DESCRIPTION FOR THE GENERAL PUBLIC

Inflammatory responses are critical for the immune system of every human being. Inflammation occurs when homeostasis of the organism is compromised by infection with a pathogenic microbe, tissue damage or autoinflammatory disease and its purpose is to alert the immune system and stimulate it to overcome the danger. Excessive inflammatory processes are dangerous (lead to development of chronic inflammatory diseases, e.g. Alzheimer's, arthritis, periodontitis and many others) and need to be tightly controlled. Inflammation is primarily developed by phagocytic cells: macrophages and dendritic cells. Studying inflammatory reactions of these cells at a molecular level allows for better understanding of the roles of the immune system (in particular innate immune system) and designing new targeted therapies. Inflammatory responses begin with activation of intracellular NOD-like receptors, which assemble in multimolecular enzyme complexes called inflammasomes. These in turn are responsible for processing and release of pro-inflammatory cytokines - mediators that alert and activate other cells. Different inflammasomes undergo strict and multilevel regulation processes which are incompletely understood. NLRP3 is the most universal of them.

NLRP3 is activated in a "canonical" way by many signals associated with danger and pathogens. Recently, also a novel caspase-4/5-dependent NLRP3 inflammasome activation process was described. This non-canonical inflammasome was found to regulate response towards intracellular, cytoplasmic bacterial pathogens, and inflammatory cell death (pyroptosis), but we are only beginning to understand what physiological and molecular processes this inflammasome regulates and what their implications are.

Extracellular vesicles are very small (<1 micrometer) membranous structures released from nearly all living cells, serving as messengers connecting the distant cells and organs. In human body, different cells secrete vesicles of different size and composition, what in consequence determines their specific biological roles. This is particularly important in the field of innate immunity, where immune system cells utilize vesicles as a system for quick, targeted and reliable communication to co-ordinate early responses to the danger. Extracellular vesicles are also secreted from inflammatory cells at many different conditions, although we are far from full understanding the regulatory mechanisms of their release and their roles in this process.

The project proposes to accurately determine the protein composition of extracellular vesicles secreted by human dendritic cells (primary antigen-presenting cells) and macrophages (inflammation-developing cells) upon canonical and non-canonical inflammasome activation , using mass-spectrometry-based proteomics. It is a technique to identify and quantify thousands of proteins from one biological sample. Analyzing the protein composition of the vesicles secreted under different conditions (homeostatic cells vs non-canonical and canonical inflammasome-activated cells) will allow for understanding the purposes of regulated protein secretion in the studied context, especially by identifying signaling proteins that can be delivered and activate or inhibit target cells.

The identified proteins will be subjected to bioinformatic analyses. In my previous studies I showed how bioinformatics and proteomics can be used in tandem as a system-level approach for identification and characterization of novel regulatory pathways. This methodology allows for putting large amounts of data into statistically backed biological context. This allows for tracing back the biogenesis, cargo selection and mechanisms of release of extracellular vesicles, thus providing excellent tools for discovering of previously unknown processes governing the phenomenon of vesiculation. In the context of novel, non-canonical inflammasome activation, we will attempt to discover and characterize novel molecular players that govern the signaling cascades and determine how they influence the protein composition of secreted vesicles. This will pave the way towards better understanding of both the biogenesis and biological roles of extracellular vesicles during novel inflammasome activation conditions. It will also provide a basis for future detailed studies on the crosstalk between vesiculation and inflammation in innate immunity.