Role of dendritic cell subsets in hyperlipidemia and atherosclerosis

Valorisation

Cardiovascular diseases, more than a killer

As mentioned previously, cardiovascular diseases (CVDs) are still the leading cause of mortality worldwide, accounting for 17.5 million deaths in 2012 (World Health Organization, WHO). These numbers are even expected to rise to 24 million by 2030. Besides the enormous impact on human health, CVD also has a profound effect on healthcare costs. In the United States (US) alone CVD was responsible for 17% of national health expenditures in 2010 and this percentage is expected to increase dramatically in the coming years. Between 2010 and 2030 medical costs of CVD in the US are projected to triple, from $273 billion to $818 billion (Heidenrich et al., 2011, Circulation). Importantly, CVD also impacts on the patietns social life as clinical events can lead to immobilization, brain damage etc.

CVDs and their socio-economic impact can be combatted by multiple approaches. First, primary prevention is a key determinant as reduction of behavioural risk factors such as tobacco use, unhealthy diet, obesity, physical inactivity and alcohol abuse, will in the long run strongly diminish CVD incidence (WHO). Moreover, these factors can be addressed at low cost. Second, secondary prevention in people with already established disease should be addressed. Current treatment such as lipid lowering drugs (e.g. statins, ezetimide, fibrates), antihypertensive drugs (e.g. ACE inhibitors, AT receptor antagonists, beta-blockers) and antithromobtic therapy (e.g. aspirin, clopidogrel) have shown their effectiveness. Third, costly surgery (e.g. coronary bypass, valve replacement) sometimes is an option to treat CVD (WHO) and to reduce the risk of clinical complications. Fourth, improving (early) diagnosis and fine tune the appropriate treatment groups for tailored therapy,is important as today people are often only diagnosed upon an overt clinical event. Although progress in all of these areas has been made, CVD remains the major cause of death. Therefore, development of new therapies that show effectiveness, preferably at a low cost, is required.

CVD reduction by targetting atherosclerosis

The main underlying cause of most CVDs is atherosclerosis. A better understanding of the complex disease pathology is necessary in order to discover new therapeutic options, indicating the importance of fundamental research in parallel to applied research. Here, we discuss some contributions of this thesis towards valorization possibilities and discuss future perspectives.

$CD8^+$DCs can be excluded as interesting therapeutic target for atherosclerosis

The role of the immune system in atherosclerosis has been studied extensively, however research on dendritic cells (DCs) is relatively new. DCs hold potential as therapeutic target as they play a major function in regulating the immune system. However, they are a heterogenous population of cells and each subset probably contributes to the disease in a different way. Except for pDCs, little is known regarding DC subset contribution to atherosclerosis. This thesis provides new insight in the DC biology in atherosclerosis, as our findings in **Chapter 4** indicate that $CD8^+$DCs and possibly cross-presentation are at most minor contributors to atherosclerosis progression. In addition, DCs have been implicated in regulating cholesterol homeostasis, we here show that $CD8^+$DCs are not the DC subset responsible for this effect. Altogether, our study suggested that $CD8^+$DC can be exluded as interesting therapeutic target option for atherosclerosis treatment.

Quaking as potential therapy for inflammatory and auto-immune diseases

In **Chapter 7**, we show that QKI deficiency was able to reduce atherosclerosis, suggesting it has potential as a new target for treatment of vascular disease. Moreover, loss of QKI augmentated Treg and reduced Th17 T cell numbers. Both cell types and their balance are important in inflammation as well as auto-immune disease, rendering QKI also a possible candidate for the treatment of these disorders.

Quaking isoforms are ubiquitously expressed and influence a wide range of cellular functions, making it difficult to specifically target certain cell types for therapy without inducing undesireable side effects. Therefore, identifying the

disease-relevant targets of Quaking will help to develop specific, efficient and safe therapy. We are currently analyzing RNA sequencing data that will provide us this new information. Moreover, delivery systems, like viral vectors, have greatly improved over the past years. Specific targeting of certain cell types by use of specially designed viral vectors may reduce the risk for off target effects.

A possibility to implement Quaking targeting drugs into therapy may involve Quaking (or the Quaking target) gene therapy, in analogy to miRNA regulation or CRISPR/Cas9-based genome engineering in the treatment of cardiovascular, inflammatory or auto-immune diseases (Rincon et al., 2015, Cardiovascular Research). miRNAs have emerged as potential therapeutic targets in CVD as they are able to mediate quantitative and coordinate changes to the transcriptome of disease relevant gene sets. RNA-binding proteins like Quaking represent an additional level of control as they are also able to qualitatively influence the transcriptome. However, this approach also has its limitations as Quaking is ubiquitously expressed and is involved in the regulation of many critical processes (neural development and maintenance, myeloid cell regulation, smooth and cardiac muscle cell regulation). Therefore, influencing specific Quaking targets will be required in order to minimize side effects.

Influencing CD40 signaling as a new generation of atherosclerosis therapy

Inhibition of CD40-CD40L interactions strongly reduces atherosclerosis. However, complete inhibition of CD40-CD40L signaling is not therapeutically feasible as long-term treatment will compromise systemic immune responses and was shown to entail thromboembolic complications (Lutgens E, 2010, J Exp Med). Therefore, more specific approaches which induce fewer and less severe side effects are required. Cell-specific targeting could reduce side effects and CD40 signaling on both platelets and leukocytes were shown responsible for the beneficial effect on atherosclerosis. DCs are interesting candidates for CD40 treatment as they strongly express these molecules and are able to influence immune reponses. However, as we showed that altering CD40 activity can have a profound effect on the autoimmune control (as reflected by the gastro-intestinal inflammation in CD11c-LMPca) , more specific approaches are necessary (**Chapter 8**). In that respect, interference in CD40 TRAF6-

signaling, genetically or with targeted small molecule drugs, was shown to confer an almost equal atheroprotective effect as seen with complete CD40 deficiency. Therefore, these drugs may hold promise for the development of a new effective therapy for atherosclerosis.

Hypercholesterolemia, more than a risk factor for CVD

Dendritic cells play a crucial role in host immune responses to pathogens**. Chapter 5 and 6** show that hypercholesterolemia profoundly impacts DC function, therefore people suffering from hypercholesterolemia are not only at risk for developing CVD, but are possibly also more vulnerable to viral and bacterial infections. Treating these patients with lipid-lowering drugs may therefore not only benefit CVD outcome but also restore patient's defense against pathogens.

Implications of thesis findings for cancer therapy

A possible role for Quaking in DC-based cancer vaccination

Cancer immunotherapy, at least in part, focusses on designing vaccines to promote strong tumor specific T cell responses in order to eradicate tumors. DCs as the most potent antigen presenting cells play key roles in this process.. In the past, various strategies of DC-based immunotherapy were adopted in clinical studies, however clinical responses remain relatively low (van Lint, 2014, Cancer immunology, immunotherapy). Besides antigen choice also the immune state of the dendritic cell is of great importance. Better outcome can therefore be achieved by enhancing the maturation state of the DCs, for example by co-electroporation of antigen with mRNA encoding for CD40L, CD70 and a constitutive active form of TLR4 (van Lint, 2014, Cancer immunology, immunotherapy). As our results in **Chapter 7**, indicate that Quaking influences DC cytokine production and likely also maturation, Quaking could be an interesting candidate to improve DC activation state for DC immunotherapy.

A role for constitutive CD40 signaling in tumor-DC vaccination?

As mentioned previously, the immune state of the DC is a cricital factor for succesfull tumor-DC vaccination. As CD40 signaling is important in DC maturation and function, the constitutive CD40 signaling chimeric LMP/CD40 protein may provide a beneficial contribution in DC activation required for effective DC immunotherapy (**Chapter 8**). Currently, scientist are using electroporation of mRNA of CD40 or CD80 along with the Ag loading in DCs to boost the immune state of DCs. Electroporation of LMP-CD40 mRNA might improve these results.

In conclusion, noticeable progression has been made in the treament of atherosclerosis. However, as CVD remains the major cause of death we need to continue our efforts. With the inflammatory component of atherosclerosis pathogenesis being well established, developing new drugs that influence atherosclerosis specific immune responses is of importance.