atherosclerosis

Atherosclerosis newsletter

Simona Negrini and Arnold von Eckardstein

Atherosclerosis is now present on Facebook (Atherosclerosis - Journal of the European Atherosclerosis Society) and Twitter (@ATHjournal).

Join our social media community and follow us!

Endothelial dysfunction, invasion of blood borne monocytes into the arterial wall, their subsequent transformation into macrophages and conversion into foam cells by the uptake of lipoproteins are pivotal for the initiation and progression of atherosclerosis. This last issue of Atherosclerosis in 2019 contains several interesting articles on the biology of endothelial cells and macrophages.

Volume 291, Issue December 2019

Macrophage fatty acid metabolism and atherosclerosis: The rise of PUFAs

Among the pathways involved in the regulation of macrophage functions, the metabolism of unsaturated fatty acids is central. Indeed, unsaturated fatty acids act as precursors of bioactive molecules such as prostaglandins, leukotrienes, resolvins and related compounds. As components of phospholipids, they have a pivotal role in cell biology by regulating membrane fluidity and membrane-associated cellular processes. Finally, polyunsaturated fatty acids (PUFAs) are also endowed with ligand properties for numerous membrane or nuclear receptors. Although myeloid cells are dependent on the metabolic context for the uptake of essential fatty acids (FAs), recent studies showed that these cells autonomously handle the synthesis of n-3 and n-6 long chain PUFAs such as arachidonic acid and eicosapentaenoic acid. Moreover, targeting PUFA metabolism in macrophages influences pathological processes, including atherosclerosis, by modulating macrophage functions. Omics evidence also supports a role for macrophage PUFA metabolism in the development of cardiometabolic diseases in humans.

Currently, there is a renewed interest in the role of n-3/n-6 PUFAs and their oxygenated derivatives in the onset of atherosclerosis and plaque rupture. Purified n-3 FA supplementation appears as a potential strategy in the treatment and prevention of cardiovascular diseases. In this context, the

ability of immune cells to handle and to synthesize very long chain PUFA must absolutely be integrated and better understood. All these issues are discussed in this review by Ménégaut and colleagues.

Cysteamine inhibits lysosomal oxidation of low density lipoprotein in human macrophages and reduces atherosclerosis in mice

Wen et al. previously showed that low density lipoprotein (LDL) aggregated by vortexing is internalised by macrophages and oxidised by iron in lysosomes to form the advanced lipid/protein oxidation product ceroid. In this study, they used sphingomyelinase-aggregated LDL, a more pathophysiological form of aggregated LDL, to study lysosomal oxidation of LDL and its inhibition by antioxidants, including cysteamine (2-aminoethanethiol), which concentrates in lysosomes by several orders of magnitude. They also investigated the effect of cysteamine on atherosclerosis in mice.

LDL was incubated with sphingomyelinase, which increased its average particle diameter from 26 to 170 nm, and was then incubated for up to 7 days with human monocyte-derived macrophages. LDL receptor-deficient mice were fed a Western diet and some of them were given cysteamine in their drinking water. The extent of atherosclerosis in the aortic root and the rest of the aorta was measured.

Confocal microscopy revealed lipid accumulation in lysosomes in the cultured macrophages. Large amounts of ceroid were produced, which colocalised with the lysosomal marker LAMP2. Cysteamine inhibited the lysosomal oxidation of LDL in cultured macrophages and reduced atherosclerosis in LDL receptor knockout mice.

These results support the hypothesis that lysosomal oxidation of LDL is important in atherosclerosis and antioxidant drugs that concentrate in lysosomes might provide a novel therapy for this disease.

MicroRNA-21 deficiency attenuated atherogenesis and decreased macrophage infiltration by targeting *Dusp-8*

Atherosclerosis is a chronic inflammatory disorder mediated by macrophage activation. MicroRNA-21 (miR-21) is a key regulator of the macrophage inflammatory response. However, the functional role

of miR-21 in atherogenesis is unclear. Gao et al. used both *in vivo* and *in vitro* models to elucidate the association between miR-21 and atherogenesis.

miR-21 was significantly upregulated in mouse atherosclerotic plaques and peripheral monocytes from patients with coronary artery disease. Compared with *miR-21*$^{+/+}$*apoE*$^{-/-}$ mice, double knockout (DKO) mice showed less atherosclerotic lesions, reduced presence of macrophages, decreased smooth muscle cells (SMC) and collagen content in the aorta. Bone marrow-derived macrophages (BMDMs) from DKO mice exhibit impaired migration induced by chemokine (C–C motif) ligand 2 (CCL2) and a weakened macrophage-endothelium interaction activated by tumor necrosis factor-α (TNF-α). However, atherogenic inflammatory cytokine secretion was not affected by miR-21 *in vitro* or *in vivo*. Additionally, miR-21 knockdown in BMDMs directly de-repressed the expression of dual specificity protein phosphatase 8 (Dusp-8), a previously validated miR-21 target in cardiac fibroblasts, which negatively regulates mitogen-activated protein kinase (MAPK) signaling.

These data demonstrate that inhibition of miR-21 may restrict the formation of atherosclerotic plaques partly by regulating macrophage migration and adhesion, while reduced SMCs and collagen content in plaques may lead to a less stable phenotype with the progression of atherosclerosis.

Neutralizing effects of anti-infliximab antibodies on synergistically-stimulated human coronary artery endothelial cells

Patients with rheumatic diseases, such as rheumatoid arthritis (RA), ankylosing spondylitis and psoriatic arthritis, have an increased risk of atherosclerosis and early development of cardiovascular disease, with up-regulated serum amyloid A (SAA), tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), which were reported to activate human coronary artery endothelial cells (HCAEC). However, their combined effects at the cellular level remain unclear. Ogrič et al. aimed to study the individual and combined effects of TNF-α, IL-1β and SAA on HCAEC, evaluate the effects of RA therapy (with the TNF-α inhibitor infliximab) on cytokine-stimulated cells, and determine whether isolated anti-infliximab antibodies from RA patients block the effects of infliximab.

HCAEC were incubated with TNF-α, IL-1β, SAA, infliximab, anti-infliximab antibodies and their combinations. The protein levels of pro- and anti-atherogenic analytes were measured in supernatants and mRNA expression was determined.

Granulocyte-macrophage colony-stimulating factor (GM-CSF), growth regulator oncogene α (GRO-α), IL-6, and IL-8 were synergistically up-regulated in triple stimulation with TNF-α, IL-1β and SAA, while their levels in solely SAA- or TNF-α-stimulated HCAEC did not increase. Interleukin 1

receptor antagonist (IL-1Ra), IL-1α, IL-10, IL-17A, vascular cell adhesion protein 1 (VCAM-1) and monocyte chemoattractant protein 1 (MCP-1), were increased, but no synergistic responses were observed in triple stimulation. Infliximab was effective in lowering the synergistic effect of IL-6, IL-8, GM-CSF and GRO-α in triple stimulation, while anti-infliximab antibodies restored the levels. The changes were confirmed at the mRNA expression level for *IL-6, IL-8* and *GM-CSF*.

Triple stimulation with TNF-α, IL-1β and SAA synergistically elevated IL-6, IL-8, GM-CSF and GRO-α release in supernatants of HCAEC, with infliximab substantially inhibiting their levels. An isolated, enriched fraction of polyclonal anti-infliximab antibodies was capable of neutralizing infliximab, in the presence of TNF-α/IL-1β/SAA. The long-term presence of anti-infliximab antibodies in the circulation of patients with chronic rheumatic diseases is potentially important for promoting the atherosclerotic process.

Aging and glycation promote erythrocyte phagocytosis by human endothelial cells: Potential impact in atherothrombosis under diabetic conditions

Atherothrombotic plaques of type 2 diabetic (T2D) patients are characterized by an increased neovascularization and intraplaque hemorrhage, representing the first cause of mortality in such patients. Catan et al. explored the hypothesis that clearance of erythrocytes may be carried out by vascular cells, and assessed the potential of human endothelial cells to bind and phagocyte *in vitro* aged and/or glycated erythrocytes and erythrocytes obtained from diabetic patients.

Fresh, aged and glycated-aged erythrocytes from healthy volunteers and T2D patients were tested for their binding and phagocytosis capacity as well as the potential functional consequences on endothelial cells (viability, proliferation and wound healing capacity). Immunohistochemistry was performed in human carotid atherothrombotic samples.

Aging and glycation of erythrocytes induced phosphatidylserine (PS) exposure and oxidative stress leading to enhanced endothelial cell binding and engulfment. Phagocytosis by endothelial cells was more pronounced with aged and glycated erythrocytes than with fresh ones. Phagocytosis was enhanced with T2D *versus* healthy erythrocytes. Furthermore, endothelial wound healing potential was significantly blunted after exposure to glycated-aged *versus* fresh erythrocytes.

Endothelial cells may play an important role in erythrocyte clearance in an atherothrombotic environment. Under diabetic conditions, erythrocyte glycation favors their engulfment by endothelial cells and may participate in endothelial dysfunction, thereby promoting vulnerable atherothrombotic plaques to rupture.

Sex-specific metabolic and functional differences in human umbilical vein endothelial cells from twin pairs

Gonadal hormones are mainly thought to account for sex and gender differences in the incidence, clinical manifestation and therapy of many cardiovascular diseases. However, intrinsic sex differences at the cellular level are mostly overlooked. Lorenz et al. assessed sex-specific metabolic and functional differences between male and female human umbilical vein endothelial cells (HUVECs).

Cellular metabolism was investigated by bioenergetic studies (Seahorse Analyser) and a metabolomic approach. Protein levels were determined by Western blots and proteome analysis. Vascular endothelial growth factor (VEGF)-stimulated cellular migration was assessed by gap closure. HUVECs from dizygotic twin pairs were used for most experiments.

No sex differences were observed in untreated cells. However, sexual dimorphisms appeared after stressing the cells by serum starvation and treatment with VEGF. Under both conditions, female cells had higher intracellular ATP and metabolite levels. A significant decline in ATP levels was observed in male cells after serum starvation. After VEGF, the ratio of glycolysis/mitochondrial respiration was higher in female cells and migration was more pronounced.

These results point to an increased stress tolerance of female cells, suggesting that female cells could have an energetic advantage over male cells under conditions of diminished nutrient supply. A more favourable energy balance of female HUVECs after serum starvation and VEGF treatment could potentially explain their stronger migratory capacity.