The sweet key: to unlocking full dendritic cell potential

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SCOPE OF THIS THESIS

To highlight the opportunities for further exploitation of glycans and lectin receptor targeting in regard to their immunotherapeutic potential, this thesis is divided into two parts; **Ligand Fitting** and **Receptor Signaling**.

Ligand Fitting investigates the multivalent aspects of glycan presentation to specific CLRs in immune therapeutic vaccine strategies. The organization of CLR receptors on the DC cell surface reflects the need of multivalent presentation of the ligand for optimal binding affinity. We selected the high mannose oligosaccharide, which is a high affinity ligand for both DC-SIGN and Langerin receptors, and we evaluated from the high mannose structure the necessary mannoside linkages and valency for high affinity binding. In Chapter 2 we investigate DC-SIGN binding to the mannoside clusters. DC-SIGN affinity was increased with higher degree of mannoside clustering. The hexavalent mannosides were selected for the elongation with a melanoma-antigen and a TLR7 ligand. We found that the strongest DC-SIGN ligand did not enhance antigen presentation. Instead, DC antigen presentation was diminished, while ligands with lower affinity improved antigen-specific T cell activation. Chapter 3 describes binding of the same mannoside library to Langerin. A similar increase in binding affinity was seen with higher degree of mannoside clustering. However, elongation of the hexavalent clusters with an antigen did not boost antigen presentation capacity. In contrast, all mannoside conjugates targeting Langerin hampered T cell activation. In Chapter 4, a synthetic *C*-mannoside was generated and compared to the endogenous *O*-mannoside. This *C*-mannoside lacks the exocyclic anomeric oxygen to render the glycosidic linkage resistant to the acidic conditions necessary for standard automated solid phase peptide synthesis (SPPS). Here, the affinity of both mannosides to DC-SIGN was again increased with higher multivalent presentation. Stimulation of DCs with the endogenous or synthetic mannoside resulted furthermore in comparable secretion of cytokines and antigen presentation to $CD4^+$ and $CD8^+$ T cells. The organization of the CLR- and TLR- targeting moieties within the synthetic long peptide vaccine conjugate did augment the measured biological effects, and could optimize the DC - T cell communication through cytokine expression and antigen presentation.

Receptor Signaling explores the altered biological processes within dendritic cells on a (phospho)proteomic and transcriptomic level. Through multivalent presentation of the glycans on a dendrimeric core, specific binding to the CLR was achieved. Chapter 5 describes the signaling of DC-SIGN on a proteomic level after triggering with high mannose oligosaccharides ($Man_{7\text{-}9}$) or $Lewis^Y$ moieties in presence of LPS. Although overlap in significantly altered proteins between both conditions were found, a set of significantly dephosphorylated proteins could be annotated to each glycan. High mannose stimulation

furthermore specifically altered pathways involved in "endolysosomal trafficking" and "DC maturation" while Lewis^{Y} stimulation did not. Chapter 6 investigates the biological DC processes affected by stimulation with sialic acids on a phosphoproteomic level. Although α2-3 and α2-6 sialic acid only differ in the linkage between the terminal sialic acid and the underlying galactose, the DC is able to discern between the two and respond accordingly. A modulatory role for α2-3 sialic acid on the TLR-4 signaling route was found, as well as α2-3 sialic acid-specific triggering of kinases and proteins in the JAK-STAT pathway in DCs, resulting in an increased IL-10:IL-12 cytokine ratio. In Chapter 7, alterations in the DC transcriptomics were assessed after moDC binding of the α2-3 sialic acid dendrimer in the presence of LPS. Transcriptomic analysis revealed differentially expressed genes involved in "antigen presentation and processing", and "T cell differentiation". A significant reduction in T_H1 differentiation upon α2-3 sialic acid-binding to moDC was observed that was attributed to inhibition of IL-23 and increased IL-10:IL-12 cytokine ratio, as well as skewing of naïve T cells towards a more regulatory T cell phenotype. Chapter 8 investigates the immune suppressive properties of the MGL on the DC phenotype. The transcriptome of human DCs was analyzed after stimulation of MGL using two tumor-associated glycans, each presented on a dendrimeric core, of which only the GalNAcβ1-4Gal binding resulted in increased secretion of IL-10. However, modifications of the glycolysis pathway, TCA cycle and oxidative phosphorylation, and a decreased metabolic activity of moDCs was confirmed after MGL triggering. Finally, Chapter 9 provides a summary and a general discussion of the findings in this thesis.