# P27 & FP – **Synergy between 15-lipoxygenase and sPLA$_2$ promotes chronic inflammation by formation of TLR4 agonists from extracellular vesicles**

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**Background:** Sterile inflammation occurs under diverse pathological conditions, such as ischemia/reperfusion or trauma and it also underlies the pathologies of diseases with chronic inflammatory conditions including atherosclerosis, autoimmune diseases and aging-related pathologies. The innate immune signaling pathways (TLRs and inflammasomes are the most extensively studied) that sense infection also contribute to the sterile inflammation. Differences in the outcome of both conditions exist, but the mechanisms have not been fully determined yet.

**Objective:** Danger-associated endogenous molecules induce innate immune response, thus making sterile inflammation medically important. During oxidative stress conditions, stress-derived EVs (stressEVs) were found to activate Toll-like receptor 4 (TLR4) with a gene profile different from lipopolysaccharide (LPS). Along with understanding of oxidative stress mechanisms and their role on TLR function we want to identify endogenous ligands, which drive inflammation in chronic diseases. Additionally, some enzymes, among them lipoxygenases (LO) and secreted phospholipase $A_2$ (sPLA$_2$s), are induced by stress and contribute to the inflammation presumably by the formation of DAMPs and their role will be exploited.

**Methodology:**

- StressEVs were isolated from HEK293 cell stimulated with 12mM A23187.
- 15-LO was immobilized to magnetic beads. SynEVs, single PLs and lysoPLs were incubated with 15-LO.
- SynEVs or oxidized synEVs (composed of 30% AAPE and 70% POPC) were incubated with porcine sPLA$_2$-IB, rh sPLA$_2$-IIA, rh sPLA$_2$-X or synovial fluid.
- qPCR on macrophages or dual luciferase test on HEK293 were performed for measurement of TLR4 activity.

**Results:** Here we show that stressEVs in comparison to LPS activate different transcription factors resulting in activation of different immune response genes. Additionally, synergistic activity of 15-LO and sPLA$_2$ is needed for the formation of TLR4 agonists, which were identified as lysophospholipids (lysoPLs) having oxidized unsaturated acyl chain. Hydroxy, hydroperoxy and keto products of 20:4 lysophosphatidylinositol (lysoPI) oxidation were determined by mass spectrometry and they activated the same gene pattern as stresses. Furthermore, sPLA$_2$ activity, which was also detected in the synovial fluid from the rheumatoid arthritis and gout patients, promoted formation of the TLR4 agonists.

**Conclusions:** We revealed the mechanism of endogenous ligand formation through activity of 15-LO and sPLA$_2$, which contribute to sterile inflammation in chronic conditions like RA with differences to classical inflammation on the signaling pathway as well as cytokine level, thereby giving option to design specific inhibitors that will limit sterile inflammation but will not globally affect systemic innate immunity.