Inflammation is inevitable for the human body to fight invading pathogens. In many cases immune cells work adequately to the threat, however, exaggerated pro- or anti-inflammatory reactions can lead to “cytokine storm” or immunoparalysis. This “life-threatening condition that arises when the body’s response to infection damages the host’s tissues” is defined as sepsis which according to WHO accounts for almost 20% of global deaths. Nowadays, during the COVID-19 pandemic among severely ill patients are also those who meet the criteria of sepsis. Therefore, efforts are undertaken to find reliable sepsis biomarkers enabling the identification of high-risk patients and to find new approaches to successfully treat the disease.

Among proteins playing important role in the development of sepsis and other inflammation-related diseases is CD14, a protein expressed predominantly on the surface of macrophages and present in a soluble form in body fluids. CD14 is involved in the activation of immune responses mainly to molecules of bacterial origin, like lipopolysaccharide (LPS). Recent studies have also linked CD14 with an inflammation induced by oxidized phospholipids (oxPAPCs) released by damaged tissues of the host. CD14 works as a transporter: when located on the cell surface binds molecules and transfers them to receptors which next initiate pro-inflammatory responses. Thus, CD14 delivers LPS to Toll-like receptor 4 (TLR4) and induces endocytosis of the activated receptor allowing the production of cytokines. oxPAPCs are transported by CD14 to the cell interior where they activate caspase -11 and the so-called inflammasome. This protein complex triggers the production of cytokines of the IL-1 family and activates gasdermin D forming pores in the plasma membrane for IL-1 release. Therefore, the cell surface level of CD14 can regulate the sensitivity of cells to pro-inflammatory stimuli and shape the magnitude of pro-inflammatory responses. This sparked our interest in poorly known mechanisms of CD14 endocytosis, the constitutive one occurring in resting cells, and the endocytosis of CD14-ligand complexes. **We aim to define mechanisms controlling constitutive and ligand-induced endocytosis of CD14 in macrophages as a target for the regulation of pro-inflammatory responses of these cells.**

CD14 is a GPI-anchored protein residing in sphingolipid/cholesterol-rich nanodomains (rafts) of the plasma membrane. Therefore, we assume that the CD14 endocytosis is initiated by changes in the lipid composition of rafts. We postulate that a local enrichment of sphingomyelin (SM)-derived ceramide (Cer) facilitates nanodomain clustering and invagination triggering thereby CD14 endocytosis, whereas phosphorylation of sphingosine to sphingosine-1-phosphate governs subsequent steps of the endocytosis. We also assume that CD14 ligation is sufficient to induce changes in protein *S*-palmitoylation which controls CD14 endocytosis in concert with the SM turnover, as proposed for so-called massive endocytosis. Therefore, we will examine the role of sphingolipids in the constitutive and LPS/oxPAPC-induced CD14 endocytosis in parallel with their contribution to pro-inflammatory signaling. This will include studies performed in living cells on co-localization of CD14 and ceramide or sphingosine with fluorescent tags in wild type cells and in those depleted of sphingomyelin synthase 2. We will proceed with an analysis of the involvement of selected proteins, also *S*-palmitoylated ones, to CD14 endocytosis and pro-inflammatory signaling. To identify S-palmitoylated proteins engaged in CD14 endocytosis, we will induce it in TLR4-deficient macrophages and follow with a quantitative mass spectrometry analysis of the palmitoylome. Last but not least we will examine the role of CD14 endocytosis and inflammasome activation for the generation and release of presepsin, a truncated form of CD14 recognized as a new marker of sepsis.

Our research will verify whether CD14 endocytosis is the master regulator of the inflammatory response of macrophages. In addition, the discovery of new mechanisms controlling CD14 endocytosis in the future may become the starting point for the development of effective regulators of pro-inflammatory responses.