PROJECT

Supervisor:	Angelo A. Manfredi
Title:	Role of Lung Megakaryocytes in models of Interstitial Lung Disease Associated with Experimental Systemic Sclerosis.
Curriculum:	BAIO
Link to the personal page of the University or relevant hospital site website:	https://research.hsr.it/en/divisions/division-of-immunology-transplantation-and-infectious-diseases/autoimmunity-and-vascular-inflammation/angelo-manfredi.html

Description of the Project (max 3,000 characters including spaces)

Background/gap of knowledge Systemic sclerosis (SSc) is a complex autoimmune disease characterized by widespread vascular abnormalities and progressive fibrosis affecting the skin, lungs, and internal organs. Despite ongoing research, therapeutic options remain limited, highlighting the critical need for translational studies that bridge basic scientific discoveries with clinical application. This is especially important given that interstitial lung disease (ILD) is the leading cause of mortality in patients with SSc.

Extracellular vesicles (EVs) are membrane-bound particles released by cells during activation or stress. EVs derived from platelets and from megakaryocytes are elevated in the circulation of SSc patients and are biologically active, contributing to hallmark features of the disease, including lung fibrosis. Their pathogenicity is at least partially mediated by the presentation of HMGB1—a potent danger-associated molecular pattern (DAMP)—to neutrophils and vascular cells.

Previous studies have shown that EVs can induce lung fibrosis in a relatively short time frame. Importantly, we observed that the concentration of lung megakaryocyte-derived EVs is

significantly higher in SSc patients with ILD compared to those without, suggesting a potential link between lung-resident megakaryocytes and fibrotic lung involvement in SSc.

Rationale and hypothesis Megakaryocytes (MKs) are traditionally known as precursors of platelets and are predominantly located in the bone marrow. However, MKs have also been identified in peripheral organs such as the lungs, liver, and spleen. While pulmonary MKs were first noted in the late 19th century, only recently have they been shown to actively produce platelets. Furthermore, lung-resident MKs exhibit a distinct immunomodulatory phenotype compared to their bone marrow counterparts, including the expression and release of higher levels of immune molecules. Despite this growing body of evidence, the origin and functional roles of lung megakaryocytes—particularly in the context of disease—remain poorly understood. Emerging data suggest these cells may play an immunoregulatory role, with potential implications in inflammation and fibrosis.

In this project, we aim to investigate the role of lung-resident megakaryocytes and their released EVs as mediators of lung injury in SSc-associated ILD. We hypothesize that lung megakaryocytes contribute to pulmonary fibrosis through the release of immunologically active EVs that promote inflammatory and fibrotic responses in the lung microenvironment.

Objectives The main goal of this project is to investigate the role of megakaryocytes (MKs) and their derived extracellular vesicles (MK-EVs) in the development of interstitial lung disease (ILD), with a particular focus on their contribution to lung fibrosis. This will be addressed using three complementary murine models of lung fibrosis. In each model, we will compare lung-resident MKs (MKs^L) to bone marrow MKs (MKs^BM), evaluate circulating MK-EVs and cytokines, and assess the involvement of HMGB1 as a key molecular effector. Animals will be longitudinally monitored from 3 to 18 months of age to detect the onset and progression of ILD. MKs^L and MKs^BM will be characterized by immunohistochemistry and RNA sequencing of paraffin-embedded sections. The concentration of circulating MK-EVs and cytokines (e.g., IL-1beta, IL-6, IL-8, IL-17, TNF-α, TGF-β) will be quantified by flow cytometry (legendPlex system) and ELISA. Expression of HMGB1 in MKs and MK-EVs will be assessed using conventional flow cytometry and ImageStream technology.

MKs^L and MKs^BM will be isolated following established protocols (Pariser DN, et al) for functional analysis of platelet and EV release, as well as transcriptomic profiling. C57BL/6J mice (Jackson Laboratory) will serve as controls across all models.

Specific aims

Aim 1: **Characterize MKs^L and MK-EVs in spontaneous ILD development using PSGL-1 knockout mice.** We will analyze PSGL-1 KO mice (B6.129-Selplgtm1Rpmc/J), which develop an SSc-like autoimmune phenotype and spontaneous ILD in ~60% of cases by 12 months of age. Lung, bone marrow, MKs^L, MKs^BM, and plasma samples will be collected at 3, 6, 12, and 18 months to evaluate fibrosis, MK profiles, MK-EVs, and cytokine levels.

Aim 2: **Investigate the dynamics of MKs and MK-EVs in bleomycin-induced lung fibrosis**. Pulmonary fibrosis will be induced in C57BL/6J mice by intranasal administration of bleomycin sulfate (0.1 mg in 50 µL/mouse), as previously described (Han X, Wang SM). Lung, BM, and blood samples will be analyzed at 7 and 14 days, and at 1 and 6 months post-treatment to assess fibrosis and MK-related changes over time.

Aim 3: **Assess the specific contribution of HMGB1 from MKs in fibrosis using HMGB1-deficient mice.** To directly test the role of HMGB1 in MK-mediated fibrosis, we will use PF4^cre/HMGB1^flox mice, which lack HMGB1 in the megakaryocytic lineage. Bleomycin-induced lung fibrosis will be performed as in Aim 2. The fibrotic response, MKs^L/MKs^BM profiles, MK-EVs, and inflammatory markers will be compared to wild-type controls to dissect the role of MK-derived HMGB1 in disease pathogenesis.

Expected outcomes

- Characterization of lung-resident megakaryocytes (MKs^L) in health and disease, including their transcriptomic and immunologic profiles compared to bone marrow MKs

- Establishment of MK-derived EVs as potential mediators of lung fibrosis, including their dynamics across disease stages and models

- Demonstration of HMGB1's involvement in MK-EV-mediated lung fibrosis, providing mechanistic insights and potentially identifying new therapeutic targets

- Development of robust techniques for isolating and analysing MKs and EVs from murine tissues, applicable to future studies

- Generation of high-impact publications and novel data to support translational research into systemic sclerosis and fibrotic lung diseases

Timeline

Year 1 – Method Optimization and Baseline Characterization

Establishment and validation of animal models (PSGL-1 KO, bleomycin-treated, and HMGB1-deficient mice). Standardization of MKs^L and MKs^BM isolation and characterization protocols. Optimization of EV isolation and flow cytometry/ImageStream analyses. Collection of baseline data in control and early-stage (3- and 6-month) PSGL-1 KO mice. Initial bleomycin treatments and short-term analysis (7–14 days) in WT mice. Begin HMGB1 knockout colony expansion and genotyping

Year 2 – Longitudinal Sampling and In-Depth Analysis

Longitudinal sampling at 12 and 18 months in PSGL-1 KO mice. Medium- and long-term post-bleomycin assessments (1–6 months) in WT and HMGB1-deficient mice. Comparative transcriptomic profiling (RNA-seq) of MKs^L and MKs^BM from each model. Quantification of cytokines and HMGB1 in plasma and MK-EVs. Functional studies of EV bioactivity and platelet generation capacity. Data analysis and identification of disease-specific signatures

Year 3 – Integration, Validation, and Manuscript Preparation

Integration of data across models to identify conserved mechanisms. Correlation of MK-EV features with fibrotic progression and cytokine profiles. Functional validation (e.g., in vitro neutrophil or endothelial cell assays, if applicable). Preparation of manuscripts for publication. Final thesis writing and submission. Presentation of findings at scientific conferences

Skills that the student should acquire (max. 600 characters including spaces):

The student will be trained to independently manage animal models and maintain required mouse lineages, including completing certified animal house training. He/She will acquire skills in organ isolation, histology, flow and ImageStream cytometry, RNAseq and transcriptomic analysis, and MK isolation from lung and bone marrow. He/She will also gain experience in experimental design, data analysis, and interpretation using appropriate statistical tools

References (max. 15)

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- Platelet microparticles sustain autophagy-associated activation of neutrophils in systemic sclerosis. Maugeri N, et al. Sci Transl Med. 2018 Jul 25;10(451):eaao3089. doi: 10.1126/scitranslmed.aao3089.
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- Lung megakaryocytes are immune modulatory cells. Pariser DN, et al. J Clin Invest. 2021 Jan 4;131(1):e137377. doi: 10.1172/JCI137377.
- Deficiency of Psgl-1 accelerates bleomycin (BLM)-induced lung fibrosis and inflammation in mice through activating PI3K/AKT. Han X, Wang SM. Biochem Biophys Res Commun. 2017 Sep 16;491(2):558-565. doi: 10.1016/j.bbrc.2017.03.003