

## TYPE 2 MACROPHAGES AND TH2 CD<sub>4</sub><sup>+</sup> CELLS IN INTERSTITIAL LUNG DISEASES (ILDs): AN OVERVIEW

*Neftali Partida-Zavala<sup>1,2</sup>, Marco Antonio Ponce-Gallegos<sup>1,2</sup>, Ivette Buendía-Roldán<sup>2</sup>, Ramcés Falfán-Valencia<sup>2</sup>*

<sup>1</sup>Universidad Autónoma de Nayarit, Unidad Académica de Medicina. Tepic, Nayarit. México. Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas. Ciudad de México, Mexico; <sup>2</sup>HLA Laboratory, Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas City, Mexico

**ABSTRACT.** Interstitial lung diseases (ILDs) are a heterogeneous group characterized mainly by damage to pulmonary parenchyma, through histopathological processes such as granulomatous pneumopathy, inflammation and fibrosis. Factors that generate susceptibility to ILDs include age, exposure to occupational and environmental compounds, genetic, family history, radiation and chemotherapy/immunomodulatory and cigarette smoke. IFN- $\gamma$ , IL-1 $\beta$ , and LPS are necessary to induce a classical activation of macrophages, whereas cytokines as IL-4 and IL-13 can induce an alternative activation in macrophages, through the JAK-STAT mediated signal transduction. M2 macrophages are identified based on the gene transcription or protein expression of a set of M2 markers. These markers include transmembrane glycoproteins, scavenger receptors, enzymes, growth factors, hormones, cytokines, and cytokine receptors with diverse and often yet unexplored functions. Fibrotic lung disorders may have a M2 polarization background. The Th2 pathway with an elevated CCL-18 (marker of M2) concentration in the bronchoalveolar lavage fluid (BALF) is linked to fibrosis in ILDs. Besides the role of M2 in tissue repair and ECM remodeling, activated fibroblasts summon and stimulate macrophages by producing MCP-1, M-CSF and other chemokines, as well as activated macrophages secrete cytokines that attract and stimulate proliferation, survival and migration of fibroblast mediated by platelet-derived growth factor (PDGF). (*Sarcoidosis Vasc Diffuse Lung Dis* 2018; 35: 98-108)

**KEY WORDS:** ILDs, fibrosis, macrophages, Th2, IL-4, IL-13

### INTRODUCTION

Interstitial lung diseases (ILDs) are a heterogeneous group characterized mainly by damage to pulmonary parenchyma, specifically the pulmonary interstitium, through histopathological processes such as granulomatous pneumopathy, inflammation

and fibrosis. Pathological changes are diverse depending on the type of disease, they can be: alveolar alterations, lymphocyte infiltration, presence of giant cells, increase of the extracellular matrix with the presence of focal areas of fibroblast proliferation. Some examples of ILDs are sarcoidosis, idiopathic pulmonary fibrosis (IPF) and hypersensitivity pneumonitis (HP) (1). Factors that generate susceptibility to ILDs include age, exposure to occupational and environmental compounds, genetic, family history, radiation and chemotherapy/immunomodulatory and cigarette smoke (2).

On the other hand, human immune system is composed of different cells that protect us from pathogens and allow homeostasis in the body; among

Received: 11 August 2017

Accepted after revision: 6 February 2018

Correspondence: Ramcés Falfán-Valencia

HLA Laboratory, Instituto Nacional de

Enfermedades Respiratorias Ismael Cosío Villegas

Ciudad de México, Mexico

E-mail: rfalfanv@iner.gob.mx

them are the macrophages, which come from bone marrow and are stimulated by monocyte colony stimulating factor (M-CSF). Macrophages can acquire certain phenotypes by activating the classical (M1) or alternatively pathway (M2) based on surface receptors, gene expression and secretion of inflammatory mediators. Some studies with knock-out mice made possible to identify an important number of genes (*iNOS*, *Arg1*, *Ym1*, *FIZZ1*) and chemokines that are involved in the activation of different pathways (3). Functions of activated macrophages include microbicidal and tumoricidal activity, phagocytosis and pinocytosis, nitric oxide generation, chemotaxis, antigen presentation, secretion of pro-inflammatory cytokines including interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-12, IL-15, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and antimicrobial peptides (cathelicidin, defensins) (4).

For the macrophages polarization and proliferation, the participation of certain molecules is necessary: interferon- $\gamma$  (IFN- $\gamma$ ), IL-1 $\beta$  and lipopolysaccharide (LPS) induce a classical activation of M1, whereas that Th2 cells are very important because produce IL-4 and IL-13, which are involved in alternative activation pathway (5). In addition they have an important role in physiology and pathophysiology in several diseases, for example, resolution of inflammation, tissue regeneration, reshuffle of the extracellular matrix and fibrotic illness (such as ILDs, liver fibrosis, etc.) (6).

## EPIDEMIOLOGY OF ILDs

A study realized in Greek population indicates that the estimated annual incidence of ILDs is 4.63 per 100,000 inhabitants and the prevalence reach 17.3 per 100,000 (7). Also, in Spain, Lopez-Campos and Rodríguez-Becerra reported an annual incidence of 3.62/100,000 cases (men 4.18/100,000/year and women 3.07 cases/100,000/year). (8). Coultas et al. described in their study that the prevalence of ILDs was 20% higher in males than females (9). In addition, in studies carried out in Germany showed up sarcoidosis and idiopathic pulmonary fibrosis (IPF) were the most frequent in that country. (10) Similar to epidemiology in another countries, in the Instituto Nacional de Enfermedades Respiratorias (INER) at Mexico City, IPF represents one of the three more common causes of morbi-mortality in ILDs (11).

Dr. Barreto-Rodríguez reported in adults during the period comprised of 2009-2013 that prevalence of ILDs at INER was of 8.7% (1,923/21,962 patients) (12). According to Martínez-Briseño et al, on an epidemiological study they analyzed mortality from ILDs in Mexico during the period comprised of 2000-2010, where 22,600 deaths were recorded during the 10 years at INER (13).

## ILDs CLASSIFICATION

There are more than 150 pathologic entities that are included in ILDs group. However, because of their complexity and heterogeneity, their classification is not a simple task (11).

The classification of ILDs is an issue that has changed over the years. To reach the current classification of ILDs, Liebow and Carrington in 1969 published the classification where ILDs are divided into: Usual interstitial pneumonia, desquamative interstitial pneumonia, bronchiolitis obliterans interstitial, pneumonia and diffuse alveolar and diffuse alveolar, lymphoid interstitial pneumonia, giant cell interstitial pneumonia. Katzenstein published in 1997 another classification of ILDs, which was similar to that published in 1969, but with the difference that there were groups of interstitial respiratory bronchiolitis lung disease, acute interstitial pneumonia and nonspecific interstitial pneumonia were aggregated. Müller and Colby in 1997 performed the classification of ILDs where the group of bronchiolitis obliterans and organizing pneumonia was added (14).

The American Thoracic Society in 2002 published a new classification clustering of interstitial lung diseases into four groups: 1) diffuse parenchymal lung disease (DPLD); 2) know-for-cause, for example secondary to drugs consumption or association to collagen - vascular diseases; 3) idiopathic interstitial pneumonias and 4) granulomatous DPLD, e.g. sarcoidosis, etc. (15).

The most recent and current classification of interstitial lung diseases was published in 2013 by the American Thoracic Society, where we can analyze in more detail and clarity the types of ILDs, mainly divided into: interstitial pneumonia idiopathic, known or associated cause, primary or associated with other diseases not well defined and in each of them there

are subdivisions. The actual classification for ILDs is presented in Figure 1.

### GENETIC SUSCEPTIBILITY TO ILDs

Genetic factors predispose to the pathophysiological development of mostly diseases, and ILDs are not the exception. There are a lot of studies of single nucleotide polymorphisms (SNPs) that have been described as a risk factor to develop an interstitial lung disease, and some of them are related to type 2 macrophages.

One of the most investigated genes is *TGFB1*. Several SNPs have been evaluated in this gene and its importance is due to codifies transforming growth factor- $\beta$  (TGF- $\beta$ ), an important regulatory cytokine. It has been seen that the presence of the proline allele in codon 10 of the *TGFB1* gene is associated with an increased deterioration in gas exchange in patients with IPF (16). However, Azmy et al. in a study realized with an Egyptian population, revealed that gene polymorphisms of TGF- $\beta$ 1 are not related with presence of ILDs (17). In addition, André PA et al in an *in vitro* study of cultured cells suggest that *BARD1* and BARD1- $\beta$  might be mediators of pleio-

tropic effects of TGF- $\beta$ 1. In particular, BARD1- $\beta$  might be a driver of proliferation and progression of pulmonary fibrosis pathogenesis and also represent an important target for treatment (18).

As it's known, IL-10 is an anti-inflammatory cytokine and contributes to fibrotic process. Ates et al described that *IL10* polymorphisms (another gene that codifies a cytokine related to M2 phenotype) are associated to lung involvement in Systemic Sclerosis (SSc), and these findings coincides with a Scala et al. [17] report, where they reported that IL10 levels are significantly higher in patients with pulmonary involvement than that in control (19, 20).

Moreover, other genes that participate in fibrotic process have been related to ILDs. Liu L et al. concluded in a study realized in Chinese patients with IPF that -156C allele for ENA-78 (now called *CXCL5*) may be a risk factor of IPF and -1596T allele for IP-10 a beneficial factor of IPF. However, the *VEGF* (+405G/C) gene polymorphism has no effect upon the predisposition to IPF (21).

In an association study realized by Li C et al, the results suggest that the polymorphism of *EGF* in the position 61A/G may be associated with sporadic ILD, with the frequency of G allele significantly increased in the ILD patient population (22). Also, in

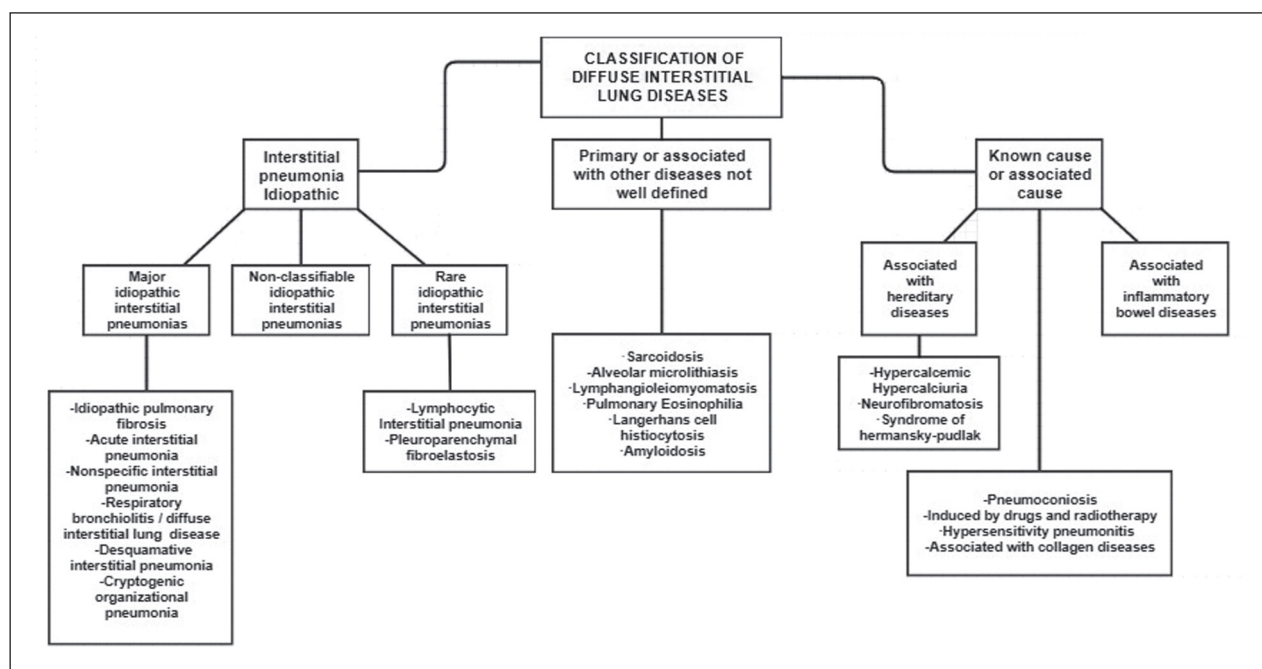


Fig. 1. Current classification of ILDs according to American Thoracic Society

patients with lung cancer treated with an inhibitor of epidermal growth factor receptor (EGFR), it has been demonstrated that can induce the development of ILD (23).

There are many studies in another kind of genes/physiopathological ways that have indicated risk factor or poor prognosis in patients with different ILDs. These are summarized in Table 1.

### M2 DIFFERENTIATION AND THE TH2 CD4+ CONTRIBUTION

Tissue macrophages are derived from monocytic phagocyte system, which committed bone marrow precursors develop into blood monocytes (40), which arise from myeloid stem cells and migrate to peripheral blood and various tissues where they differentiate. It is estimated that a healthy adult mouse contains approximately  $10^8$  macrophages that are distributed throughout the body in several organs and tissues. This cell lineage display great phenotypic and functional diversity because of their ability to adapt to their microenvironment (41).

Macrophages can differentiate into either a pro-inflammatory (M1) subtype, also known as a classically activated subtype, or an anti-inflammatory alternatively activated subtype (M2) according to the microenvironment (cytokines, antigens, etc.) (42). Also, they are a functionally heterogeneous cell population. IFN- $\gamma$ , IL-1 $\beta$ , and LPS are necessary to induce a classical activation of macrophages, whereas cytokines as IL-4 and IL-13 can induce an alternative activation in macrophages, through the JAK-STAT mediated signal transduction (3).

M1 macrophages are potent effector cells that kill microorganisms and produce proinflammatory

mediators, such as nitric oxide (NO), tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), IL-6, and IL-12. M2 macrophages produce anti-inflammatory factors like IL-10, TGF- $\beta$  and IL-1 receptor antagonist (IL-1Ra), and promote angiogenesis by VEGF, tissue remodeling and repair. Reprogramming of intracellular metabolisms is required for the proper polarization and functions of activated macrophage. M1 macrophages increase glucose consumption and lactate release, whereas M2 macrophages mainly employ oxidative glucose metabolism pathways (3).

STAT6 regulates effector Th2 responses in lung inflammation through multiple mechanisms including canonical Th2 cell differentiation and recruitment (43). The IL-4 signaling cascade through STAT6 activation is considered the canonical pathway of Th2 differentiation, as well as M2 (44). IL-4 and IL-13 each bind to two receptor complexes and have one shared receptor subunit; this allows the stimulation of either receptor activates IL-4R $\alpha$  and associated JAK1 to phosphorylate STAT6 monomers, which then homodimerize and translocate to the nucleus, where inducing expression of certain genes related to the M2 phenotype (45).

M2 macrophages are identified based on the gene transcription or protein expression of a set of M2 markers. These markers include transmembrane glycoproteins, scavenger receptors, enzymes, growth factors, hormones, cytokines, and cytokine receptors with diverse and often yet unexplored functions. The majority of these markers were defined by early studies of the M2 activation, based on the observation that their gene transcription was amplified by IL-4/IL-13 and fungal or parasite infections (collectively, in conditions associated with Th2 immune response) (46). Many of the genes associated with mouse M2 macrophages are regulated by STAT6, which are ex-

**Table 1.** Genes and polymorphisms related to principal ILDs.

Gene	ILD-Related	Reference
<i>FOXP3, RTEL1, EGF</i>	Idiopathic interstitial pneumonia	(24) (25)
<i>HLA-DRA, BTNL2, HLA-DRB1, EGF</i>	Sarcoidosis	(26) (27) (28)
<i>HSPA1B, HSPA1L, HSPA1, PARN, RTEL, TGFB1, MUC5B, EGF, BARD1, ADAM33, ACE, CXCL5</i>	Idiopathic pulmonary fibrosis	(16) (29) (30) (31) (18) (21) (32) (33) (34)
<i>HLA-DRB1*04, PSMB8</i>	Hypersensitivity pneumonitis	(35) (36)
<i>IL17F, NALP3</i>	Silicosis	(37) (38)
<i>PADI2, FOXP3</i>	Diffuse interstitial lung disease	(24) (39)

pressed when an external stimuli binds to membrane receptors, including *Retnlb*, also known as *FIZZ1*, *Arg1*, *Chil3*, also known as *Ym1* in mice, and in humans have been described *CD163*, *MRC1/CD206*, *CD200R1*, *TGM2*, *TGB1*, *IL10*, *VEGFA*, *ITGAM* (*CD11B*), *CD209*, *IL1R1*, *CHI3L1* and *ARG1* as M2 related (47). These genes in mice and humans are described in Table 2, in addition with Th2 related genes.

Some Th2-related cytokines are important to M2 polarization and contribute to pathologic stages. For example, IL-13 contributes to fibrosis in a number of chronic infectious and autoimmune diseases, and is likely involved in airway fibrosis and smooth muscle increase in asthma as well as interstitial lung disease. (52) Also, IL-13 transgene overexpression in the lung has been shown to induce persistent subepithelial fibrosis and smooth muscle hypertrophy, (53) and some of the effect of IL-13 may be influenced by STAT6-independent (54).

Reforging the role of these cytokines in Th2 and M2 differentiation and proliferation, as well as fibrotic process, a study with mice subjected to bleomycin induced pulmonary fibrosis displayed elevated IL-4 and IL-13 and therapeutic blockade was shown to reduce the pulmonary interstitial fibrosis phenotype (55). These data suggest that the IL-4/IL-13/STAT6 pathway may be a target for intervention in these severe lung diseases. *In vitro*, IL-21 significantly increase IL-4R $\alpha$  and IL-13R $\alpha$ 1 expression in macrophages, resulting in increased *FIZZ1* mRNA and arginase-1 activity. These data suggest that the IL-21R is an important amplifier of alternative macrophage activation, and could have relevance in therapies for both inflammatory and chronic fibrotic diseases (56).

In addition, a Th2 pattern (characterized by IL-4 and IL-5 synthesis) predominates in the pulmonary interstitium in patients with cryptogenic fibrosing alveolitis (CFA), a fatal and rare inflammatory lung condition marked by excessive fibroblast activation, deposition of collagen and scar formation secondary to the remodeling of the extracellular matrix (57).

It has been previously described that cigarette smoking increases the number of macrophages that are functionally impaired, because reduce phagocytic activity, produce lower levels of pro-inflammatory cytokines, and their metabolic activity is weak (58).

In human peripheral blood, monocytes are differentiated into uncommitted macrophages (M0).

However, when they migrate to different tissues, due to the stimulation of antigens coupled with the cytokine microenvironment, polarization to M1 and M2 occurs, and they are identified as CD64+CD80+ and CD11b+CD209+, respectively. Activated M1 cells secrete IP-10, IFN- $\gamma$ , IL-8, TNF- $\alpha$  and IL-1 $\beta$ , whereas M2 cells secreted IL-13, CCL17, and CCL18 (45, 59).

Based on the applied stimuli and the achieved transcriptional changes, the M2 macrophages have been classified into subdivision: M2a, M2b, and M2c. (Figure 2). (60) (61) The M2a activation is a response to IL-4 and IL-13, the M2b to immune complexes and bacterial lipopolysaccharide (LPS), and the M2c to glucocorticoids and TGF- $\beta$ . (62) (63) Since the initial discovery of M2 macrophage activation, arginase-1, is considered as a prototypic M2 marker in mice (64). Arginase-1 is an enzyme of the urea cycle, which uses the amino acid L-arginine as a substrate to produce L-ornithine and urea. Initial studies on the function of M2, have emphasized that L-ornithine may enter polyamine and collagen biosynthesis, eventually promoting fibrosis and tissue healing (51).

Recently, epigenetic has been described as an important factor that participate in the macrophage polarization. Histone methyltransferases are associated with M2 activation by repressing M1 phenotype signature genes and promoting the transcription of M2 genes (65). For these reasons, the existence of epigenetic regulation of macrophages-related genes is not only essential for the induction of their activation and participation in the inflammatory process, but is also necessary for the inhibition of the immune response and avoidance of excessive inflammation and tissue damage (66).

## PARTICIPATION OF M2 AND TH2 CD4+ IN ILDS

Fibrotic lung disorders may have a M2 polarization background. The Th2 pathway with an elevated CCL-18 (marker of M2) concentration in the bronchoalveolar lavage fluid (BALF) is linked to fibrosis in ILDs (67).

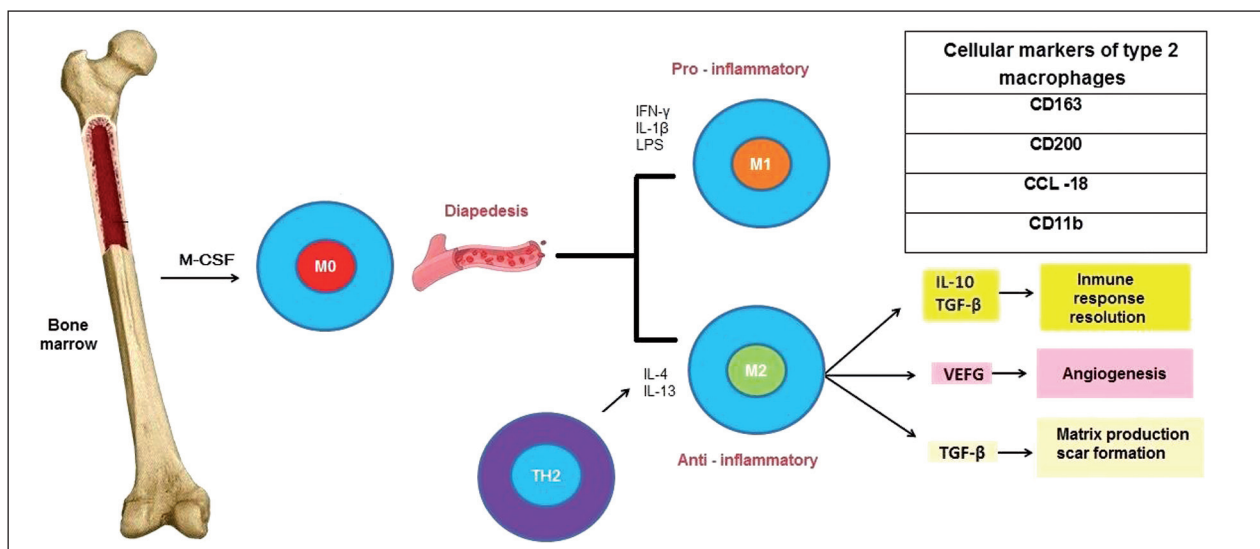
Pechkovsky et al showed in their study that BAL cells from patients with fibrotic lung disorders released higher amounts of marker proteins such as IL-1RA, CCL17, CCL18 and CCL22 compared to

**Table 2.** Principal characteristics of M2 and Th2 related genes

Gene	Encoded protein	Locus	Strand	Exons/ introns	Principal functions
<b>Type 2 Macrophages (M2)</b>					
<i>TGFB1</i>	TGF- $\beta$	19q13.2	Reverse	7/6	Regulates cell proliferation, differentiation and growth, and can modulate expression of other growth factors, including interferon gamma and tumor necrosis factor.
<i>IL10</i>	IL-10	1q32.1	Reverse	5/4	Down-regulates the expression of Th1 cytokines, MHC class II, and costimulatory molecules on macrophages. It also enhances B cell survival, proliferation and antibody production.
<i>VEGFA</i>	VEGF-A	6p21.1	Forward	9/8	Induces proliferation and migration of vascular endothelial cells, and is essential for both physiological and pathological angiogenesis.
<i>PPARG</i>	PPAR- $\alpha$	22.q13.31	Forward	14/13	For the acquisition and long-term maintenance of M2 phenotype this protein is requires. *
<i>CHI3L1</i>	Chitinase 3-like 1	1q32.1	Reverse	10/9	Chitinases catalyze the hydrolysis of chitin, which is an abundant glycopolymer found in insect exoskeletons and fungal cell walls. The protein is secreted by activated macrophages and plays a key role in inflammation and tissue remodeling.
<i>CD163</i>	CD163	12p13.31	Reverse	17/16	Member of the scavenger receptor cysteine-rich (SRCR) superfamily, and is exclusively expressed in monocytes and macrophages. It functions as an acute phase-regulated receptor involved in the clearance and endocytosis of hemoglobin/haptoglobin complexes by macrophages.
<i>MRC1</i>	MRC-1	10p12.33	Forward	30/29	The protein encoded by this gene is a type I membrane receptor that mediates the endocytosis of glycoproteins by macrophages.
<i>CD200</i>	CD200	3q13.2	Forward	8/7	The encoded protein plays an important role in immunosuppression and regulation of anti-tumor activity.
<i>TGM2</i>	TGM-2	20q11.23	Reverse	14/13	The protein encoded by this gene acts as a monomer, is induced by retinoic acid, and appears to be involved in apoptosis.
<i>ITGAM</i>	Mac-1; CD11b	16p11.2	Forward	30/29	This gene encodes the integrin alpha M chain and is necessary to form a leukocyte-specific integrin referred to as macrophage receptor 1 (Mac-1). It's important in the adherence of monocytes to stimulated endothelium.
<i>CD209</i>	CD209	19p13.2	Reverse	7/6	It's expressed in dendritic cells and macrophages. The encoded protein is involved in the innate immune system and recognizes numerous pathogens ranging to parasites to viruses.
<i>IL1R1</i>	IL-1R	2q11.2-q12.1	Forward	21/20	This gene encodes a cytokine receptor for IL-1 alpha, IL-1 beta and IL-1 receptor antagonist. It's an important mediator involved in many cytokine-induced immune and inflammatory responses.
<i>ARG1</i>	Arginase-1	6q32.2	Forward	8/7	Arginase-1 is an enzyme of the urea cycle. Initial studies on the function of M2, have emphasized that eventually this enzyme can promote fibrosis and tissue healing. †
<b>Th2 CD4+</b>					
<i>IL4</i>	IL-4	5q31.1	Forward	4/3	IL-4 has many biological roles, including the stimulation of activated B-cell and T-cell proliferation, and the differentiation of B cells into plasma cells, and it's an important factor to M2 differentiation.
<i>IL13</i>	IL-13	5q31.1	Forward	4/3	This cytokine is involved in several stages of B-cell maturation and differentiation. It up-regulates CD23 and MHC class II expression, and promotes IgE isotype switching of B cells. Also, this cytokine down-regulates macrophage activity.
<i>GATA3</i>	GATA-3	10p14	Forward	8/7	This gene is an important regulator of Th2 development and plays an important role in endothelial cell biology.

Data from Ensembl (48) and National Center for Biotechnology Information (49). \*Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance (50)

† Arginase: an emerging key player in the mammalian immune system (51)



**Fig. 2.** The polarization of type 2 macrophages is a process involving several molecules. The involvement of Th2 cells are critical for the activation of M2, through the secretion of IL-4, IL-13.

**Note:** Activated type 2 macrophages secrete molecules that have various functions in the human body such as resolution of the immune response, angiogenesis, scar formation. There are several cell markers to identify M2, which are shown in the table upper in figure.

**Abbreviation:** M-CSF, monocyte colony stimulating factor; M1, type 1 macrophages; M2, type 2 macrophages; M0, monocyte.

cells from healthy donors. Authors concluded that there is evidence for M2 polarization in fibrotic lung diseases. In addition, they showed that alveolar macrophages express markers related to M2, whereby they strongly support that alternative activation of macrophages is a pathologic mechanism in pulmonary fibrosis and that the potential manipulation of the M2 polarization process in alveolar macrophages is a highly interesting novel therapeutic approach to be analyzed (68). Otherwise, Paweł-Wojtan et al described that in BALF of patients with different ILDs there is no evidence of defined polarization of alveolar macrophages (AM). However, there is evidence of the important role of CD40+ cells in sarcoidosis and the role of CD163 positive cells in fibrotic diffuse lung diseases (M2 phenotype) (69).

In the healthy lung, at least two macrophage populations are present; AM and interstitial macrophages (IM). AM are located in the airway space and express high levels of CD11c and low levels of CD11b. These play a central role in recycling surfactant molecules produced by alveolar epithelial cells. IMs reside in the lung parenchyma, highly express CD11b, and have low surface expression of CD11c. IM are in the parenchyma to influence pulmonary fibrotic processes. However, there is a little

of information regarding their role in human lung fibrosis. Experimental model in mice, using bleomycin model, induces the fibrotic phase, IMs have been shown to acquire a pro-fibrotic phenotype, characterized by elevated expression of the M2 marker, CD206 (also known as mannose receptor) (70).

Likewise, in the normal injury-repair response macrophages readily acquire a phenotype which promotes fibroproliferation. AM in particular have been shown to be involved in extracellular matrix (ECM) processing through secretion of matrix metalloproteases or by direct uptake of collagen. (71) Besides the role of M2 in tissue repair and ECM remodeling, activated fibroblasts summon and stimulate macrophages by producing MCP-1, M-CSF and other chemokines, as well as activated macrophages secrete cytokines that attract and stimulate proliferation, survival and migration of fibroblast mediated by platelet-derived growth factor (PDGF). It has been demonstrated that AM recovered from idiopathic pulmonary fibrosis (IPF) patients spontaneously produce PDGF, which contribute to fibrotic process. (72, 73)

Studies realized with the administration of drugs in animal models have been helpful to elucidate pathologic mechanisms of fibrosis. For exam-

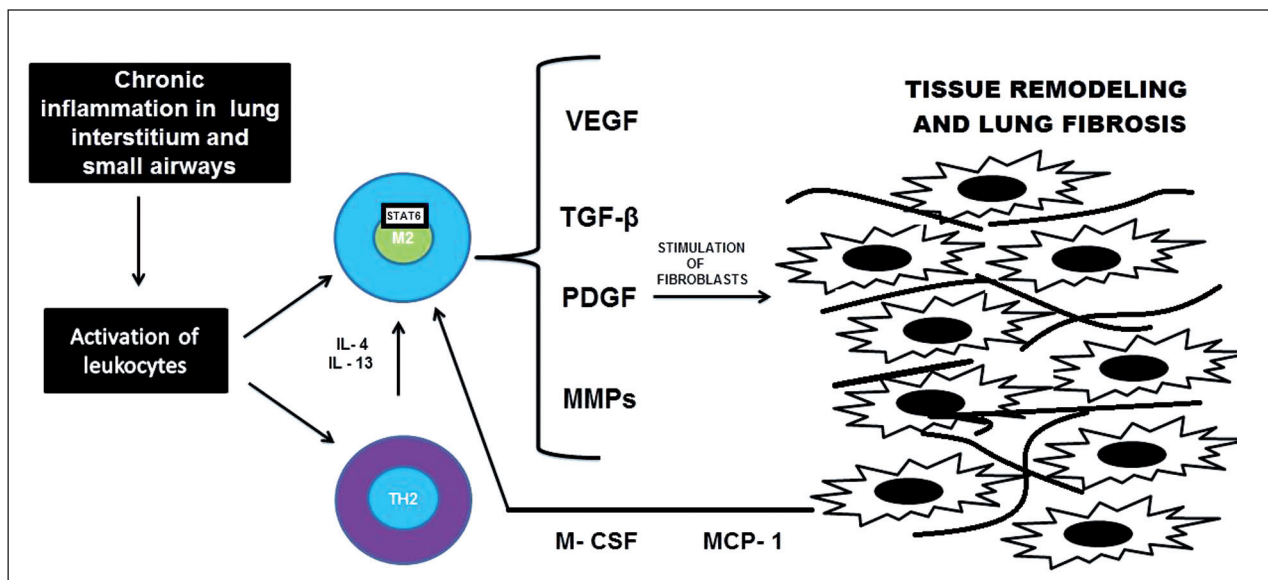
ple, Gibbons MA et al performed a study where they observed depletion of Ly6Chi circulating monocytes by systemic administration of Liposomal Clodronate (biphosphonate), after 5 days of Bleomycin administration, which resulted in reduced fibrotic responses in mice, as well as a lower number of M2 macrophages. These findings strongly suggest that the macrophages play an important role in the fibrosis process (74).

Zhou et al revealed in their study that Chitinase 3-Like 1 (*CHI3L1*) is elevated in the BAL of IPF patients and is expressed in pulmonary macrophages, concomitant with CCL18 and iNOS. In the murine model, Chi3l1 has been shown to play a protective role during the establishment of disease injury by reducing inflammatory responses and a profibrotic role in the tissue repair phase, characterized by M2 macrophage activation, fibroblast proliferation, and lung matrix deposition. (75)

In a study realized by Schupp JC et al, the results suggest that acute exacerbation in idiopathic pulmonary fibrosis is not an incidental event but rather driven by cellular mechanisms including M2

macrophage activation. Also, in patients with acute exacerbation of IPF levels of Chi3l1 are increased, which suggest a pathological role of M2 (75, 76).

Other studies realized in TGF- $\beta$  reported elevated BAL levels during pulmonary fibrosis, including in patients with IPF, sarcoidosis and in silicosis. The Overexpression of active TGF- $\beta$  in murine lungs originates pulmonary fibrosis, whence pulmonary macrophages appear to be crucial for the development of this process, because macrophage diminution ameliorates the disease pulmonary fibrosis (77, 78). Also, patients with Hermansky-Pudlak syndrome type 1 ([HPS-1] an autosomal recessive disorder), who have defective biogenesis of lysosome-related organelles, develop and accelerated form of progressive fibrotic lung disease, that is associated with increased alveolar macrophage activity. This is supported for a study realized by Rouhani et al who found higher levels of MCP-1, MIP-1a, GM-CSF, and M-CSF in epithelial lining fluid (ELF) in patients with HPS-1 than healthy subjects (79). Figure 3 shows the pathogenic mechanisms in fibrotic lung diseases, mediated by Th2 CD4+ cells and M2.



**Fig. 2. Figure 3.** M2 play an important role in the pathophysiology of ILDs, due to the secretion of certain molecules that induce changes at the lung level.

**Note.** The M2 are activated by IL-4 and IL-13. STAT6 is involved in the transcription of certain genes that induce the formation of molecules, especially PDGF that stimulates the proliferation of fibroblasts that cause a process of tissue remodeling and fibrosis in the lungs. MCP-1 and M-CSF induce a new cycle, stimulating the polarization of M2.

**Abbreviation:** M-CSF, monocyte colony stimulating factor; MCP-1, monocyte chemoattractant protein 1; PDGF platelet-derived growth factor; VEGF, vascular endothelial growth factor; MMPs, matrix metalloproteinases; TGF- $\beta$ , transforming growth factor beta.



## Conclusions

There are many studies that show an active role of type 2 macrophages in the pathogenesis of fibrotic diseases, including ILDs. However, as well as there are promising results, other studies do not have concrete results about this, becoming controversial results. Fibrotic process involves diverse types of cells, including Th2 CD4+, fibroblasts, Tregs CD4+ and activated macrophages by the alternative pathway, which have an anti-inflammatory function and profibrotic phenotype. Also, there are evidence that alveolar and interstitial macrophages, in fibrotic ILDs, has polarization to M2 and its products (TGF- $\beta$ , VEGF, Arg1, among others) play a key role in diseases development. Furthermore, functional studies are required to establish the role in normal conditions and in ILDs of M2 and Th2 CD4+ cells.

## REFERENCES

- Travis WD, Costabel U, Hansell DM, King TE, et al. An Official American Thoracic Society/European Respiratory Society Statement: Update of the International Multidisciplinary Classification of the Idiopathic Interstitial Pneumonias. *Am J Respir Crit Care Med* 2013; 188(6): 733-48.
- Mori S, Koga Y, Sugimoto M. Different risk factors between interstitial lung disease and airway disease in rheumatoid arthritis. *Respir Med* 2012; 106: 1591-9.
- Zhu L, Zhao Q, Yang T, Ding W, Zhao Y. Cellular Metabolism and Macrophage Functional Polarization. *Int Rev Immunol* 2015; 34(1): 82-100.
- Duthie F, O'sullivan ED, Hughes J. The Diverse Function of Macrophages in Renal Disease. *Kidney Int Rep* 2016; 1: 204-9.
- Van Dyken SJ, Locksley RM. Interleukin-4- and Interleukin-13-Mediated Alternatively Activated Macrophages: Roles in Homeostasis and Disease. *Annu Rev Immunol* 2013; 31(1): 317-43.
- Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment. 2014; 6: 1-13.
- Karakatsani A, Papakosta D, Rapti A, et al. Epidemiology of interstitial lung diseases in Greece. *Respir Med* 2009; 103(8): 1122-9.
- López-Campos JL, Rodríguez-Becerra E, Neumosur Task Group. Registry of Interstitial Lung Diseases. Incidence of interstitial lung diseases in the south of Spain 1998-2000: the RENIA study. *Eur J Epidemiol* 2004; 19(2): 155-61.
- Kreuter M, Herth FJF, Wacker M, et al. Exploring Clinical and Epidemiological Characteristics of Interstitial Lung Diseases: Rationale, Aims, and Design of a Nationwide Prospective Registry - The EXCITING-ILD Registry. *Biomed Res Int* 2015; 2015: 1-9.
- Schweisfurth H. Report by the Scientific Working Group for Therapy of Lung Diseases: German Fibrosis Register with initial results. *Pneumologie* 1996; 50(12): 899-901.
- Mejía M, Buendía-Roldán I, Mateos-Toledo H, et al. Primer Consenso Mexicano sobre Fibrosis Pulmonar Idiopática. *Neumol Cir Torax* 2016; 75(1): 32-51.
- Barreto-Rodríguez JO, Mejía M, Buendía-Roldán I. The importance of applying diagnostic criteria from consensus 2011 to diagnose idiopathic pulmonary fibrosis (IPF) in a referral site. *Pulm Crit Care Med Pulm Crit* 2016; 1(1): 11-4.
- Martínez-Briseño D, García-Sancho C, Fernández-Plata R, Franco-Marina F, Torre-Bouscuolet L, Pérez-Padilla JR. Tendencia de la mortalidad por enfermedades intersticiales en México, período 2000-2010. *Neumol Cir Torax* 2014; 73(3): 179-184.
- Kim DS, Collard HR, King TE, Jr. Classification and natural history of the idiopathic interstitial pneumonias. *Proc Am Thorac Soc* 2006; 3(4): 285-92.
- International S, Consensus M. American Thoracic Society American Thoracic Society/European Respiratory Society. International Multidisciplinary Consensus. Classification of the Idiopathic Interstitial Pneumonias. *Am J Respir Crit Care Med* 2002; 165: 277-304.
- Xaubert A, Marin-Arguedas A, Lario S, et al. Transforming Growth Factor- $\beta$  1 Gene Polymorphisms Are Associated with Disease Progression in Idiopathic Pulmonary Fibrosis. *Am J Respir Crit Care Med* 2003; 168(4): 431-5.
- Azmy RM, Helmy R, Helbawy E, Abd A, Dawood ER, Dahdaouh S El. Transforming growth factor  $\beta$ 1 polymorphism and serum levels in Egyptian patients with interstitial lung diseases. *Egypt J Chest Dis Tuberc* 2017; 66: 487-95.
- André P-A, Prêle CM, Vierkotten S, et al. BARD1 mediates TGF- $\beta$  signaling in pulmonary fibrosis. *Respir Res* 2015; 16(1): 118.
- Ates Ö, Müsellim B, Öngen G, Topal-Sarıkaya A. Association between "interleukin" 10 gene (IL10) polymorphisms and systemic sclerosis with interstitial lung involvement. *Rheumatol Int* 2008; 28(11): 1123-6.
- Scala E, Pallotta S, Frezzolini A, et al. Cytokine and chemokine levels in systemic sclerosis: relationship with cutaneous and internal organ involvement. *Clin Exp Immunol* 2004; 138(3): 540-6.
- Liu L, Dai H-P, Xiao B, Zhang S, Ban C-J, Xin P. Association of ENA-78, IP-10 and VEGF gene polymorphism with idiopathic pulmonary fibrosis. *Zhonghua Yi Xue Za Zhi* 2009; 89(38): 2690-4.
- Li C, Wei R, Jones-Hall YL, Vittal R, Zhang M, Liu W. Epidermal Growth Factor Receptor (EGFR) Pathway Genes and Interstitial Lung Disease: An Association Study. *Sci Rep* 2015; 4(1): 4893.
- Kudoh S, Kato H, Nishiwaki Y, et al. Interstitial Lung Disease in Japanese Patients with Lung Cancer. *Am J Respir Crit Care Med* 2008; 177(12): 1348-57.
- Yao J, Zhang T, Zhang L, Han K, Zhang L. *FOXP3* polymorphisms in interstitial lung disease among Chinese Han population: A genetic association study. *Clin Respir J* 2017.
- Cogan JD, Kropski JA, Zhao M, et al. Rare Variants in *RTEL1* Are Associated with Familial Interstitial Pneumonia. *Am J Respir Crit Care Med* 2015; 191(6): 646-55.
- Wolin A, Lahtela EL, Anttila V, et al. SNP Variants in Major Histocompatibility Complex Are Associated with Sarcoidosis Susceptibility—A Joint Analysis in Four European Populations. *Front Immunol* 2017; 8: 422.
- Morais A, Lima B, Peixoto MJ, Alves H, Marques A, Delgado L. *BTNL2* gene polymorphism associations with susceptibility and phenotype expression in sarcoidosis. 2012; 106: 1771-7.
- Maver A, Medica I, Salobir B, et al. Peroxisome proliferator-activated receptor gamma/Pro12Ala polymorphism and peroxisome proliferator-activated receptor gamma coactivator-1 alpha/Gly482Ser polymorphism in patients with sarcoidosis. *Sarcoidosis, Vasc Diffus lung Dis Off J WASOG* 2008; 25(1): 29-35.
- Aquino-Gálvez A, González-Ávila G, Pérez-Rodríguez M, et al. Analysis of heat shock protein 70 gene polymorphisms in Mexican patients with idiopathic pulmonary fibrosis. *BMC Pulm Med* 2015; 15: 129.
- Peljto AL, Selman M, Kim DS, et al. The MUC5B Promoter Polymorphism Is Associated With Idiopathic Pulmonary Fibrosis in a Mexican Cohort but Is Rare Among Asian Ancestries. *Chest* 2015; 147(2): 460-4.

31. Stuart BD, Choi J, Zaidi S, et al. Exome sequencing links mutations in PARN and RTEL1 with familial pulmonary fibrosis and telomere shortening. *Nat Genet* 2015; 47(5): 512-7.
32. Uh ST, Kim TH, Shim EY, et al. Angiotensin-Converting Enzyme (ACE) Gene Polymorphisms are Associated with Idiopathic Pulmonary Fibrosis. *Lung* 2013; 191(4): 345-51.
33. Li X, Li N, Ban C, Zhu M, Xiao B, Dai H. Idiopathic pulmonary fibrosis in relation to gene polymorphisms of transforming growth factor- $\beta$ 1 and plasminogen activator inhibitor 1. *Chin Med J* 2011; 124(13): 1923-7.
34. Zhang HP, Zou J, Xie P, Gao F, Mu HJ. Association of HLA and cytokine gene polymorphisms with idiopathic pulmonary fibrosis. *Kaohsiung J Med Sci* 2015; 31(12): 613-20.
35. Falfán-Valencia R, Camarena Á, Pineda CL, et al. Genetic susceptibility to multicase hypersensitivity pneumonitis is associated with the TNF-238 GG genotype of the promoter region and HLA-DRB1\*04 bearing HLA haplotypes. *Respir Med* 2014; 108(1): 211-7.
36. Camarena Á, Aquino-Galvez A, Falfán-Valencia R, et al. PSMB8 (LMP7) but not PSMB9 (LMP2) gene polymorphisms are associated to pigeon breeder's hypersensitivity pneumonitis. *Respir Med* 2010; 104(6): 889-94.
37. Chen Y, Fan XY, Jin YL, et al. Association between polymorphisms of interleukin-17A and interleukin-17F genes and silicosis susceptibility in Chinese Han people. *Asian Pac J Cancer Prev* 2014; 15(20): 8775-8.
38. Weng S, Wang L, Rong Y, et al. Effects of the Interactions between Dust Exposure and Genetic Polymorphisms in Nalp3, Caspase-1, and IL-1 $\beta$  on the Risk of Silicosis: A Case-Control Study. *PLoS One* 2015; 10(10): e0140952.
39. Shaw M, Collins BF, Ho LA, Raghu G. Rheumatoid Arthritis-Associated Lung Disease. *Eur Respir Rev* 2015; 24: 1-16.
40. Davies LC, Jenkins SJ, Allen JE, Taylor PR. Tissue-resident macrophages. *Nat Immunol* 2013 Sep; 14(10): 986-95.
41. Trouplin V, Bouchet N, Gorvel L, Conti F, Mottola G, Ghigo E. Bone Marrow-derived Macrophage. *J Vis Exp* 2013; 81: 1-6.
42. Jaguin M, Houlbert N, Fardel O, Lecqueur V. Polarization profiles of human M-CSF-generated macrophages and comparison of M1-markers in classically activated macrophages from GM-CSF and M-CSF origin. *Cell Immunol* 2013; 281(1): 51-61.
43. Dickinson JD, Alevy Y, Malvin NP, Patel KK, Gunsten SP, Holtzman MJ, et al. IL13 activates autophagy to regulate secretion in airway epithelial cells. *Autophagy* 2016; 12(2): 397-409.
44. Goenka S, Kaplan MH. Transcriptional regulation by STAT6. *Immunol Res* 2011; 50(1): 87-96.
45. Doherty T, Walford HH, Doherty TA. STAT6 and lung inflammation. *JAK-STAT* 2013; 2.
46. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, et al. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity* 2014; 41(1): 14-20.
47. Lawrence T, Natoli G. Transcriptional regulation of macrophage polarization: enabling diversity with identity. *Nat Rev Immunol* 2011; 11(11): 750-61.
48. Ensembl genome browser 89 [Internet]. [cited 2017 Aug 6]. Available from: <http://www.ensembl.org/index.html>
49. Home - Gene - NCBI [Internet]. [cited 2017 Aug 6]. Available from: <https://www.ncbi.nlm.nih.gov/gene/>
50. Odegaard JI, Ricardo-Gonzalez RR, Goforth MH, et al. Macrophage-specific PPAR $\gamma$  controls alternative activation and improves insulin resistance. *Nature* [Internet] 2007; 447(7148): 1116-20.
51. Munder M. Arginase: an emerging key player in the mammalian immune system. *Br J Pharmacol* 2009; 158(3): 638-51.
52. Doherty T, Broide D. Cytokines and growth factors in airway remodeling in asthma. *Curr Opin Immunol* 2007; 19(6): 676-80.
53. Fulkerson PC, Fischetti CA, Rothenberg ME. Eosinophils and CCR3 Regulate Interleukin-13 Transgene-Induced Pulmonary Remodeling. *Am J Pathol* 2006; 169(6): 2117-26.
54. Fichtner-Feigl S, Strober W, Kawakami K, Puri RK, Kitani A. IL-13 signaling through the IL-13 $\alpha$ 2 receptor is involved in induction of TGF- $\beta$ 1 production and fibrosis. *Nat Med* 2006; 12(1): 99-106.
55. Jakubzick C, Kunkel SL, Puri RK, Hogaboam CM. Therapeutic Targeting of IL-4- and IL-13-Responsive Cells in Pulmonary Fibrosis. *Immunol Res* 2004; 30(3): 339-50.
56. Pesce J, Kaviratne M, Ramalingam TR, et al. The IL-21 receptor augments Th2 effector function and alternative macrophage activation. *J Clin Invest* 2006; 116(7): 2044-55.
57. Wallace WAH, Ramage EA, Lamb D, Howie Department of pathology SEM. A type 2 (Th2-like) pattern of immune response predominates in the pulmonary interstitium of patients with cryptogenic fibrosing alveolitis (CFA). *Clin Exp Immunol* 1995; 101: 436-41.
58. Sopori M. Science and society: Effects of cigarette smoke on the immune system. *Nat Rev Immunol* 2002; 2(5): 372-7.
59. Tarique AA, Logan J, Thomas E, Holt PG, Sly PD, Fantino E. Phenotypic, Functional, and Plasticity Features of Classical and Alternatively Activated Human Macrophages. *Am J Respir Cell Mol Biol* 2015; 53(5): 676-88.
60. Colin S, Chinetti-Gbaguidi G, Staels B. Macrophage phenotypes in atherosclerosis. *Immunol Rev* 2014; 262(1): 153-66.
61. Ferrante CJ, Leibovich SJ. Regulation of Macrophage Polarization and Wound Healing. *Adv wound care* 2012; 1(1): 10-6.
62. Wang Q, Ni H, Lan L, Wei X, Xiang R, Wang Y. Fra-1 proto-oncogene regulates IL-6 expression in macrophages and promotes the generation of M2d macrophages. *Cell Res* 2010; 20(6): 701-12.
63. Ferrante CJ, Pinhal-Enfield G, Elson G, et al. The adenosine-dependent angiogenic switch of macrophages to an M2-like phenotype is independent of Interleukin-4 receptor alpha (IL4R $\alpha$ ) signaling. *Inflammation* 2013; 36(4): 921-931.
64. Stempin CC, Dulgerian LR, Garrido V V, Cerban FM. Arginase in parasitic infections: macrophage activation, immunosuppression, and intracellular signals. *J Biomed Biotechnol* 2010; 2010: 683485.
65. Kapellos TS, Iqbal AJ. Epigenetic Control of Macrophage Polarisation and Soluble Mediator Gene Expression during Inflammation. *Mediators Inflamm* 2016; 2016: 6591703.
66. Natoli G. Specialized Chromatin Patterns in the Control of Inflammatory Gene Expression. In: *Current topics in microbiology and immunology* 2011; 349: 61-72.
67. Renzoni E, Srihari V, Sestini P. Pathogenesis of idiopathic pulmonary fibrosis: review of recent findings. *F1000Prime Rep* 2014; 6: 69.
68. Pechkovsky D V., Prasse A, Kollert F, Engel KMY, Dentler J, Luttmann W, et al. Alternatively activated alveolar macrophages in pulmonary fibrosis—mediator production and intracellular signal transduction. *Clin Immunol* 2010; 137(1): 89-101.
69. Wojtan P, Mierzejewski M, Osińska I, Domagała-Kulawik J. Macrophage polarization in interstitial lung diseases. *Cent Eur J Immunol eur j Immunol* 2016; 41(412): 159-64.
70. Redente EF, Jacobsen KM, Solomon JJ, et al. Age and sex dimorphisms contribute to the severity of bleomycin-induced lung injury and fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2011; 301(4): L510-8.
71. Dancer RCA, Wood AM, Thickett DR. Metalloproteinases in idiopathic pulmonary fibrosis. *Eur Respir J* 2011; 38(6): 1461-7.
72. Wynn T. Cellular and molecular mechanisms of fibrosis. *J Pathol* 2008; 214(2): 199-210.
73. Trapnell BC, Crystal RG. Upregulation of Platelet-derived Growth Factor-A and -B Gene Expression in Alveolar Macrophages of Individuals with Idiopathic Pulmonary Fibrosis. *Clin Invest* [Internet] 1990; 85: 2023-7.
74. Gibbons MA, MacKinnon AC, Ramachandran P, et al. Ly6C hi Monocytes Direct Alternatively Activated Profibrotic Macrophage Regulation of Lung Fibrosis. *Am J Respir Crit Care Med* 2011; 184(5): 569-81.
75. Zhou Y, Peng H, Sun H, et al. Chitinase 3-Like 1 Suppresses Injury

- and Promotes Fibroproliferative Responses in Mammalian Lung Fibrosis. *Sci Transl Med* 2014; 6(240): 240ra76.
76. Schupp JC, Binder H, Jäger B, et al. Macrophage Activation in Acute Exacerbation of Idiopathic Pulmonary Fibrosis. *PLoS One* 2015; 10(1): e0116775.
77. Bonniaud P, Kolb M, Galt T, et al. Smad3 null mice develop airspace enlargement and are resistant to TGF-beta-mediated pulmonary fibrosis. *J Immunol* 2004; 173(3): 2099-108.
78. Murray LA, Chen Q, Kramer MS, et al. TGF-beta driven lung fibrosis is macrophage dependent and blocked by Serum amyloid P. *Int J Biochem Cell Biol* 2011; 43(1): 154-62.
79. Rouhani FN, Brantly ML, Markello TC, et al. Alveolar Macrophage Dysregulation in Hermansky-Pudlak Syndrome Type 1. *Am J Respir Crit Care Med* 2009; 180(11): 1114-21.