Abstract: **P563**

**Relevance of C3-complement activation in atherosclerotic plaques and in the lipid-loaded smooth muscle cell phenotype**

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**Topic(s):**
Atherosclerosis, Cerebrovascular Diseases, Aneurysm, Restenosis

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**Background:** Non-structural elements of the vascular extracellular matrix (ECM) are thought to regulate progression of the atherosclerotic lesions. During the atherotrombotic process innate-immunity is a major component. The confluence of the classical, lectin and alternative pathways activating the inflammatory complement system is the C3-System (C3-S), an inflammatory pathway that has been found increased at the onset of acute myocardial infarction. The C3-S is the trigger of a cascade regulated by components such as the factor H/CFHR family that lead to active products such as C3b, C3a, and the cleavage of C5 into C5a and C5b. The anaphylatoxins C3a and C5a can induce chemotaxis of different cell types, while C5b is part of the terminal complex C5b-9, responsible of cell death.

**Purpose:** Investigate whether C3-S changes are found in the ECM of human atherosclerotic plaques of different severity and the effects of its cleavage products on the VSMC phenotype and function.

**Methods:** Segments of human aorta with atherosclerotic lesions (AT) and without atherosclerosis (nAT) obtained from sudden death cases were sequentially extracted to obtain the ECM protein-fraction. Proteomic studies were performed by 2D-electrophoresis and mass-spectrometry. Phenotype and migration of human vascular smooth muscle cells (hVSMC) were studied in vitro, in cells cultured with/without 100µg/mL human aggregated LDL (agLDL) in the presence/absence of purified C3-derived products. Protein and mRNA levels were analyzed by western-blots and RT-PCR. Cell migration was defined using a wound repair model.

**Results:** The ECM of human aortas is enriched in active components of the C3-S with a significantly different proteomic-signature in AT. C3 signal was more abundant in ECM of AT-arteries and western blot analysis demonstrated a 3-fold increase in its active cleaved product C3b (p<0.05) compared with nAT-ECM. In addition, CFHR1 and CFHR5 increased 2.5- and 3.8-fold, respectively in AT-arteries. In contrast, Factor-H consistently detected in ECM, did not differ between AT- and nAT. Isolated hVSMC expressed C3 and C5 as well as the anaphylatoxin receptor C3αR as demonstrated by RT-PCR and western-blots. C3-levels were significantly upregulated (1.7-fold) in cells exposed to aggregated-LDL (100 µg/ml). Using a model of wound-repair, the segment C3α (1µM) significantly prevented the inhibitory effect of agLDL on hVSMC migration, without showing any effect on cell migration in the absence of LDL.

**Conclusions:** Our results demonstrated for the first time the presence and differential abundance of active products of C3-System in the ECM of atherosclerotic lesions, and the capacity of C3-derived products to modulate the migratory and repair function of VSMC that is impaired by LDL. These results suggest the C3-complement-pathway as a novel player in vascular remodeling and in the progression of advanced human atherosclerotic lesions.
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