

Description for the general public

The inflammatory response to pathogens which attack the human and animal organisms is necessary to overcome the infection. One of the most important cell types responsible for this process are macrophages expressing receptors that recognize molecules of microbial origin, e.g., lipopolysaccharide (LPS). LPS, the main component of the outer membrane of Gram-negative bacteria, is bound by cell surface protein CD14 and next activates Toll like receptor 4 (TLR4). The receptor triggers two signaling pathways leading to the production of proinflammatory cytokines aiming at eradication of bacteria. The first signaling pathway is triggered by TLR4 localized in the plasma membrane and the main cytokine produced in this pathway is TNF- α . Subsequently, the TLR4/LPS complex is internalized under the control of CD14 and in endosomes the receptor maintains its signaling activity. Endosome maturation and translocation of TLR4 to lysosomes terminates the signaling. The TLR4 signaling has to be strictly controlled since exaggerated inflammatory responses to LPS cause serious illnesses such as severe sepsis and septic shock, which are fatal in 30-50% of cases. Recent studies, also showed that TLR4-dependent prolonged immune responses to a low doses of LPS, which are released from the gut microflora and translocate to the blood, contribute to the development of obesity, cardiovascular diseases and diabetes.

To avoid the detrimental effects of exaggerated inflammatory reactions the TLR4 signaling has to be strictly controlled. Among mechanisms by which cells can regulate inflammation triggered by LPS-activated TLR4 are degradation of the receptor and recycling of TLR4 and CD14 to the plasma membrane. These processes could be facilitated by retromer, a heteropentameric protein complex which controls the endosomes-to-Golgi trafficking of receptors which bind lysosomal hydrolases in the Golgi. Next, the receptor/hydrolases are transported to endosomes assuring subsequent lysosomal degradation of proteins. Therefore, the proper functioning of the lysosomes could be important in the degradation of TLR4, termination of its signaling and preventing exaggerated production of proinflammatory cytokines by macrophages in response to LPS. Moreover, retromer is also involved in recycling of plasma membrane receptors from endosomes and therefore it could affect the magnitude of TLR4 signaling by involvement in recycling of TLR4 and CD14. **The above facts prompted us to ask if retromer could terminate the inflammatory responses of macrophages to LPS by increasing TLR4 degradation and/or affect the transport of TLR4 and CD14 back to the plasma membrane.** We will also search for molecular mechanism which can control retromer activity in LPS-stimulated cells. We will focus on the turnover of a lipid, phosphatidylinositol 4-monophosphate [PI(4)P], because one of retromer constituents interacts with this lipid in endosomes and this interactions can modulate the retromer activity. Having established the involvement of retromer in TLR4 signaling we will examine whether retromer activity can affect the inflammatory responses induced by LPS released from gut commensal bacteria which are opportunistic pathogens. In healthy organism, LPS derived from these bacteria induces moderate inflammatory responses. In distinct conditions (surgeries, immunosuppression, radiotherapy) even commensal microbiota can induce exaggerated inflammatory responses. For these studies, we will apply LPS from *Hafnia alvei* and *Bacteroides thetaiotamicron*, the bacteria naturally colonizing the intestine and belonging to opportunistic pathogens. We will check whether insufficient retromer activity can contribute to upregulation of inflammatory reactions of macrophages to those forms of LPS.

Our results will for the first time provide comprehensive data on retromer-dependent regulation of LPS-induced inflammatory responses of macrophages and they will also specify the role of phosphatidylinositol turnover in retromer functioning in these cells. The role of retromer in LPS-induced responses of macrophages has not been studied, therefore our work will be original and ground-breaking. The studies will broaden our knowledge on the regulation of TLR4 signaling and reveal physiological meaning of retromer dysfunction in inflammatory response to opportunistic pathogens. Extending this knowledge can help in the future develop effective drugs that can prevent excessive inflammatory response and thereby decrease the mortality of sepsis and diseases in which activation of TLR4 is important.