ImmunoTools *special* Award 2019

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Targeting specific myeloid cell populations in fatal multiorgan inflammation and fibrogenesis in systemic sclerosis

Background:

Systemic Sclerosis (SSc) is a complex autoimmune disease with a chronic and progressive course. The pathogenesis is dominated by activation of innate and adaptive immunity with distinct autoantibodies production, vascular changes and fibrosis of skin and internal organs. Although skin fibrosis is the distinguishing criterion, the pathological changes in lungs, heart, kidneys and intestinal tract determine the clinical outcome and poor prognosis. Fibrogenesis is a multistage process, seen as the result of deregulated tissue repair responses, in which aberrantly sustained production of cytokines, growth factors and angiogenic factors tilt tissue homeostasis towards excessive accumulation of extracellular matrix (ECM), activation of fibroblasts and differentiation into pathological ECM-secreting myofibroblasts.

Dysregulated innate and adaptive immune responses are major contributors in the progression of fibrotic diseases. Myeloid cells, as part of innate immunity, are the first responders in case of an organ/tissue injury. Acute inflammatory reactions are important in triggering fibrosis in many different organ systems. Circulating monocytes infiltrate the internal organs in SSc and play a central role in activating and propagating acute inflammation responses followed by pathological fibrosis and organ dysfunction, also in SSc.

Objective

With this project we aim to perform a comprehensive mapping of myeloid lineage cells in multiple organ inflammation and to identify rare pathologic cells on a single-cell level with high dimensional analysis in mouse models and patient-derived cells/tissues. Such complex

data will be processed by mathematical algorithms and combined with clinical observations of patients with SSc.

Project description

Characterization of macrophages using the Fra-2 transgenic mouse model:

In a first step, bone marrow derived macrophages differentiated *in vitro* with murine M-CSF and peritoneal macrophages (PM) from the Fra-2 transgenic (tg) mouse model will be characterized using myeloid specific markers from ImmunoTools. The isolated macrophages will be polarized *in vitro* with LPS or IL-4 to classically activated M1 resp. alternatively activated M2 macrophages to investigate their response to pro- and anti-inflammatory stimuli. The macrophage cytokine profile will be assessed using standard ELISA kits from ImmunoTools for macrophage specific cytokines, such as IL-6 and TNF-α.

In parallel, we would like to phenotype and identify different myeloid populations of Fra-2 tg mice and litter mate controls in organs affected in systemic sclerosis (SSc), such as the heart, lung and skin, to name a few. The myeloid lineage mapping will be performed using various myeloid specific markers from ImmunoTools. Based on the analysis, cell populations of interest will be selected and sorted to perform functional *in vitro* assays, including the detection of pro-inflammatory cytokine release by standard ELISAs from ImmunoTools. The specific myeloid populations will be further characterized by single cell RNA-sequencing to study differences on the gene expression level.

Characterization of macrophages from SSc patients:

Finally, we want to correlate our findings from the Fra-2 tg animal model with human data using blood, lung, skin and heart biopsies from SSc patients and healthy controls. The phenotype of the human myeloid cells in blood will be characterized by flow cytometry and the same functional *in vitro* assays will be performed to evaluate the heterogeneity of myeloid populations. The phenotype of the human myeloid cells in skin, lung and heart biopsies will be evaluated by special transcriptomics and ChipCytometry to identify deregulated genes and proteins at the single cell level in intact human tissues.

Expected outcome

The contribution of innate immune cells, such as myeloid cells, to the development and progression of SSc is still not fully understood. We hope to describe the so far unknown characteristics of myeloid cells in different organs involved in fatal multi organ inflammation. We assume that our findings will serve as a basis for developing new diagnostic tools and further for personalized, precise and effective therapies tailored to the individual patient, decreasing the economic burden and improving the quality of daily life.

Reagents: **ImmunoTools** reagents helpful in conducting the above-mentioned studies.

ImmunoTools *special* AWARD for **Amela Majdancic** includes 25 reagents:

mouse ELISA-sets IL-6, TNFalpha

recombinant mouse cytokines: rm M-CSF, rm IFNgamma, rm IL-4

FITC - conjugated anti-mouse CD45

PE - conjugated anti-mouse CD19, CD80

APC - conjugated anti-mouse CD11b

human ELISA-sets IL-6, TNFalpha

recombinant human cytokines: rh IL-4, rh M-CSF

DETAILS more AWARDS