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Alternatively activated alveolar macrophages in pulmonary fibrosis—mediator production and intracellular signal transduction

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Abstract Activated macrophages have been characterized as M1 and M2 according to their inflammatory response pattern. Here we analyzed the M2 marker expression and intracellular signal transduction in the course of cytokine-driven differentiation. We found elevated spontaneous production of the chemokines CCL17, CCL18 and CCL22 and increased expression of CD206 by alveolar macrophages from patients with lung fibrosis. Stimulation of normal human AM with Th2 cytokines IL-4 and/or IL-10 *in vitro* revealed IL-4 as the most powerful inducer of M2-phenotype in AM and monocytes. Importantly, IL-10 enhanced IL-4-induced expression of CCL18 and IL-1RA in a synergistic fashion. IL-4/IL-10 stimulation induces a strong activation of STAT3 in AM from fibrosis patients. These results suggest an important role for M2 polarized AM in the pathogenesis of pulmonary fibrosis and indicate that both IL-4 and IL-10 account for human AM phenotype shift to M2, as seen in patients with fibrotic interstitial lung diseases.

Abbreviations: AM, alveolar macrophages; BAL, bronchoalveolar lavage; CCL, CC-chemokine ligand; IL, interleukin; IPF, idiopathic pulmonary fibrosis; M2, alternatively activated macrophages; SAR, sarcoidosis; SSc, systemic sclerosis; STAT, signal transducer and activator of transcription; TGF-β, transforming growth factor-β.

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Introduction

Pulmonary fibrosis evolves from a variety of different interstitial lung diseases and idiopathic pulmonary fibrosis (IPF) is the most common form of fibrotic lung diseases [1]. Systemic sclerosis (SSc) is a complex systemic disorder characterized by uncontrolled deposition of collagen and other matrix proteins and is often associated with fibrotic lung remodeling. Sarcoidosis (SAR) is a granulomatous disease of unknown origin primarily affecting the lung and the lymphatic system and may develop into pulmonary fibrosis. Spontaneous remission occurs in nearly two-thirds of sarcoidosis patients; however, chronic progressive disease frequently results in pulmonary fibrosis. While patients with sarcoidosis of the radiological types I and II do not show signs of fibrotic remodeling, patients with sarcoidosis of the radiological type III and IV show it increasingly [2,3]. Clinically, patients with fibrotic lung disease disclose progressive dyspnea and worsening of pulmonary function, which often lead to disability or even patients' death [4,5].

Despite the obvious etiological and pathological differences, fibrotic processes in all of the above-mentioned diseases share common cell biologic mechanisms as exaggerated wound healing ultimately leading to an excess of fibroblast replication [6], increased production of transforming growth factor-β (TGF-β) [7], abundant collagen production and deposition [8], and a Th2-cell cytokine milieu in the lung characterized by increased release of IL-4, IL-13 and IL-10 [9–13]. Human alveolar macrophages (AM) are able to express several mediators involved in fibrotic processes, and disclose an increased expression of IL-10, IL-13, and PDGF in patients with IPF and SSc [9,13,14]. These data suggest that AM may play a pivotal role in pulmonary fibrosis [15–17].

Recently we have shown that CCL18 production by alveolar macrophages from patients with fibrotic lung diseases is highly up-regulated and reflects fibrotic progress in pulmonary fibrosis and systemic sclerosis[18–20]. In analogy to the dichotomic characterization of Th cells (Th1, Th2), macrophages were characterized by their marked phenotypic heterogeneity depending on their microenvironmental stimulation. Classical activation by microbial agents and/or Th1 cytokines, in particular by IFN-γ, is mainly associated with the production of TNF, IL-12, IL-8, IL-6, and IL-1; the expression of NOS2 and the down-regulation of CD14 expression [21,22]. These classically activated macrophages (also called M1) are found e.g. in SAR and tuberculosis [23–25]. More recently, it has been shown that macrophages stimulated by the Th2 cytokines IL-4, and IL-13 or IL-10 disclose a different activation pathway called alternative activation [26–29]. It has been reported that alternatively activated macrophages play a critical role in allergy and parasitic infections [reviewed in 28] and appear to be involved in the control of tissue repair [30]. This M2 macrophage phenotype has also been characterized by a specific expression pattern of membrane receptors, including scavenger receptors A and B, mannose receptor (CD206), CD36, CD163, chemokine receptors (CXCR1, CXCR2, CCR2) and expression of several cytokines and chemokines, including IL-1 receptor antagonist (IL-1RA), IL-10, CCL1, CCL17, CCL18, and CCL22 and arginase expression [28,29].

The purpose of this study was to examine the functional status and phenotype of AM in a variety of diseases associated with pulmonary fibrosis including IPF, SSc, and SAR and to compare these observations with AM from healthy controls. In particular, we were interested in the activation pattern of AM induced by the aforementioned Th2 cytokine milieu. In addition, we addressed the signal transduction mechanisms involved in disease-associated Th2-driven alternative macrophage activation. We observed that AM from the vast majority of patients with fibrotic lung disease show a characteristic expression pattern of chemokines, cytokines and surface markers. Moreover, they appear to differ from AM of healthy individuals in their intracellular signal transduction in response to the Th2 cytokines IL-4 and IL-10, generally characterized by activation of signal transducers and activators of transcription (STAT) 6 or STAT3, respectively [31,32]. Macrophages from fibrosis patients but not from healthy donors specifically respond to IL-4 and IL-10 with a profound, and in case of STAT3, synergistic activation of STAT molecules. These results strongly support the view that induction and maintenance of an M2 phenotype of human AM is a specific feature in pulmonary fibrosis and suggests an important role of M2 polarized AM in the pathogenesis of fibrosing disorders.

Materials and methods

Subjects

In total, 180 patients and healthy volunteers underwent bronchoscopy with bronchoalveolar lavage (BAL). We included 33 patients with IPF, diagnosed according to a recently published consensus statement of the American Thoracic Society and the European Respiratory Society [4]. Eighteen subjects meeting the American Rheumatism Association diagnostic criteria [33] for the diagnosis of SSc were recruited. Additionally, 102 patients with SAR were included in the study. Diagnosis of pulmonary SAR was based on clinical and radiological data with the histological confirmation in lymph nodes or lung biopsies as suggested by a recent consensus statement [3]. SAR patients were categorized according to radiological types of diseases. In total, 32 patients with sarcoidosis radiologic type I (bilateral hilar lymphadenopathy without parenchymal involvement, SAR I), 35 patients with sarcoidosis type II (bilateral hilar lymphadenopathy with parenchymal involvement, SAR II), 23 patients with type III (pulmonary infiltrates with or without hilar lymphadenopathy, SAR III), and 12 patients with type IV (end-stage pulmonary fibrosis without hilar lymphadenopathy, SAR IV) were analyzed. None of the patients was treated with any anti-inflammatory or immunosuppressive drug for at least 2 months prior to investigation. Twenty-seven volunteers served as healthy controls (C). Demographic characteristics, respiratory function tests and BAL cytology data of the study populations are depicted in Table 1. The study was approved by the local ethics committee.

BAL cell isolation and culture

BAL cell isolation and culture were performed as previously described [34]. Cell differentials were determined using