Lipopolysacharide (LPS) is a major component of the outer membrane of Gram-negative bacteria. During bacterial infection, LPS strongly induces the pro-inflammatory reaction of the organism, which facilitates combating the infection. Macrophages are the primary target of LPS. They carry specific receptors recognizing distinct molecules of pathogens: LPS is recognized mainly by plasma membrane proteins, CD14 and TLR4/MD2 receptor complex. LPS-activated TLR4 triggers two pro-inflammatory signaling pathways leading to production of numerous inflammatory reaction mediators, including cytokines. Stimulation of macrophages has to be tightly controlled since an exaggerated response to LPS can lead to systemic inflammation called sepsis. Severe sepsis and septic shock, which are fatal in 30-50% of cases, occasionally develop as a result of bacterial infection especially in patients exposed to surgical procedures, anti-cancer and immunosuppressive therapies. Moreover, the high-fat westernized diet promotes the appearance in the blood of low doses of LPS originating from the gut microbiota. This induces chronic low-grade inflammation which is linked with the development of, i.a., type II diabetes and atherosclerosis.

Our project aims at characterizing a previously unrecognized mechanism controlling the pro-inflammatory reaction of macrophages. This mechanism relies on the regulation of functioning of an enzyme, diacylglycerol kinase epsilon (DGKε), which modulates the levels of distinct lipids in cells, phosphatidylinositols. Our earlier studies have revealed that one of these lipids, phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$], accumulates in the plasma membrane of LPS-stimulated macrophages. Notably, $PI(4,5)P_2$ is well known to control the pro-inflammatory signaling pathways triggered by LPS. This is because $PI(4,5)P_2$ and its derivatives affect the activity and localization of numerous proteins participating in the signaling cascades leading from the LPS-activated TLR4 receptor to the nucleus and inducing expression of genes encoding cytokines. The diverse phosphatidylinositols undergo interchanges and one such change involving the hydrolysis of $PI(4,5)P_2$ is also important for the signaling to proceed. After hydrolysis, $PI(4,5)P_2$ has to be replenished. This is achieved via the so-called phosphatidyinositol (PI) cycle. In this cycle, DGKε catalyzes phosphorylation of diacyglycerol formed by hydrolysis of $PI(4,5)P_2$. The phosphatidic acid formed can then be recycled to PI and eventually to $PI(4,5)P_2$.

In our project we plan to reveal that the modification of DGKε kinase called palmitoylation is a key modulator of the enzyme functioning in LPS-stimulated macrophages. Thereby, DGKε palmitoylation can be crucial for the $PI(4,5)P_2$ turnover in macrophages exposed to LPS. Our recent proteomic studies have revealed that under the influence of LPS the level of palmitoylated DGKε increases in macrophages. Palmitoylation consists in the attachment of a fatty acid residue - palmitic acid residue - to a protein. This modification drastically changes the properties of the protein, it facilitates its binding to membranes, changes the enzymatic activity and localization in cells. **We hypothesize that palmitoylation determines the involvement of DGKε in the PI cycle in macrophages stimulated with LPS, and is thereby crucial for the pro-inflammatory signaling to proceed with full intensity**.

Palmitoylation of DGKε has not been studies before. Our hypothesis is, however, supported by literature data on the structure and functioning of DGKε and by our data pointing to DGKε palmitoylation. At first, we plan to find out which amino acid residues of DGKε are modified by the attachment of the palmitic acid residue. Next we will examine whether the palmitoylation is required for the DGKε involvement in pro-inflammatory signaling in macrophages. For this, we will obtain DGKε variants with the palmitoylation site(s) disabled by mutation and introduce them to cells, also to cells previously depleted of endogenous DGKε by lentivirus-driven expression of specific RNA species. We will examine whether in such cells the course of the two signaling pathways of TLR4 will change and whether the level of studied lipids will be affected. In parallel, we want to learn how LPS affects palmitoylation of DGKε, what is the role of this modification for the DGKε binding to the membrane, its activity and cellular localization. Enzyme(s) catalyzing DGKε palmitoylation will be identified. **We posit that palmitoylation is a signal for the proper cellular location of DGK, i.e., its association with the plasma membrane and/or the endoplasmic reticulum. This, in turn, enables the DGKε participation in the PI cycle and thereby in the pro-inflammatory signaling induced by LPS. Thus, DGKε palmitoylation could be an important element controlling the pro-inflammatory signaling pathways.** Our research will be based on modern techniques, starting with the use of mass spectrometry to identify palmitoylated amino acids of DGKε, biochemical analyses of DGKε palmitoylation using so-called click chemistry, immunoassays for analysis of pro-inflammatory responses of macrophages, lipid measurements and confocal microscopy.

Taken together, the proposed studies will for the first time provide comprehensive data on palmitoylation of DGKε and reveal how the palmitoylation modulates the reaction of macrophages to LPS. These subjects have not been studied to date, therefore our work will be original and ground-breaking. The studies will substantially broaden our understanding of the regulation of pro-inflammatory reactions of macrophages. This knowledge can help in the future to develop effective drugs preventing excessive inflammatory responses to LPS. Moreover, dysfunction of DGKε has been implicated in several diseases (e.g., kidney disease), therefore our studies will supply novel information useful in a context broader than sepsis.