Disarray at the membrane

Valorization

Socioeconomic impact

Cardiovascular diseases (CVDs) are the leading cause of morbidity and mortality worldwide, accounting for ~31% of all global deaths [1]. However, with the onset of metabolic disorders, including obesity and type 2 diabetes, the prevalence and incidence of CVDs is expected to rise. Currently, CVDs represent a major economic burden accounting for €210 billion a year in healthcare costs in Europe alone [2]. Furthermore, as CVDs are often not instantly lethal, patients may require life-long treatment and suffer from severe disabilities. Moreover, CVDs may also indirectly have great impact for instance on people which are close to the afflicted patient.

Approximately 80% of all CVD-related deaths is attributable to coronary heart disease and stroke, which have atherosclerosis as underlying pathology [1]. Atherosclerosis is a multifactorial lipid-driven chronic inflammatory disease of the medium- and large-sized arteries. Atherosclerosis develops during childhood and adolescence, and can remain clinically silent for decades. Risk factors include a family history of CVD, aging, hyperlipidemia, hypertension, diabetes and life-style factors such as stress, tobacco smoke, lack of physical activity and diets that are high in saturated and trans fatty acids, cholesterol, salt, and sugar. Currently available treatments involve surgical interventions or risk factor management through lifestyle changes and/or medications, such as 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, also commonly known as statins [3]. Statins have been the cornerstone in the treatment of atherosclerosis, however, despite their effectiveness in lowering LDL-cholesterol and preventing CVD events, there still remains a ~75% residual CVD risk that remains unresolved [4]. In addition, some patients are able to tolerate only low doses of statins, while other are unable to tolerate statin therapy at all [5]. Novel drugs, including lipid-lowering proprotein convertase subtilisin/kexin type 9 [6] and anti-inflammatory interleukin 1β [7] inhibitors, show great promise in reducing CVD events on top of statin treatment, however they too are insufficient in fully preventing CVD-related deaths.

The socioeconomic burden caused by atherosclerosis raises demand for new innovative treatment strategies. Currently, the progression from an early fatty streak lesion to a rupture-prone atherosclerotic plaque is still incompletely understood. This implies that fundamental research is essential to improve our understanding of the atherosclerotic disease processes, which can yield new insights that will aid in the development of novel therapies for treating atherosclerosis, and thus prevent the majority of the CVD-related deaths.

High density lipoproteins

Raising high-density lipoprotein (HDL) cholesterol (HDL-C) has gained a lot of interest due to the inverse relationship between serum HDL-C levels and the risk of CVD events [8-10]. However, recent clinical trials using HDL-C raising agents, such as nicotinic acid, fibrates and cholesterylester transfer protein inhibitors, with the exception of anacetrapib, have failed to show any beneficial effect on CVD outcome [11-15]. While anacetrapib doubled blood levels of HDL-C, the reduction in CVD risk on top of statin treatment of anacetrapib was mainly ascribed to its low-density lipoprotein cholesterol lowering effect [15]. Based on these observations, research has been refocused towards HDL particle functionality, more specifically its anti-atherogenic/-inflammatory functions, rather than its cholesterol content. A myriad of studies have shown that HDL exerts anti-inflammatory effects in endothelial cells (ECs) and vascular smooth muscle cells (VSMCs), two important vascular cell types which are intricately involved in the development and progression of atherosclerosis [16]. However, in contrast to the aforementioned cell types, we showed that

HDL exerts pro-inflammatory effects in macrophages (**chapter 2**), the main inflammatory cell type in atherosclerotic lesions [17]. Therefore, HDL can either augment or inhibit the progression of a disease, depending on the cell type and cellular process it modulates.
In ECs, HDL exerts many anti-atherogenic effects, including the improvement of endothelial function and repair, as well as the attenuation of leukocyte recruitment by reducing the expression of adhesion molecules and chemokine [16]. Moreover, infusion of reconstituted HDL increased capillary density and blood flow recovery in a murine ischemic hind-limb model of angiogenesis [18]. However, due to its pro-angiogenic potential, HDL might also promote pathological neovascularization in atherosclerotic lesions, thereby promoting plaque instability, however, this remains to be determined.
In the case of VSMCs, HDL can inhibit their proliferation and thus limit neointimal hyperplasia [19]. However, reduced VSMC proliferation in atherosclerotic lesions may cause plaque instability and rupture [20]. On the other hand, HDL also reduces chemokine secretion by VSMCs [19], which may limit leukocyte recruitment and atherosclerosis progression [21].
In macrophages, HDL activates pro-inflammatory signaling (**chapter 2**), which has been associated with atherosclerotic plaque instability [22]. Moreover, we also found that HDL enhanced bacterial phagocytosis (**chapter 2**), a process which shows a high degree of overlap with efferocytosis, the phagocytosis of apoptotic cells [23,24]. However, in contrast to bacterial phagocytosis, efferocytosis dampens the inflammatory response by removing immunogenic debris and stimulating the production of anti-inflammatory mediators [21]. Moreover, we have shown that HDL can aid in the clearance of bacterial infections. Interestingly, bacterial infections have been shown to accelerate atherosclerosis development in mice [25,26]. Therefore, HDL may limit the exacerbation of atherosclerosis induced by bacteria.
Collectively, HDL can exert both pro- and anti-atherogenic effects in the aforementioned cell types and it will be the net effect of HDL on the investigated cell types that will dictate the course of the disease.

Studies in preclinical animal models, especially mice, show that raising plasma HDL levels can inhibit or even reverse both early and advanced atherosclerosis development. Furthermore, infusion of reconstituted HDL reduces plaque volume size in humans [27], suggesting that the net effect of HDL is anti-atherogenic. However, all (pre)clinical studies on HDL to date have relied on surrogate markers for atherosclerotic plaque stability, including lesion size/volume and lipid-, macrophage- and collagen content, rather than on clinical manifestations, such as myocardial infarction or stroke. Therefore, the pro-inflammatory effects of HDL in macrophages might still be relevant in terms of plaque rupture and subsequent atherothrombotic events. Reconstituted HDL CER-001 and CSL-112, which were developed by Cerenis Therapeutics and CSL Behring, respectively, are currently used in clinical trials as a treatment for coronary heart disease. The results of these studies will provide evidence whether raising plasma HDL levels is a viable therapeutic strategy against CVDs and whether the pro-inflammatory effects of HDL in macrophages are clinically relevant in the management of CVD by HDL raising therapies. In addition, based on our findings, HDL therapy may be useful in patients with persistent or recurrent bacterial infections.

Transmembrane proteases

In contrast to HDL, the use of A Disintegrin And Metalloproteinases (ADAM)8 and 10 or signal peptide-peptidase-like (SPPL) 2a and 2b as therapeutic targets is still far removed from clinical application. First of all, under homeostatic conditions, protease activity is delicately balanced to accommodate the needs of the cell or tissue. However, disturbances in proteolytic activity through a dysregulation in transcriptional control, alternative splicing, post-translational modifications, subcellular trafficking and localization, protein-protein interactions, the presence/absence of natural inhibitors, and in the abundance of both protease and substrate can have dramatic effects resulting in the development and/or progression of a disease [28]. Second, the design of protease specific inhibitors appears to be rather challenging, because they are designed to block the active site of an enzyme, which contains residues that are often well conserved in a family of proteases, as is the case with members of the metalloprotease family, like ADAMs and matrix metalloproteases. Third, most if not all proteases have more than one substrate rendering them capable of affecting multiple pathways, however the net result seems to depend on the cell type and context. Previously, we have shown that myeloid ADAM10 promotes atherosclerotic plaque instability by stimulating a pro-degradative/inflammatory phenotype in macrophages [29]. In contrast, endothelial ADAM10 is atheroprotective by limiting pathological intraplaque neovascularization and hemorrhage (**chapter 6**). Furthermore, ADAM10 can enhance the migration and invasiveness of cancer cells thereby promoting cancer metastasis [30]. Collectively, targeting ADAM10 will warrant a cell type- and disease-specific approach. For atherosclerosis, this will entail the inhibition of ADAM10 activity specifically in atherosclerotic plaque macrophages through targeted delivery of ADAM10 enzyme inhibitors or antisense oligonucleotides, using for example ligand-targeted nanoparticles as a delivery system [31]. This has to be accomplished without affecting macrophages in other tissues, since reducing the pro-inflammatory response of macrophages will hamper their response to micro-organisms and their antitumor activity for example [32]. Furthermore, using a similar strategy as for plaque macrophages, reducing ADAM10 activity in cancer cells will limit their metastatic potential. On the other hand, increasing ADAM10 activity specifically in endothelial cells may limit atherosclerosis development, however, to date, there is no therapeutic that can increase the proteolytic activity of a specific protease. While overexpression of a protease can increase the abundance of a protein, it does not mean that the protein is proteolytically active.

In contrast to ADAM10, ADAM8 is upregulated in many inflammatory diseases and is not required under homeostatic conditions in adulthood, at least in mice [33-37]. This makes ADAM8 an excellent target for intervention. Indeed, inhibiting ADAM8 function attenuates bronchial hyperresponsiveness and inflammation in murine asthmatic models [38], as well as pancreatic tumor burden and invasiveness in mice [39]. However, we show that ADAM8 is not involved in atherosclerosis development (**chapter 5**), thus ADAM8 is not a viable target for intervention in the treatment of atherosclerosis.

Unlike ADAM proteases, research on SPPL2 proteases is still in its infancy. We have shown that SPPL2a/b limit pro-atherogenic LOX-1 signaling and their expression in the non-hematopoietic compartment protects against atherosclerosis development (**chapter 7**). Interestingly, deletion of SPPL2a alone results in an overt immunological phenotype, characterized by depletion of mature B cells, suggesting that SPPL2a might be a viable therapeutic target in B cell-dependent autoimmune diseases [40]. In addition, B cell depletion was shown to reduce atherosclerosis development [41], suggesting that SPPL2a deletion in the hematopoietic, which also includes leukocytes, may limit atherogenesis, opposite to its effect in the non-hematopoietic compartment. However, this requires

further investigation. Given the fact that LOX-1 is also implicated in many other diseases [42-44] and the full substrate repertoire of SPPL2a/b is still unresolved [40], it remains to be determined whether targeting SPPL2a/b can be beneficial in other diseases besides atherosclerosis.

Conclusion

In conclusion, while raising plasma HDL levels is a promising therapeutic strategy in the treatment of atherosclerosis, caution is warranted in its implementation in the clinic due to potential adverse effects in macrophages. Furthermore, although ADAM8 is not a viable target for intervention, ADAM10 requires a cell type- and disease-specific approach for it to be exploited as a therapeutic target against atherosclerosis. Lastly, targeting SPPL2a/b in atherosclerosis requires further investigation before it can be regarded as a suitable target for intervention.

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