GENERAL PUBLIC DESCRIPTION

The etiology of atherosclerosis, in particular the factors inducing its early stages (inflammation in arterial wall) are not well understood. The “infection hypothesis” states that bacterial and viral factors, classically associated with transient infections, may circulate in the blood, contributing to atherosclerosis development, however, this interdependence is not thoroughly studied. It has been observed that patients with leaky gut syndrome (in which microbial flora may reach the bloodstream through compromised gut barriers) and periodontal diseases (where dental plaque bacteria damage the gums and access the bloodstream) are more prone to atherosclerosis and related cardiovascular diseases.

The cells of vascular endothelium, although not considered as immune system cells, are equipped with primary mechanisms allowing for sensing the pathogenic factors and alarming the specialized immune cells. Their exact roles in development of inflammatory responses in vascular wall are not fully established. We hypothesize that small outer membrane vesicles (OMV) produced by Gram-negative bacteria, carrying lipopolysaccharide (LPS) can be taken up by endothelial cells exerting inflammation and secretion of immunomodulatory extracellular vesicles (EV).

In leukocytes, the inflammatory process begins with activation of intracellular receptors called NOD-like receptors (NLR), which upon sensing the signal form large molecular platforms with enzymatic activity. Activated inflammasomes are critical for processing the pro-inflammatory cytokines (IL-1β and IL-18) – small proteins that are secreted to alarm and activate various other cells and co-ordinate immune responses. Several inflammasomes are known to exist, each of them is subject to strict molecular regulation, however we have still not fully elucidated their functions. NLRP3 is the best characterized inflammasome. Recent years provided novel insights into mechanisms of NLRP3 activation, which is dependent on caspases-4/5. This so called “non-canonical inflammasome” turned out to mediate critical functions in intracellular bacteria detection, reacting to the lipopolysaccharide (LPS) in cellular cytosol, delivered e.g. by OMV. Caspases-4/5 play critical roles also in pyroptosis (pro-inflammatory cell death), although we are only at the beginning of our way to fully understand the implications these novel discoveries impose.

Our preliminary results indicate that these cells express caspase-4 and are capable of sensing the OMV and secrete proinflammatory IL-18. This detection correlates with secretion of EV. We hypothesize that these immunomodulatory EV are capable of transferring pro-inflammatory signals to local macrophages, which are essential in early atherogenesis.

In the proposed studies we plan to apply mass spectrometry (MS) combined with bioinformatics for in-depth analysis of protein secreted on extracellular vescicles of endothelial cells upon intracellular LPS delivery and OMV. The analysis of modulation of vesicle secretion during caspase-4-mediated response will allow for identification of biological processes driving the immune responses and intracellular signaling involved in regulation of endothelial inflammation. The study may reveal novel cellular responses in early stages of atherosclerosis, and propose novel targets for antiatherosclerotic therapies. Demonstrating that OMV (circulating in the blood of patients with leaky gut and periodontosis) may activate caspase-4 and IL-18 secretion in vascular endothelium cells would be a major breakthrough in understanding vascular inflammatory mechanisms and explain why leaky gut or periodontitis patients frequently develop atherosclerosis. Endothelial extracellular vesicles may be the signaling factors driving atherogenesis. Our studies have the potential to reveal novel mechanisms that may help us understand the observed correlation between intestinal dysbiosis, endotoxemia and periodontosis on one hand and increased risk of cardiovascular diseases, and fundamentally contribute to development of novel antiatherosclerotic therapies.