ImmunoTools *FlowISiAM* Award 2024

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Unveiling the Diagnostic Potential of Circulating Monocytes in Fibrosing Interstitial Lung Disease

Background

Interstitial lung disease (ILD) is a group of diffuse lung disorder characterized by a variable degree of inflammation and fibrosis of lung parenchyma and it is associated with substantial morbidity and mortality (1). Patients with predominantly fibrotic disease have a poor prognosis, with a median survival of only 5 years after being diagnosed with idiopathic pulmonary fibrosis (IPF) (2). While IPF is the prototypical progressive fibrosing ILD (F-ILD), a high proportion of patients with other ILD subtypes develop a F-ILD phenotype (3).

Often, pulmonary fibrosis can be definitively diagnosed only by examining a lung tissue biopsy (3). However, the clinical application of this invasive technique has been limited by relative complexity, cost, and risk of acute deterioration (3). Therefore, there is an urgent need to enhance the accuracy and reliability of pulmonary fibrosis diagnosis, monitor disease progression, and predict treatment outcomes through non-invasive approaches (3). One promising approach is the detection of fibrotic biomarkers (DNA, RNAs, proteins, peptides, metabolites, extracellular vesicles) in biological fluids like blood or bronchoalveolar lavage (BAL) (3).

Traditionally, fibrosis is defined as the excessive and pathologic deposition of extracellular matrix (ECM) during wound healing. F-ILD was believed to be mainly caused by repetitive injuries to the alveolar epithelium (4). This process induces epithelial dysregulation, promoting fibrosis and senescence (4). Moreover, cellular dysregulation may also occur in other cell types, including fibroblasts and endothelial cells (4). Several studies indicated that after tissue damage and reparative proliferation processes, these cells can secrete soluble factors that are released from the lung into blood.

In the epithelial context, recent evidence identifies mucins and integrins as key effectors in fibrotic processes observed in F-ILD (5,6). One of these, Krebs von den Lungen-6 (KL-6 or MUC1), has a pro-fibrotic and anti-apoptotic effects on lung fibroblasts (3,5). However, in recent years, several circulating mucins have been found to be elevated in IPF, including cancer antigen (CA) -125 (MUC16) (3,5). This marker is expressed in small amounts by pulmonary epithelium in states of health, but are secreted in abundance by epithelial cells in patients with F-ILD and are considered markers of epithelial damage (3,5). On the other hand, αvβ6 integrin is a cell surface protein found specifically on epithelial cells and is upregulated after injury, and data from humans found that high expression were present in the lungs of patients with IPF and were associated with worse outcomes (6).

In the endothelial context, endothelin-1 (ET-1) is a molecule mainly expressed by the vascular endothelium, and a profibrotic role has been described in patients with autoimmune diseases who develop F-ILD (7). In the fibroblast context, it's worth noting that the inflammatory response recruits these cells and activates a subset of cells called myofibroblasts, which deposit ECM in the form of collagen and other proteins (4). It has been reported that myofibroblasts express high levels of α-smooth muscle actin (α-SMA) and fibroblast activation protein (FAP), which are associated with inflammation and fibrosis in F-ILD (8,9).

Our research aims to explore a novel diagnostic avenue - the detection and quantification of KL-6, CA-125, ET-1, αvβ6 integrin, α-SMA and FAP within circulating monocytes. Monocytes play a crucial role in the immune system, with phagocytosis being one of their key functions (10). Phagocytosis, a process by which monocytes engulf and digest cellular debris and foreign substances, influences the differentiation potential and functional characteristics of monocytes (10). In the context of phagocytosis, monocytes can express markers that render them competent for phagocytosis, such as CD14, CD206, and CD163 (10). Moreover, CD209 has been identified as a marker associated with phagocytosis capacity and directly correlates with the amount of uptake particles (10).

Circulating monocytes have been suggested to play an important role in the pathogenesis of F-ILD (11). Aberrantly activated monocyte levels have been found in IPF patients and circulating monocytes can produce ECM leading to progressive pulmonary fibrosis (4). Moreover, murine models of F-ILD demonstrated that recruited monocyte-derived lung macrophages with activated phenotype ($CD14^+$ $CD163^+$ $CD206^+$ $CD209^+$) contribute to the progression of F-ILD (12-13).

Our proposed research will investigate the potential of these phagocyted damaged cells as a novel biomarker for F-ILD, aiming to enhance the diagnostic process and improve patient outcomes. This approach could revolutionize F-ILD diagnostics, providing a non-invasive, accurate, and reliable method for early detection and monitoring. Specifically, we aim to detect tumor antigens in circulating monocytes that originate from phagocyted damaged cells by tissue

macrophages. This could provide a more detailed understanding of the lung microenvironment and potentially lead to the development of new therapeutic strategies.

Aims

In this project we will investigate: the phenotype of monocytes and the extent to which monocytes that have phagocytosed damaged cells recirculate into the bloodstream and BAL. As a next step, we will investigate whether a monocyte-based diagnostic approach can be developed.

Experimental Design & Methods

1. Sample Collection: Collect peripheral blood samples and BAL from F-ILD patients. Ensure that the patients have been diagnosed without or with different stages of fibrosis to have a diverse sample set.
2. Mononuclear cell isolation and cytometry phenotyping from peripheral blood and BAL.
3. Phagocytosis Verification: Verify the phagocytosis process in monocytes by specific markers (CD163, CD206, CD209).
4. Antigens and KL-6 Detection: Detect peptides derived from tumor cells within the monocytes using flowISAM. Peptides could be obtained from: KL-6, CA-125, ET-1, αvβ6 integrin, α-SMA and FAP.
5. Data Analysis: Analyze the data collected from the antigen detection step. Look for patterns or correlations between the presence/absence of specific antigens and the stage or type of F-ILD.

Cooperation partner

The group of Dr. Osuna-Gómez and Dr. Castillo will work together with ImmunoTools to adjust the experimental and instrumental set-up to conduct *FlowISiAM* analysis at the IIB-Sant Pau (Barcelona). ImmunoTools and its partner SME, INVIGATE, will share specific know-how for computer-aided scoring from *FlowISiAM* raw data for optimal test results. ImmunoTools' partner SME, INVIGATE, will help on the task of developing peptide specific monoclonal antibodies and will support the initial evaluation. With our collaborators we will explore the identification of specific markers that could facilitate early detection of peptides from F-ILD in circulating cells by *FlowISiAM* testing. They envisage to create good preconditions for a joint research grant application.

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