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Endothelial NLRP3-Inflammasome impairs vascular function via microparticles

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Background:
In recent years inflammation has emerged to the centre of attention of cardiovascular research. One of its key figures is the NLRP3-inflammasome a multimeric protein complex that stimulates inflammatory responses in atherogenesis through proinflammatory cytokines like caspases IL-1ß and -18. It is activated by danger signals such as cholesterol crystals, oxidized LDL, ATP or uric acids. Microparticles (MP) are extracellular vesicles that are released by activated or apoptotic cells. They are known as a vector for the intercellular transfer of biological information. The body of evidence indicates that endothelial microparticles contribute to the development and complications in atherosclerosis. With this study we sought to elucidate the effects microparticles, that are discharged by inflammasome activated endothelial cells, exert on arterial vascular cells.

Methods and Results:
RT-PCR experiments showed that activation of human coronary artery endothelial cells (HCAEC) with LPS and Nigericin leads to NLRP3-inflammasome-specific upregulation of NLRP3 and IL1ß. Analysis of the supernatant of aforementioned cells via westernblot revealed release of cleaved caspase-1 while donor cells undergo pyroptosis. FACS and electronmicroscopy experiments revealed time dependent release of endothelial microparticles (EMP) by inflammasome activated HCAEC, while western blot demonstrated that EMP enclose active caspase-1. Fluorescence microscopic imaging illustrated time dependent incorporation of EMP by HCAEC. Stimulation of HCAEC with EMP revealed detrimental biological effects on recipient cells as viability assay and scratch assay showed decreased viability and proliferation/migration, cytotoxicity assay showed increased cytotoxicity and RT-PCR experiments showed increased expression of NALP3, IL-1ß, VCAM and ICAM. The fact that treatment of recipient cells with the NLRP3-Inhibitor isoliquiritigenin (ILG), heat-inactivation of EMP and rupturing the EMP-membrane by freezing is able to diminish harmful effects EMP exert on recipient cells shown by viability assay, scratch assay and microscopic imaging underlines detrimental effects being exerted by EMP-encapsuled inflamasome-components.

Conclusion:
Our findings verify that MP released from inflammasome-activated endothelial cells are incorporated by vascular cells which in turn sustain a reduction of cell viability, migration and proliferation. EMP effectuate activation of the NLRP3-inflammasme in their target cells. The cytotoxic effects of EMP are suppressed by inhibitors of the NLRP3-inflammasome and affection of EMP-membrane. Our results emphasize the immunological role of endothelial cells and indicate that inflammasome activation is transferable through microparticle-associated communication. This in turn facilitates cell death and possibly initiates a vicious cycle of inflammation suggesting a role in the advancement of atherosclerosis.