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Atherosclerosis is an inflammatory disease, involving tissue recruitment and activation of inflammatory and vascular cells. During the last years, several in vitro and in vivo studies, focused on atherosclerosis and its acute and chronic complications have been performed in our Lab. We investigated in mice the effects of chemokine receptor knockout and anti-chemokine treatments on the pathogenesis of atherosclerosis. As we demonstrated in ApoE-/- mice prone to develop atherosclerosis, the progression of atherosclerotic lesions correlates very well with an increase of pro-inflammatory chemokine and chemokine receptor expression within aortas. Furthermore, in a recent collaborative study with Professor Christian Weber (Institute for Cardiovascular Molecular Biology, University Hospital of Aachen, Germany), we demonstrated that genetic deletion of the chemokine receptor CCR5 but not CCR1 protects from the development of atherosclerosis, associated with a more stable atherosclerotic plaque phenotype, with reduced infiltration of Th1-type immune responses. In addition, we have shown that treatment with a chemokine analogous antagonist (Met-RANTES) markedly reduces the onset of atherosclerosis in our mouse model of atherosclerosis. We further studied in a clinically more relevant experimental setting if treatment with a RANTES antagonist could inhibit the progression of already established atherosclerotic lesions. Indeed, we found that treatment with the antagonist [^{44}AANA^{47}]-RANTES limits atherosclerotic plaque formation, which was associated with reduced infiltration of macrophages and T lymphocytes and reduced production of matrix metalloproteinase MMP-9. Using two mouse models of myocardial injury, we demonstrated that the selective anti-inflammatory treatments targeting chemokines (neutralizing anti-CCL5 antibody) reduced myocardial ischemia/repefusion injury and improved the post-infarction remodelling. Finally, in a cohort of subjects with severe carotid stenosis, we showed that different mediators of systemic and intraplaque inflammation were associated with plaque vulnerability. Importantly, circulating levels of an auto-antibody (Anti-Apoliprotein A1 IgG) were shown as directly related with the metalloprotease release and collagen digestion within human and mouse atherosclerotic plaques.

Based on various reports demonstrating immunomodulatory properties of cannabinoids, we recently shown that oral administration of THC resulted in the significant inhibition of disease progression. Furthermore, we detected the cannabinoid receptor CB_2, the main cannabinoid receptor expressed on immune cells, in both human and mouse atherosclerotic plaques. Our data demonstrated that oral treatment with a low dose of THC, through its immunomodulatory effects on inflammatory cells, is a potent inhibitor of atherosclerosis progression in a mouse model.

In vitro, we have shown that statins (lipid-lowering drugs) modulate chemokine and chemokine receptor expression in human macrophages and endothelial cells. Furthermore, we have recently shown that C-reactive protein (CRP) induces proinflammatory activities in human adherent monocytes. On the other hand, TNF-alpha induces chemotaxis and adhesion molecules expression in human neutrophils. We are now investigating the possible pharmacological modulation of these pro atherosclerotic activities.

Several in vivo projects investigating the role of chemokines and leukocytes in atherogenesis and myocardial infarction are actually ongoing in the Division of Cardiology, with some preliminary encouraging results. Prof. Mach and Dr. Montecucco are members of the "Atheroremo Consortium" that obtained in 2008 a grant (EU FP7, Grant number 201668) by the European Commission. Importantly, they benefit of the support of the Swiss National Foundation (SNF). Main collaborations include the research group of Prof. Da silva and Prof. Robson (University of Minas Gerais, Brazil), Dr. C. Monaco (Imperial College, London, UK), Prof. F. Dallegri and Prof. A. Nencioni (University of Genoa, Italy), Prof. Stergiopulos, (EPFL, Lausanne, Switzerland), Dr. N. Vuilleumier and Dr. P. Roux-Lombard (Geneva University Hospital, Switzerland), Dr. Y. Gasche and Dr. Copin (University of Geneva, Switzerland).

Connexins in cardiovascular disease

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Coronary heart disease is the leading cause of morbidity and mortality in Europe. Atherosclerosis, an inflammatory disease of large and medium-sized arteries, is the most important underlying cause of coronary heart disease. Atherosclerosis involves the formation of intimal lesions that are characterized by a dysfunctional endothelium, inflammation, lipid accumulation, cell death and fibrosis. The distribution of atherosclerotic plaques is highly characteristic in human; the lesions develop predominantly near side branches of arteries, where blood flow is disturbed. The most severe clinical events follow the rupture of a plaque and sudden thrombotic occlusion of the affected artery. Then, treatment consists of procedures that allow the rapid return of blood flow to the ischemic myocardium to rescue heart muscle. Reperfusion, however, may paradoxically lead to further complications resulting from an inappropriate inflammatory response in the microcirculation.

The work in our laboratory is focused primarily on the role of connexins in atherosclerotic disease. Connexins form gap junctions, clusters of intercellular channels synchronizing responses in multi-cellular organisms through the direct exchange of ions, small metabolites and second messengers between adjacent cells. We have previously shown beneficial effects on both progression and composition of the atherosclerotic lesions in mice with reduced levels of Cx43. In contrast to the atherogenic role of Cx43, Cx37 hemichannels in macrophages and endothelial Cx40 gap junction channels are atheroprotective. Our current work concentrates on the shear stress-dependent regulation of Cx37 and Cx40 in healthy endothelium and atherosclerotic vessels as well as on the role of connexins in atherosclerosis-derived complications, such as arterial thrombosis and reperfusion injury. Finally, we like to understand the role of connexins in the development of lymphatic vasculature as well as the role that these vessels may play in the development of atherosclerotic plaques.

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Division of Cardiology; Myocardial Biology group

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Mechanisms and consequences of impaired stimulation of myocardial glucose uptake in the metabolic syndrome

The capacity of the heart muscle to derive energy from a wide variety of substrates provides the myocardium with remarkable adaptability in the face of the ever-changing metabolic status of the organism. Among the myocardial substrates, glucose accounts for less than 25% of the energy production under normal conditions. Glucose is however unique among myocardial substrates because 1) energy can be obtained from glucose through glycolysis even in situations of ischemia, such as occurs during coronary occlusion and 2) ATP obtained from glycolysis, although scarce, is of paramount importance for the maintenance of ionic homeostasis. The importance of stimulation of glucose metabolism for post-ischemic recovery is illustrated by the poor recovery of GLUT4-null hearts, which are incapable of stimulation of glucose transport.

The Western diet is characterized by overconsumption of fatty and sugary food, leading to a worldwide epidemic of obesity and type II diabetes, termed the metabolic syndrome or "diabesity". The increased cardiovascular risk associated with the metabolic syndrome is usually explained by the higher prevalence of atherosclerosis and thus of myocardial infarction. However, experimental and clinical data suggest that the metabolic syndrome also increases the severity of infarcts. In the metabolic syndrome circulating glucose, fatty acids (FA), lipoproteins and proinflammatory cytokines are increased, chronically exposing the myocardium to an altered metabolic milieu. We recently observed that FA, Very-Low Density Lipoproteins (VLDL) and the cytokine Cardiotrophin-1 (CT-1) impair the stimulation of glucose transport into cardiomyocytes by physiologic – insulin – or pathologic – metabolic stress - stimulators.

Our lab is currently working towards the goals of 1) determine the cellular mechanisms of myocardial stimulated glucose transport impairment and 2) evaluate the consequences of this impairment in situations of myocardial metabolic stress.

To these ends, isolated adult rat cardiomyocytes are exposed *in vitro* to cytokines and metabolites previously identified to impair glucose metabolism in cardiomyocytes. Glucose metabolism in cardiomyocytes is assessed in response to insulin, chemically induced metabolic stress or simulated ischemia. Intracellular signaling and events leading to increased or impaired glucose transport are investigated by biochemical and cell imaging methods. Also, the capacity of the cardiomyocytes to withstand prolonged metabolic stress is evaluated.

The functional consequences of impaired glucose transport will be further assessed in isolated perfused heart experiments, wherein myocardial function can be studied together with metabolism, both in normal and ischemia-reperfusion conditions.

Although the impact of the metabolic syndrome on cardiovascular morbidity is well known, research has mostly focused on the development of atherosclerosis. Our research could shed light on how the metabolic syndrome directly affects one of the tissues that most severely suffers from atherosclerosis-induced events, the myocardium.

Summary of research (group Steffens)

Role of the endocannabinoid system in atherosclerosis

Atherosclerosis and its major adverse cardiovascular events, heart disease and stroke, are the leading cause of morbidity and mortality worldwide. It is an inflammatory disease of the arteries, characterized by lesions containing immune cells, smooth muscle cells, lipids and extracellular matrix. Both innate and adaptive immunity are involved in atherosclerosis. In recent years, exciting discoveries have revolutionized our current understanding of the molecular pathways underlying the disease, providing potential new targets for clinical therapy.

A dysregulation of the endocannabinoid system has been linked to a variety of pathologic conditions, including atherosclerosis and its related cardiovascular risk factors, obesity, dyslipidemia and diabetes. The endocannabinoid system comprises at least two distinct membrane receptors, CB_1 and CB_2, their endogenous ligands (named endocannabinoids) as well as enzymes for ligand biosynthesis and inactivation. It is well established that endocannabinoids are synthesized and released ''on demand'' and that this process can be regulated both physiologically and under pathological conditions.

As to cardiovascular disease, blocking of CB_1 receptors reduces several cardiometabolic risk factors in rodents and humans, indicating a potential relevance for the process of atherosclerosis. A modulation of endocannabinoid levels was reported in patients with coronary artery disease as well as in atherosclerotic mice. The first evidence for a causal role of CB_1 activation in atherosclerosis has been provided in mice treated with a pharmacological CB_1 receptor antagonist. In vitro, CB_1 antagonism mediates anti-inflammatory effects in macrophages and smooth muscle cells. We have shown that the phytocannabinoid delta-9-tetrahydrocannabinol inhibited atherosclerotic plaque progression in mice, mainly by inhibiting macrophage recruitment. The effect was inhibited by pharmacological CB_2 antagonism. However, some controversy exists about the effects of genetic CB_2 deficiency in atherosclerotic mice.

In the future, we would like to clarify whether the activated endocannabinoid system in atherosclerosis indeed plays a causal role to increase the risk of acute thrombotic events or rather counterbalances atherogenic processes. The existing data so far suggest opposing effects of CB_1 and CB_2 activation in the pathogenesis of atherosclerosis; however, the underlying cellular and molecular mechanisms remain largely elusive and deserve further investigations.