Englisch summary for laymen

Sweet modulation of the immune response

Where does cancer originates from? Each day cells in our body are being damaged. This damage normally will be repaired, but if the damage is too big, the cells will be eliminated by the immune system. When damaged cells are not recognized properly, it can lead to uncontrolled growth. These uncontrolled growing cells form a tumor. Why aren't the uncontrolled harmful cells recognized by the immune system? Because cancer cells are not foreign, but do originate from self, and look a lot like normal cells. Due to this, the immune defence has sometimes troubles to recognize the harmful cells and to induce a good immune response against the tumorcells.

Our immunesystem protects us from everything that is harmful to our body, this includes bacteria, viruses and yeast, but also damaged and old cells. The immune system comprises of different types of cells with each their own task. Dendritic cells (DCs) play a central role in the immune system. DCs are capabel to detect harmful particles and activate the T-cells (the soldiers of our immune system) to attack these particles.

How does that work precisely? The DC are localized on strategic places of the body which come in contact with the outer surface, like for example the skin and the mucous membranes. There, they scan their surroundings with several receptors. These receptors recognize components of these harmful particles. There are different groups of receptors. For example: there are the Toll-like receptors that recognize bacterial- or viral components, and say to the DC: Watch out, danger. Next to this, there are the C-type lectins which recognize sugar structures on the surface of a particle, and after recognition take them up in the DC. After uptake of the particle, the DC cuts the particle in pieces and brings them to the surface of the DC to show them to the T-cells. Because of this, the T-cell becomes activated and will perform their effector function.

T-cells can be subdivided in different classes. T-cells which are important for the killing of a tumor are the cytotoxic $CD8^+$ T-cells who attack the tumor, and the Th1 $CD4^+$ T-cells who help the cytotoxic $CD8^+$ T-cells to perform their function.

DCs can activate these $CD8^+$ T-cells through a mechanism called cross-presentation. Naïve $CD4^+$ T-cells can be activated through a process called MHC-class II presentation.

This thesis describes a model to stimulate the DCs as such, that they activate the T-cells to attack a existing tumor, instead of ignoring it.

How do we do that? To stimulate the DCs we use proteins that are specific for tumorcells and do not come to expression on healthy cells, the so-called tumorantigens. To these tumorantigens we coupled a flag, certain sugar-moieties, and gave these to the DCs. With these tumorantigen-sugar structure conjugates we analysed whether we could influence the immune response, inducing antitumor responses.

In more detail: in the studies described in this thesis we use the model antigen OVA. It has been described that OVA binds the C-type lectin Mannose receptor, and when high concentrations of antigen are used with an additional Toll-like receptor signal that cross-presentation is induced. Till date it was not known which sugar-structures OVA had on its surface which bound the Mannose receptor. We have described that +/- 20% of the OVA-molecules brings high-mannose structures to expression, which bind the Mannose receptor **(chapter 3)**.

In **chapter 2** is described which sugars bind to the C-type lectins MGL1 and MGL2. We found that GalNAc-structures bind MGL2, similar as for the human MGL. In contrast to the human MGL, MGL2 has no specificity for galactose, TF-antigen en Core2 O-glycans. MGL1 recognizes the sugar-structures Lewis^X and Lewis^A, and has no overlapping specificity with human MGL. Next to the sugar-specificity of MGL1 and MGL2, we also described that MGL2 binds sugar structures, like GalNac, which are associated with tumors. Due to this the DCs that express MGL2 can interact with tumorcells, via GalNAc.

We coupled sugar-structures who bear specificity for either MGL1 or MGL2 to OVA. Using these OVA-sugar structure conjugates we have asked different questions: are these conjugates taken up more efficiently and what is the impact of these sugar-structures on the induced immune response.

We showed that Lewis^X-conjugated antigen was more efficiently taken up by BC through MGL1 compared to not conjugated OVA **(chapter 3)**. The proliferation of CD4$^+$ T-cells was not influenced; however, naïve CD4$^+$ T-cells where activated to become effector Th-1 cells. Next to this, uptake of Lewis^X-conjugated antigen resulted in enhanced proliferation of CD8$^+$ T-cells *in vitro* compared to non-conjugated OVA. Furthermore, after immunisation *in vivo* more IFN-γ producing antigen-specific CD8$^+$ T-cells where observed. Several intracellular routes have been described for cross-presentation. One depends on TAP-mo-

lecules, another relies on the endosomal protease Cathepsin S. We showed that the cross-presentation of OVA-Lewis^X conjugates does not depend on either pathways. Moreover, the cross-presentation of OVA-Lewis^X is independent of Toll-like receptor signalling, in contrast to native OVA.

We inverstigated whether a ligand for Toll-Like Receptor 4 (LPS) could improve MGL1-induced cross-presentation **(chapter 4)**. Suprisingly, we found that LPS abrogates MGL-1 induced cross-presentation. This seems to be due to that OVA-Lewis^X in the presence of LPS is taken up via a second uptake receptor which lead to a intracellular rerouting of antigen which does not lead to cross-presentation to CD8$^+$ T-cells.

In **chapter 5** I described that targeting antigen to MGL2 via OVA-GalNAc conjugates results in improved cross-presentation to CD8$^+$ T-cells and the induction of CD4$^+$ Th-1 cells.

In **chapter 6** is decribed that the addition of other ligands of the Mannose receptor induced increased cross-presentation. The modification of OVA with triGlcNAc or SulfoLe^A-structures induced efficient CD8$^+$ T-cell proliferation compared to native OVA. Moreover, Toll-like receptor 4 ligand LPS improved OVA-triGlcNAc en OVA-SulfoLe^A induced cross-presentation significantly.

In conclusion: in this thesis I showed that sugar-structures improve the recognition of tumorantigens by DCs, and that this leads to better activation of the T-cells. This could potentially lead to better antitumor immune responses and the breakdown of the tumor. The coupling of sugars to tumorantigens should be considered in the design of novel cancer immunotherapies.

DCs do not only regulate the immunity against tumors. But also the defence against pathogens and they prevent auto-immunity. Due to this, immunotherapy via sugar conjugated antigens cannot only be used in the battle against cancer but potentially also in the defence against pathogens and auto-immunity. For this, the conditions and the components of the vaccin should be modified to the kind of immune response needed.