PROJECT 1

DoS: Angelo A. Manfredi

Title: Mitochondrial DAMPs in vascular inflammation

Curriculum: Basic and Applied Immunology and Oncology

Link to OSR/UniSR personal page:
https://research.hsr.it/en/divisions/division-of-immunology-transplantation-and-infectious-diseases/autoimmunity-and-vascular-inflammation/angelo-manfredi.html

Project description (*Number of characters*, *including spaces*: 2.000 - 3.000):

Background and biological questions. Immunomodulatory therapies for systemic autoimmune diseases characterized by extensive vascular inflammation such as systemic sclerosis and systemic lupus erythematosus have so far yielded disappointing results, probably due to our lack of information on the causes and the mechanisms that maintain and amplify the disease. Recently, we have learned that the mitochondria, organelles that derive from eubacteria that initiated a symbiosis with ancestral host cells produce, can be extruded from eukaryotic cells after extreme stress. Extracellular mitochondria act as potent Damage Associated Molecular Patterns (DAMPs) since they express microbial-like mediators, such as DNA with unmethylated CpG motifs, N-formylated proteins and lipids, which in turn activate pattern recognition receptors on leukocytes. Thus mitochondria outside the cell are seen as bacteria from the cells of the innate immune system and cause robust inflammation.

Hypothesis.Based on preliminary results, we propose that mitochondria are released in the blood of autoimmune patients as a consequence of initial vascular injury, mostly from endogenous sources such as platelets and endothelial cells. As a consequence of a relative failure in their early local and systemic clearance they accumulate in the blood, activate leuckocytes triggering the production of reactive oxygen species, cytokines and neutrophil extracellular traps, influence coagulation, alter the endothelial barriers and eventually sustain maladaptive tissue remodelling with loss of organ function.

Experimental system. Mitochondria will be purified from platelets and endothelial cells, characterized phenotypically and functionally in vitro, verified for their ability to cause inflammation, autoimmunity and tissue remodeling after injection in experimental mice, in the absence or in the presence of signals that feed or hinder their clearance.

Aims. Goal of this project is to obtain evidence that the mitochondria: I. are sufficient to induce systemic vascular inflammation, purifying and characterizing them upon injection in immunodeficient mice; II. are necessary, decreasing the severity of experimental autoimmunity by interfering with the release of mitochondria or accelerating their clearance; III. can directly influence with intravascular homeostasis, regulating the generation of thrombin and the activation state and the function of peripheral leukocytes, neutrophils in particular.

Skills to be acquired by the student:

Flow cytometric, biochemical and functional characterization of human and mouse mitochondria isolated from various sources (platelets, parenchymal tissues). Handling of immune-deficient mouse system for xenotransplantation of mitochondria. In vivo assessment of experimental autoimmunity and vascular inflammation. Experiment design and implementation, critical analysis of experimental data.

References (max. 3)

Maugeri N, Capobianco A, Rovere-Querini P, Ramirez GA, Tombetti E, Valle PD, Monno A, D'Alberti V, Gasparri AM, Franchini S, D'Angelo A, Bianchi ME, Manfredi AA. Platelet microparticles sustain autophagy-associated activation of neutrophils in systemic sclerosis. *Sci Transl Med*. 2018 Jul 25;10(451). pii: eaao3089. doi: 10.1126/scitranslmed.aao3089.

Manfredi, A.A., Rovere-Querini, P. The mitochondrion - A Trojan horse that kicks off inflammation? (2010) *New England Journal of Medicine,* 362 (22), pp. 2132-213.