and TNFα by AM from IPF patients compare to HC [54]. Moreover, the authors found a positive correlation between TNFα production by AM, and neutrophil count in BALF [54], suggesting a role for TNFα in neutrophils recruitment in IPF. In addition to TNFα, the potent neutrophils chemoattractant IL-8/CXCL8 has been found to be increased in IPF patients and HP patients by 2.2 and 2.5 fold respectively, whereas its concentration in the BALF of sarcoidosis patients drop by 70% [33]. Interestingly the authors observed that the expression pattern of IL-8/CXCL8 and other cytokines such as IL-1β, TNFα and IL-6 in the BALF of IPF patients was similar to the profile found in the supernatant of the AM isolated from those BALF. Thus, suggesting that AM are the primary source of BALF cytokines and chemokines in IPF [33]. Consistent with an increase AM-derived IL-8/CXCL8 in IPF, another study has shown that AM from IPF patients express higher quantities of this chemokine which correlates with neutrophils count and disease severity [55]. The same way an earlier study showed an increased ability of AM from IPF patients to attract neutrophils in vitro and showed that this could be due to the stimulation of AM IgG receptors by immune complexes present in the BALF of these patients [56]. In another study, it has been shown that the BALF concentration of the CC chemokines CCL2, 3 and 4 were significantly increased in IPF patients compared with HC [30]. Moreover, the authors showed that a positive correlation existed between the concentration of CCL3 in the BALF and the neutrophil count, whereas a negative correlation was found between the concentration of the potent monocyte chemoattractant CCL2/MCP-1 and the PaO2 [30].

Another study, analysing the secretory activity of BALF cells from IPF patients, showed that they secrete higher amount of CCL2, 17, 18, 22, IL-8, IL-1β and IL-1Ra than those from HC individuals [31; 57]. Interestingly, in one of these studies, the authors showed that AE-IPF was associated with further increases in the concentration of the same mediators and the recruitment of the same cells rather than with a change in the inflammatory cells landscape [31]. Among those mediators the chemokines CCL18/PARC has attracted much attention. Indeed, its level has been found to be increased in the BALF, lung tissue and serum of patients with many ILD including IPF [58]. Moreover, AM have been shown to be the main source of this chemokine in many of these ILD including IPF, sarcoidosis and systemic sclerosis [57; 59; 60]. In another study, involving 72 IPF patients, a significantly higher mortality rate was associated with serum concentrations of CCL18 above 150mg/ml [61]. Interestingly, in vitro experiments showed that AM production of CCL18 was stimulated by, co-culture with fibroblasts, Th2 cytokines (i.e., Here IL-13 and IL-4), and IL-10 [57]. Moreover, the authors showed that binding to collagen of AM stimulated with Th2 cytokines further increases their expression of CCL18. More importantly, supernatants of AM from IPF patients were able to stimulate the production of collagens by lung fibroblasts partly through CCL18 stimulation [57]. In line with the idea of a Th2 polarisation of the immune system in IPF, the Th2 cytokines IL-4 and IL-13 were both increased in the BALF of IPF patients [34]. Those of the Th1 cytokines (i.e., IL-12p40, INF-γ) and Th17 (i.e., IL-23 and IL-17A) on the other hand showed no change compared to HC [34; 35]. Moreover IL-13 concentration inversely correlated with FVC% (r=-0.47, P=0.043) and DLCO% (r=-0.58, P=0.014) in IPF patients [34]. Immunostaining of both IL-13 and IL-13Rα1/2 on IPF lung sections revealed a strong expression by AM, endothelial cells, smooth muscle cells, and the bronchiolar epithelium [34].

Beyond the cytokines and chemokines mentioned above, many macrophage-related mediators potentially involved in IPF pathogeny have been identified, including members of the matrix metalloproteinases (MMP), as well as several angiogenic [62] and growth factors [63; 64]. In particular, AM have been shown to be a great source of many MMP (i.e., MMP-1, -7, -8, -10) in IPF lungs. Moreover, increases of these MMP in the serum or BALF of IPF patients have often been negatively correlated with disease progression and survival. For review see [60; 65]. In addition to MMP, two matricellular proteins (i.e., Osteopontin and Periostin) released by and, influencing the function of monocytes and macrophages, have also been shown to be increased in the serum and/or BALF of IPF patients. Indications from experimental models, suggest their direct contribution to lung fibrosis development [52; 60; 66]. Macrophages have such an important impact on IPF physiopathology that some of their mediators are candidate biomarkers in IPF. Those include CCL18, IL-8/CXCL8, CCL2, S100A9, and MIF [67]. Table 2 summarises the variations in BALF and serum concentration of many mediators related to the monocyte/macrophage lineage in patients with IPF. In conclusion, it is clear that macrophages and in lesser extent monocytes contribute greatly to the changes in mediators observed in the BALF and serum of patients with IPF. Whether they have a positive or negative impact on IPF development is the topic of the next chapter.

| | BALF | | Serum | |
|---|---|---|---|---|
| **Mediators** | **Changes** | **References** | **Changes** | **References** |
| IL-1β | ↑↑ | [33,68] | ↑ | [68] |
| TNFα | ↑ | [33] | → | [69] |
| INF-γ | → | [34] | ↓ | [70] |
| IL-6 | ↑ | [33] | ↑ | [69] |
| IL-4 | ↑ | [34] | → | [70] |
| IL-10 | ↓ | [71] | ↑↓ | [69,70] |
| IL-12p40 | → | [35] | ↑ | [70] |
| IL-13 | ↑↑↑ | [34,72,73] | ↑↑↑ | [72-74] |
| IL-23 | → | [35] | - | - |
| TGF-β1 | ↑§ | [75] | ↑→ | [69,76] |
| M-CSF | ↑ | [77] | - | - |
| GM-CSF | - | - | ↑ | [78] |
| IL-1Ra | ↑↑ | [53,68] | ↓ | [68] |
| CCL2/MCP-1 | ↑↑↑↑↑ | [30,35,53,79,80] | ↑↑ | [77,79] |
| CCL3 | ↑↑ | [30,80] | - | - |
| CCL4 | ↑ | [30] | - | - |
| CCL5/RANTES | → | [35] | - | - |
| CCL18/PARC | ↑↑ → | [35,58,61] | ↑ | [58,61] |
| CCL22/MDC | ↑↑ | [35,80] | - | - |
| CXCL8/IL-8 | ↑↑ | [33,35] | ↑↑ | [78] [70] |
| CXCL10/IP10 | → | [81] | → | [81] |
| CXCL5/ENA-78 | ↑ | [81] | → | [81] |
| sICAM1 | - | - | ↑↑↑ | [82-84] |
| MMP-1 | ↑→ | [85,86] | ↑↑ | [85,87] |
| MMP-2 | ↑ | [88] | ↑ | [87] |
| MMP-3 | ↑ | [88] | → | [87] |
| MMP-7 | ↑↑↑ | [85,88,89] | ↑↑ | [87,90] |
| MMP8 | ↑↑↑↑ | [35,65,86,88] | ↑ | [65,87] |
| MMP9 | ↑↑↑ | [35,86,88] | → | [87] |
| MMP10 | - | - | ↑ | [87] |
| MMP12 | - | - | ↑ | [87] |
| TIMP-1 | ↑→ | [86,88] | - | - |
| TIMP-2 | ↑ | [88] | - | - |
| VEGF | ↓↑*↑ | [35,88,91] | → | [78] |
| PAI-1 | ↑↑↑ | [35,92,93] | → | [94] |
| Angiopietin-2 | → | [95] | → | [78] |
| S100A9 | ↑ | [96] | → | [96] |
| YKL-40 | ↑↑ | [97,98] | ↑↑ | [97,98] |
| Osteopontin | ↑ | [99] | ↑ | [50] |

***Table 2:*** *Variation of monocyte/macrophage-related mediator's in the BALF and serum of IPF patients.*

**: Rapidly progressive IPF;* §: Active TGF-*β1; ↑: upregulated; →: Stable; ↓: Downregulated. Each arrow represents one reference.*

## The role of the monocyte/macrophage lineage in experimental lung fibrosis models

Despite the observation of changes in immune cell numbers and phenotype in IPF patients, it is difficult to conclude as for their contribution to the disease progression. The use of animal models, especially rodents is a way to bypass this disadvantage by allowing the depletion of specific cell populations or genes during experimental lung fibrosis. One of the most common model, of lung fibrosis, is a model induced by a single intratracheal administration of the chemotherapeutic agent bleomycin, often in rodents [100]. However, this model is criticised, because it is characterised by a very acute and transitory inflammatory phase, followed by a repair phase which is almost entirely resolved after a few weeks. IPF, on the other hand, is a chronic disease with low/mild inflammation in which the fibrotic process is developing inexorably probably over decades. Modified versions of the bleomycin model, involving repetitive intra-tracheal instillation of bleomycin [101] or the use of aged male mice [102], have been shown to mimic more closely IPF development. More recently, a few models relying on targeted injury of type II epithelial cells, fibroblastic autonomous effects, and targeted genetic defects, have been developed [103]. However, by nature the root cause of IPF is unknown, and many suspect it to be multifactorial. Therefore, it is almost impossible to create a single model recapitulating all the aspect of IPF pathophysiology. Moreover, the recent marketing of new IPF treatments (i.e., Pirfenidone, Nintedanib) discovered using the bleomycin model support its relevance to the disease.

Using the bleomycin model, Wei *et al*., have observed an increase in circulating Ly6Chi monocytes from day one to twenty-one after bleomycin instillation. This increase was later followed, by an increase in M2 alveolar and interstitial macrophages at day 21 during the fibrotic phase. During the same period, they observed a concomitant decrease in M1 macrophages [104]. Consistent with a role for the monocyte/macrophage lineage in lung fibrosis and IPF, mice deficient for the monocyte/macrophage chemoattractant CCL2 and M-CSF, are protected against bleomycin-induced lung fibrosis [77]. The respective contribution of interstitial and alveolar macrophages to lung fibrosis progression is not yet fully understood. In a recent article, it has been shown that administration of the mineralocorticoid receptor antagonist spironolactone inhibits lung fibrosis development in the mouse bleomycin model. Further analysis showed that mineralocorticoid administration was associated with a decrease in blood $Ly6^{Chi}$ monocytes expansion and the number of alternatively activated macrophages (AAM) ($F4/80^{+}$/ $Cd11c^{+}$/$CD206^{+}$ cells) in the alveoli, whereas the interstitial macrophage population ($F4/80^{+}$/ $Cd11c^{-}$ cells) remained unchanged [105].

Whereas monocytes-derived interstitial macrophages doesn't seem to play a key role in experimental lung fibrosis, recent evidence suggested that CCR2+ tissue monocytes also known as monocytic myeloid-derived suppressor cells (M-MDSCs) are a source of TGF-β1 in a mouse model of silica induced-lung fibrosis [106]. The authors also showed that co-culture of those lung M-MDSCs with fibroblasts resulted in the release by the later of collagens and the metalloproteinase inhibitor TIMP1. Finally, using mice deficient for CCR2 in the monocyte/macrophage lineage (i.e., LysMCreCCR2loxP/loxP mice) the authors showed that the loss of M-MDSCs was associated with decrease TGF-β1, TIMP1 and collagen expression in the lung of mice treated with silica [106]. Overall, these data highlight the role of the M-MDSCs in the creation of a non-degrading collagen microenvironment in experimental lung fibrosis.

Recently Lin *et al*., highlighted the role of PAR-1 in the recruitment of macrophages in response to bleomycin instillation [107]. They showed that the macrophage-dependent induction of fibroblast migration, differentiation and secretion of collagen, require PAR-1 activation at the surface of the fibroblasts by macrophage-derived FXa [107].

In a recent article Li *et al*., showed that similar to what observed in the BALF and lung tissue of IPF patients [108], bleomycin-induced lung fibrosis in mice is associated with a rise of IL-33 expression. They showed that IL-33 expression was induced in AM and induces an M2 polarisation characterised by the release of IL-13, TGF-β and the expansion of type 2 innate lymphoid cells [109]. Conversely, blockage of IL-33 signalling or depletion of macrophage impairs fibrosis development in this model [109]. Recently the IL-1R-associated Kinase-M (IRAK-M), a MyD88-dependent inhibitor has been shown to be necessary for the development of AAM during bleomycin-induced lung fibrosis. Indeed macrophages from fibrotic mice deficient in IRAK-M had impaired IL-13 secretion reducing their ability to stimulate fibroblast expression of α-SMA and collagens [110]. Another regulator of M2 macrophages, the MMP28, has been shown to promote AAM

dependant lung fibrosis in the bleomycin model [111]. Interestingly, the macrophage suppressor Paired Immunoglobulin-like Receptor B (PIR-B) has been shown to negatively regulate AAM differentiation in response to IL-4 *in vitro* [112], and mice deficient for PIR-B present increase macrophage expression of the alternatively activated markers OPN, MMP12 and Relm-α [112]. The central contribution of AAM to the development of bleomycin-induced lung fibrosis has been suggested by TNFα therapy a well-known M1 macrophage activator. TNFα therapy was associated with decreased AAM, and reduced fibrosis [113]. These results indicate that activators of the M1 macrophage program could represent a therapeutic target in lung fibrosis and probably most fibrotic diseases.

The contribution of monocytes to lung fibrosis is not limited to the bleomycin model. Indeed, in a model of lung fibrosis induced by targeted injury of type II epithelial cells, Osterholzer *et al*., observed an increase in circulating Ly6Chi monocytes and non-resident exudate macrophages characterised by cd11b expression. They further showed that CCR2 deficient mice presented a reduced accumulation of AAM and fibrosis in this model [114].

Many other articles published over the years have involved lung macrophages in experimental lung fibrosis. However, for the sake of concision, I will end this chapter by focusing on three critical recent publications.

In early 2016 Cao *et al*., used an improved model of IPF based on repetitive instillation of bleomycin in the mice. Using this model, they showed the critical role played by a hematopoietic-vascular niche in alveolar repair and lung fibrosis. Indeed, they showed that repeated lung injuries by bleomycin instillation was associated with, sustained suppression of CXCR7 expression by the pulmonary capillary endothelial cells (PCEC), and the recruitment of VEGFR-1+ perivascular macrophages [115]. The interaction between the PCEC and the VEGFR-1+ macrophages resulted in the up-regulation of the Notch ligand Jagged1 (encoded by Jag1) in PCEC in a Wnt/β-catenin dependent fashion, leading to increased Notch signalling in perivascular fibroblasts and increase fibrosis. Conditional deletion of the VEGFR-1 in the monocyte/macrophage lineage using genetically engineered mice (i.e., Vegfr1ΔLysM/ΔLysM mice) or the adoptive transfer of VEGFR-1 deficient monocytes in wild-type mice led in both cases to a significant reduction of lung fibrosis compared to control animals [115]. However, whether such a mechanism exists in humans and is involved in IPF pathogeny remains to be demonstrated. Flow Cytometry analysis or immunohistochemistry staining of AM from IPF tissue or sections should help answer this question.

Earlier this year, in an effort to identify a monocyte population, specifically recruited to the lung during the fibrotic phase of the bleomycin-induced lung fibrosis model, Satoh *et al.*, described an atypical monocyte playing a critical role in experimental lung and liver fibrosis and named segregated-nucleus-containing atypical monocytes (SatM) [116]. This new cell type has been phenotypically characterized as Ceacam1+, Msr1$^{+}$, Ly6C$^{-}$, F4/80$^{-}$, Mac1$^{+}$ monocytes. The authors showed that the SatM express granulocytic markers and is dependent for its development upon the transcription factor CCAAT/enhancer binding protein β (C/EBPβ) [116]. Interestingly the conditional deletion of C/EBPβ in the haematopoietic compartment was associated with a lack of SatM and the inhibition of bleomycin-induced fibrosis but not inflammation. However, the adoptive transfer of WT SatM into these mice restored the fibrotic development. The SatM have been found to be derived from a new Ly6C−FcεRI+ granulocyte/macrophage progenitor in which the expression of C/EBPβ is required to produce both the immediate SatM progenitor and the fully differentiated SatM [116]. It is not clear whether there is a human equivalent of the SatM. Using microarray analysis of the SatM transcriptome, the authors characterised their molecular signature. Recently Villani *et al*., using single-cell RNA-sequencing discovered new subsets of human monocytes. The search for human orthologues of genes associated with the SatM in single-cell RNA-sequencing data from peripheral blood CD14$^{+}$ cells of IPF patients could potentially lead to the discovery of a human equivalent of the SatM. This article also suggests that C/EBPβ pharmacological inhibitors could be of interest in preventing experimental lung and liver fibrosis and on the long-term in the treatment of IPF and other fibrotic diseases.

As discussed in the introduction, lung macrophages are a heterogeneous population, due to differences in localisation, and origin. Whereas the critical impact of environmental factors and therefore macrophage location on their phenotype and function has recently been demonstrated, the effect of macrophage origin (i.e., resident/foetal derived or Exudate/Monocyte-derived), on their phenotype and

function in health and disease is not well understood. In a recent study, published in the Journal of Experimental Medicine, Misharin *et al*., investigated the respective contribution of TRM and MDM, to lung fibrosis induced by intratracheal instillation of bleomycin in the mouse. Using a combination of engineered mouse strains and bone marrow transplantation, they have been able to generate mice devoid of either population. They found that monocyte-derived but not tissue-resident alveolar macrophages contribute to lung fibrosis in this model [117]. Using transcriptomic analysis of flow-sorted cells, they observed distinct expression profile of profibrotic genes between both populations during lung fibrosis development. Interestingly, they noticed that MDM persists in the lung up to one year after fibrosis resolution and are becoming increasingly similar to TRM. Indeed, comparison of their respective transcriptome ten months after initiation of lung fibrosis revealed only 330 differentially expressed genes [117]. It is not clear, however, whether the initial phenotypic distinction was due to the ongoing process of monocyte to macrophage differentiation or whether the reduced differences at ten months was due to the lack of pathological signal in this model at this time point. In other words, it will be interesting to determine whether there is a persistent epigenetic signature in macrophages linked to their origins, dictating their responses to the pathological lung milieu found in experimental fibrosis and IPF. An analysis of the transcriptome of TRM, and MDM shortly after bleomycin re-challenge of mice initially challenged ten months earlier could offer the beginning of an answer to this question. Last but not least, some of the profibrotic genes identified in MDM was also up-regulated in human AM from ILD lungs including some IPF samples [117]. In conclusion, this article supports the targeting of MDM in the treatment of ILD including IPF [117]. Consequently, the research of drugs targeting the monocyte to macrophage differentiation process while preserving TRM and monocytes should be encouraged as well as their testing in experimental models of lung fibrosis.

## Effects of past, present and prospective IPF treatments on the monocyte/macrophage biology

### The corticosteroids

A few years ago, the standard treatment for IPF included a tritherapy of, prednisone (corticosteroid and immunosuppressant), azathioprine (immunosuppressant), and N-acetyl cysteine (mucolytic agent) that was classically and with some success used in the treatment of non-specific interstitial pneumonia [3; 118]. However, a clinical trial assessing the effectiveness of this treatment showed it was associated with an increased risk of death or hospitalisation [119]. More recently an analysis of the benefice of corticosteroids treatments in acute exacerbation of IPF (AE-IPF) also concluded to a higher risk of mortality in the treated group [120]. These studies had a profound impact on the scientific community perception of IPF pathogeny, and IPF is no longer considered as a chronic inflammatory disease [121]. This conclusion, however, is based on a truncated view of inflammation, reducing it to its acute phase. Many fibrotic conditions linked to inflammation such as atherosclerosis evolve silently over decades in the absence of acute tissue damages or inflammation. Whereas anti-inflammatory steroids are very efficient at blocking the production of acute inflammatory mediators such as IL-1β, TNFα, INFγ and IL-6, they failed to prevent the expression of others. Indeed, in a recent article Khalaf et al., demonstrated that AM from COPD patients stimulated with the bacteria Non-typeable Haemophilus influenza (NTHi) was resistant to the immunosuppression induced by the corticosteroid dexamethasone. The authors found that whereas dexamethasone treatment of AM inhibits TNFα, IL-6 and IL-10 production induced by NTHi it fails to block that of the chemokines CXCL8 [122]. This resistance of CXCL8 expression to dexamethasone treatment was due to the activation by NTHi of the p38 MAPK pathway. However, whereas dexamethasone or the p38MAPK inhibitor BIRB-796 did not affect CXCL8 expression induced by NTHi in AM, a combination of both drugs successfully decreased it [122]. Interestingly, the potent neutrophil chemoattractant IL-8/CXCL8 has been shown to be up-regulated in IPF but not sarcoidosis in several studies [35; 78]. Moreover, an old report by Carre et al., established a positive and negative correlation between IL-8/CXCL8 expression by AM, and respectively the number of neutrophils in the BALF of IPF patients and their $PaO_2$ [55]. These results suggest that corticosteroids in IPF treatment might fail to limit the release of CXCL8 by AM and the subsequent recruitment of neutrophils to the lung. Beyond CXCL8 it is probable that other inflammatory mediators released by AM in IPF shares the same type of regulation and shows corticosteroid resistance as well. Indeed, an old article by Lacronique et al., showed that AM from IPF patients, although expressing glucocorticoid receptors, failed to decrease their secretion of fibronectin and other AM-derived growth factors [123]. The resistance of IPF macrophages to corticosteroid-induced apoptosis has also been reported and might contribute to the increased AM count seen in IPF patients BALF [40]. Moreover, another study

comparing the BALF cellularity of IPF patients treated or not with corticosteroids failed to identify any change in macrophages and neutrophils number in the treated group confirming their resistance to this treatment [37]. The same way, the administration of high doses of the corticosteroid methylprednisolone in a rat model of bleomycin-induced lung fibrosis failed to block the increased expression of the profibrotic cytokine TGF-β in AM [124].

Not only steroids fail to inhibit some part of the inflammatory response of AM, but recent data also suggest that they might promote the generation of AAM or M2 macrophage. Indeed, in a mouse model of acute lung injury induced by intravenous administration of lipopolysaccharide (LPS, 5mg/kg), administration of the glucocorticoids methylprednisolone (5mg/kg) was found to attenuate acute lung injury presumably by promoting type 2 macrophage expansion and their release of IL-10 and TGF-β [125]. Moreover, using the mouse model of bleomycin-induced lung fibrosis researchers showed that dexamethasone does not alter the severity of lung fibrosis in this model despite reducing the number of TRM, lymphocytes and eosinophils [126]. Interestingly, they found that dexamethasone treatment has no impact on MDM supporting data from Misharin et al., suggesting their implication in lung fibrosis and IPF. In conclusion, the failure of anti-inflammatory steroids to stop IPF progression cannot be used to rule out a contribution of these cells to IPF pathogeny. The articles presented above suggest instead: 1) a failure of these treatments in suppressing, AM releases of critical mediators (e.g. CXCL8, fibronectin, Growth Factors), and the recruitment of MDM. 2) their promotion of a pro-fibrotic AAM phenotype.

### Pirfenidone

Pirfenidone (5-methyl-1-phenyl-2- (1H)-pyridone) is a small molecule known for its anti-fibrotic properties (i.e., inhibition of procollagen I and II, PDGF isoforms, TGF-β, FGF, IL-13 expression in the mouse model of bleomycin-induced lung fibrosis), but whose exact mechanism of action remains elusive [127].

Based on its observed anti-fibrotic action in experimental lung fibrosis, several clinical trials have been conducted to evaluate its safety and effectiveness in IPF treatment. Most of these clinical trials concluded to a significant benefit of Pirfenidone treatment due to a significant reduction in FVC% decline, and a significant increase in progression-free survival (improved survival in the treated group) [128]. These results prompted its market for the treatment of patients suffering from IPF in Japan, Europe, and USA respectively in 2008, 2011 and 2014.

Interestingly, some data suggest that Pirfenidone might slow down IPF progression and lung fibrosis by targeting the monocyte/macrophage lineage. Indeed, it has been recently demonstrated in a murine model of chronic, graft versus host disease-induced bronchiolitis obliterans, that Pirfenidone administration inhibits disease progression by limiting macrophage infiltration and TGF-β production [129]. The authors concluded that the macrophages were a major source of TGF-β in this model and that the reduction observed in Pirfenidone treated animals was due to a decrease in macrophage number and capacity to secrete TGF-β [129]. Moreover, Pirfenidone has also been shown to decrease macrophage infiltration in nephrectomised rats [130].

In the mouse model of bleomycin-induced lung fibrosis, Pirfenidone treatment is associated with a reduction of CCL2/MCP-1 production offering a molecular basis for its inhibition of the monocyte/macrophage lineage in lung fibrosis [131]. This result was confirmed in another study, published in 2014, and showing that Pirfenidone treatment inhibits CCL2/MCP-1 expression and reduces BALF macrophage count in the same model [132]. Direct *in vitro* evaluation of Pirfenidone effect on macrophages (i.e., J774 macrophages) migration showed its ability to significantly block the chemoattraction induced by IL-17A and MCP-1/CCL2 [129]. One hypothesis to explain such effects could be the inhibition by Pirfenidone of the CCL2/MCP-1 receptor CCR2. Indeed, it has been shown *in vitro* that Pirfenidone reduces fibrocytes migration toward CCL2 by lowering CCR2 expression in these cells [132].

Altogether, these data suggest that Pirfenidone inhibits experimental lung fibrosis and other fibrotic disorders in part through inhibition of the monocyte/macrophage lineage function implying that such mechanism could account for its effect on IPF progression.

## Nintedanib

Nintedanib is a small molecule that was originally designed as an ATP-competitive inhibitor of the proangiogenic and tyrosine kinase receptors, Fibroblast Growth Factor Receptor-1 (FGFR-1) and vascular endothelial growth factor receptor-2 (VEGFR-2) to serve as an anticancer drug [133]. It was later found to also inhibit the receptor for Platelet-derived Growth factors alpha and beta (PDGFRα and β) prompting its assessment as a potential treatment in IPF [133]. Besides, Nintedanib has been shown to also inhibit VEGFR-1 and 3 as well as FGFR-3 [134].

The potential benefit of Nintedanib in IPF treatment has been assessed in the "To Improve Pulmonary Fibrosis with BIBF 1120" (TOMORROW) clinical trial [135], and the INPULSIS-1, and INPULSIS-2 trials [136]. In all these studies, Nintedanib treatment showed its ability to improve FVC% against the placebo treatment over a one year period [128].

Several lines of evidence suggest that Nintedanib benefice in IPF could be linked to the inhibition of the monocyte/macrophage lineage function in the lung. Indeed, it has been shown that macrophages and epithelial cells are the primary sources of PDGF in the lung [137; 138]. Moreover AM from IPF patients releases around four times more PDGF than those from HC [139]. In addition, pulmonary macrophages are also a significant source of ligands (i.e., FGF-2, VEGF) for the other targets of Nintedanib such as the FGF2R [140], and the VEGFR- [133; 141]. In light of the publication by Cao *et al.*, presented above, involving VEGF-R1+ macrophages in experimental lung fibrosis, one might think that if such mechanism was present in IPF, it's inhibition by Nintedanib could contribute to its effectiveness in IPF.

Earlier this year, Huang *et al*., using the FRA2 mouse model of pulmonary hypertension associated with systemic sclerosis, showed that Nintedanib treatment resulted in the normalisation of VEGF serum level, reduced M2 polarisation of monocytes, and decreased numbers of M2 macrophages in the lung [142]

Moreover, in an abstract, recently presented at the American Thoracic Society (ATS) conference 2017, Tandon et al., studied the effect of Nintedanib on macrophage polarisation *in vitro* and *in vivo* using the mouse model of bleomycin. The authors showed that Nintedanib inhibits the M2 polarisation of both murine bone marrow-derived macrophages and the human monocytic cell line THP-1 in response to IL-4/IL-13 or IL-4/IL-13/IL-6 stimulation as assessed by the measurement of arginase activity and CCL18 production respectively. The authors further demonstrated the ability of Nintedanib to inhibit the CSF1 receptor tyrosine phosphorylation at a concentration of 0.3μM. *In vivo*, Nintedanib inhibition of bleomycin-induced lung fibrosis was associated with a decreased number of macrophages expressing the AAM markers YM1 and arginase [143].

Pirfenidone has also been shown to inhibit the expression of the cytokine Il-1β in the lung of bleomycin-treated mice [144]. Interestingly, IL-1β is a cytokine well known to drive the development of fibrosis in many organs including the lung [145]. Furthermore, AM from IPF patients have been shown to release an increased amount of IL-1β compared to those from HC [54]. Moreover, BALF macrophages from IPF patients have been shown to present an imbalance in the IL-1Ra/IL-1β ratio, resulting in increased IL-1β activity [146]. Therefore, the inhibition of the IL-1β signalling by Nintedanib is another potential mechanism by which it can inhibit macrophages contribution to lung fibrosis.

The same way, interstitial macrophages from IPF patients have been shown to express higher levels of the MMP inhibitor TIMP-1 whose expression is also inhibited by Nintedanib in experimental lung fibrosis [144; 147]. However, unlike what observed with Pirfenidone, Nintedanib treatment in mouse models of lung fibrosis induced by bleomycin or silica particles instillation failed to significantly reduce the number of BAL macrophages [144].

Further studies are required to determine the impact of Nintedanib on the monocyte/macrophage lineage recruitment and polarisation in the lung with regard to tissular location (interstitial vs alveolar) and origin (TRM vs MDM).

### The PI3K/mTOR pathway inhibitor GSK2126458

Based on some similarities between IPF and lung cancers pathogeny, Mercer et al., recently evaluated the effectiveness in IPF of a novel, potent PI3K/mammalian target of rapamycin (mTOR) inhibitor named GSK2126458 and currently in clinical trial for application in oncology [148]. The study showed by immunofluorescence on tissue sections from IPF patients that, AKT phosphorylation on serine 473 and threonine 308 was present in fibroblasts of the fibroblastic foci, AM and the bronchiolar epithelium of IPF sections. Moreover, the phosphorylation of AKT was confirmed in the BALF cells from IPF patients (mainly AM) and was inhibited in these cells by treatment with GSK2126458 [148]. Interestingly, recent data showed that monomeric collagen type I binding to AM from IPF patients through CD204 induces PI3K signalling, supporting an M2 polarisation characterised by the release of CCL18, IL-1RA and CCL2 [149]. Therefore, PI3K/mTOR inhibitors could represent a novel therapeutic avenue in IPF involving the targeting of the monocyte/macrophage lineage function. However, as discussed above lung macrophages are dependent for their development upon the GM-CSF/GM-CSFR pathway, and inhibition of this pathway by autoantibodies or genetic defects leads to the development of pulmonary alveolar proteinosis [26]. Unfortunately, GM-CSF triggers three central pathways in macrophages the JAK/Stat, the Ras/mitogen-activated protein kinase, and the PI-3 kinase (PI-3K)-Akt pathways [150]. Therefore, studies should be conducted to assess the risk of pulmonary alveolar proteinosis associated with this treatment and the best administration route to ensure preferential targeting of the MDM.

### The Mitogen-activated protein kinase Kinase Kinase19 (MAP3K19)

MAP3K19 is a newly described MAP 3-kinase that has been shown to be expressed mainly in the lung and trachea, and more specifically in the bronchial/alveolar epithelium (type II epithelial cells), interstitial/alveolar macrophages and neutrophils of both human and mouse lung [151]. In an early publication, MAP3K19 has been shown to be up-regulated in rice cultivated under high salt condition [152]. Thereafter, MAP3K19 have been shown to regulate the nuclear translocation of activated smad2/3 following TGF-β stimulation in the monocytic cell line THP-1 and the type II epithelial cell line A549 [153]. Beyond TGF-β signalling, MAP3K19 was also found to be activated in numerous cell types in response to oxidative stress, making it a probable molecular mediator between environmental stressors and the development of lung fibrosis [151]. MAP3K19 has been shown to be overexpressed in AM from both IPF, and COPD patients compare to HC [151; 153]. MAP3K19 was found to be up-regulated in bleomycin-induced lung fibrosis, and its pharmacological inhibition (Molecule: US patent number 60/096308) was able to diminish the development of lung fibrosis in the mouse bleomycin model [153]. The authors also showed that MAP3K19 synergise with Pirfenidone and Nintedanib in the repression of pro-fibrotic genes at the transcriptional and translational level [153]. Further study should determine the role of MAP3K19 in the differentiation of the AAM induced by TGF-β and other inducers of AAM.

## Concluding Remarks

IPF is a devastating disease affecting a growing number of individuals worldwide, and whose prognostic remains poor despite the recent marketing of Pirfenidone and Nintedanib as treatments [121]. Due to the ineffectiveness of anti-inflammatory therapies in IPF, the concept of dysregulated epithelial-mesenchymal interactions has replaced the initial idea of chronic inflammation as the driving force of fibrosis in this disease [154]. Therefore, in the past few years, the research effort has been mainly concentrated on the targeting of epithelial-mesenchymal interactions. In this review, we presented convergent data highlighting the activation, of the monocyte/macrophage lineage in IPF and their critical contribution to the changes in BALF mediator concentration, and the recruitment of neutrophils. Moreover, several aspects of their function such as, efferocytosis have been found to be impaired in IPF patients. A body of evidence suggests a monocyte-like phenotype of these cells in the BALF of IPF patients that could sign the active recruitment of MDM in this disease or a defective monocyte to macrophage differentiation. Furthermore, recent publications highlighted a detrimental contribution of the monocyte/macrophage lineage in experimental lung fibrosis supporting their possible contribution to IPF pathogeny. Finally, we showed evidence that corticosteroids treatments in IPF failed to inhibit the activation of the monocyte/macrophage lineage consistently and could favour their differentiation toward the profibrotic AAM phenotype. Therefore, the failure of anti-inflammatory treatments in IPF cannot

be used to rule out their involvement in the disease pathogeny. Interestingly, several lines of evidence suggest that current IPF treatments (i.e., Pirfenidone and Nintedanib) and new prospective drugs target some aspect of the monocyte/macrophage function related to fibrogenesis. However broad targeting of the monocyte/macrophage lineage in human have been associated with significant side effects, especially in the gut [117]. In recent years nano- and micro-based inhaled drug delivery systems allowing specific targeting of AM have been developed and could be used to bypass such side effects [155]. Efforts are also required to identify mechanisms specific to monocyte recruitment to the lung that could serve as therapeutic targets in IPF and other lung inflammatory diseases. Overall the data presented in this review support the targeting of the lung monocyte/macrophage lineage as a promising strategy in IPF treatment.

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