ImmunoTools *special* Award 2018

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Activation and dysfunction of cardiac endothelium by autoreactive CD4$^+$ T helper cell subsets

Background

Mechanisms controlling induction and progression of inflammatory heart diseases are poorly understood. Endothelial and microvascular endothelial cells (MVECs) lining of capillaries and microcapillaries are the key cellular component of the endothelial barrier and represent highly selective permeability system that controls entry of blood proteins and cells into the tissue. During inflammation, endothelial cells become activated and acquire new properties. The ability to interact with circulating leukocytes and the decrease of endothelial barrier function represent the most prominent features of activated endothelium. Although microvascular endothelial activation is an important step in the induction and progression of tissue-specific inflammation, surprisingly little is known about activation of MVECs in inflammatory heart diseases.

Effector heart-specific CD4$^+$ T lymphocytes play important role in the development of the heart muscle inflammation - myocarditis. Activation of naïve CD4$^+$ T cells through T cell receptor leads to expansion of effector pool of antigen-specific effector lymphocytes. During activation, proinflammatory cytokines polarize effector CD4$^+$ T cells into the specific T helper (Th) subtypes Th1, Th2, Th17 or Th22. These specific Th subsets produce various cytokines and chemokines, which have been implicated in different aspects of autoimmune diseases. Although Th cells orchestrate most aspects of the immune response in autoimmune myocarditis, their role in endothelial activation and subsequent recruitment of immune cells remains largely speculative.

Hypothesis

Autoreactive CD4$^+$ T cells are critically involved in the activation of endothelium initiating subsequent inflammatory response. This process is mediated mostly by tumor necrosis factor α (TNF-α) secreted by Th cells, but other cytokines play modulatory role in it.

Objectives

TNF-α represents the best characterized cytokine involved in endothelial activation and dysfunction. During immune response, TNF-α is produced mainly by $CD4^+$ T cells and monocytes, but also by endothelial cells. Our aim is to compare how different subsets of effector $CD4^+$ T cells (Th1, Th2, Th17 and Th22) affect activation and dysfunction of MVECs. Using TNF-α deficient Th cells, we will specifically address the role of TNF-α produced by $CD4^+$ T cells in the context of endothelial activation.

Project description

Primary mouse MVECs will be stimulated with TNF-α (positive control) or with different subsets of activated T-lymphocytes isolated from Balb/c mice. Lymphocytes will be sorted using flow cytometry as effector ($CD4^+CD44^+CD62L^-CD25^-$) and naïve ($CD4^+CD44^+CD62L^+CD25^-$) populations. Naïve lymphocytes will be used to generate Th1, Th2, Th17 and Th22 subsets following standard protocols by using the specific combinations of antibodies and cytokines. Th cells will be stimulated using anti-CD3/CD28 beads alone (to generate conditioned media) or directly in co-cultures with MVECs. In the alternative approach, we will use $CD4^+$ T cells isolated from the TCR-M transgenic mice, which express transgenic T cell receptor recognizing exclusively heart-specific alpha myosin heavy chain peptide (α-MyHC) antigen. In these approaches, TCR-M $CD4^+$ T cells will be stimulated with α-MyHC antigen in the presence of antigen presenting cells.

Activation of MVECs will be measured as an increase in surface expression of adhesion molecules (ICAM-1, VCAM and P-selectin) by flow cytometry. To address MVEC activation in the functional assay, we will employ the BioFlux system, which produces constant shear flow with electropneumatic pumps in microcapillaries and is combined with the fluorescence microscope. Using the BioFlux system we will analyse and quantify adhesion (and transmigration) of fluorescence-labelled leukocytes to activated MVECs growing in microcapillaries. Furthermore, measurements of NO and ROS using the specific fluorogenic probes will be applied to address endothelial dysfunction of MVECs. Cytokines produced by lymphocytes and endothelial cells will be measured using ELISA.

Reagents provided by the ImmunoTools will be used to differentiate $CD4^+$ naïve T cells into the specific lineages (Th subsets) and for stimulation of MVECs. Labeled antibodies will be used for flow cytometry analysis and for sorting of lymphocytes.

Expected relevance

Our aim is to identify which subset of Th lymphocytes and which cytokine profile cause activation and dysfunction of MVECs. This will allow to identify molecular mediators of these pathogenic processes. Obtained results will broaden our understanding of autoimmune processes in the heart and will open new perspectives for development of novel treatment strategies against inflammatory-mediated heart diseases in the future.

ImmunoTools *special* AWARD for **Filip Rolski** includes 25 reagents

FITC - conjugated anti- mouse CD3, CD44, CD134

PE - conjugated anti- mouse CD62L, CD80

APC - conjugated anti- mouse CD4, CD11b, CD19

recombinant mouse cytokines**:** rm Il-13, rm IFN gamma, rm IL-4, rm IL-6, rm IL-17A, rm VEGFA, rm IL-11, rm IL-22

mouse ELISA-set (for one 96 plate): rm TNF-a, rm IL-17A

recombinant human cytokines**:** rh IL-11

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