CD14 turnover participates in regulation of inflammatory response in murine macrophages.

Introduction and Objectives
CD14 is a GPI-anchored protein involved in transfer of lipopolysaccharide, Gram - bacteria endotoxin, from LBP to TLR4 receptor, and thereby in induction of the inflammatory response of myeloid cells. Even though CD14 molecule does not contain the intracellular and the transmembrane domain, the protein is crucial for TLR4 signaling and endocytosis. Moreover, recent studies indicate that soluble fragment of CD14 (presepsin) can be a marker of sepsis. Therefore, mechanisms regulating CD14 synthesis, turnover and degradation are crucial in combating infection. We focused our attention on a role of retrograde rout and retromer complex in CD14 turnover and regulation of the inflammatory response of macrophages to LPS.
Methods
We performed recirculation assay to analyze turnover of TLR4 and CD14 in peritoneal macrophages, J774 cells and HEK293 overexpressing CD14 and TLR4. Moreover, we used ELISA and qPCR to evaluate levels of cytokines and immunoblotting to follow changes in protein levels in peritoneal macrophages and J774 cells. To checked whether retromer complex is involved in turnover of CD14 form late endosomes to the trans-Golgi network and the plasma membrane, we silenced SNX1 gene expression in macrophages.
Results
We found that a pool of CD14 molecules recycled between the plasma membrane and cytoplasmic compartments and LPS decreased level of the recycled protein. We used Retro-2, a retrograde transport inhibitor, to check whether this recycling is important in CD14 turnover. We found that Retro-2 decreased the surface level of CD14 and reduced the level of the recycled protein and only slightly affected TLR4 level. Simultaneously, Retro-2 decreased LPS-induced cytokine production. Silencing of retromer component, SNX1, reduced the cell surface level of CD14 and diminuted recycling of the protein not affecting expression of Cd14. Finally, silencing of SNX1 attenuated the LPS-induced pro-inflammatory response of macrophages.
Conclusion
Collectively our data suggest that retrograde transport is important for CD14 recycling and regulation of the LPS-induced inflammatory response.

Keywords : CD14, retrograde transport, LPS
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