The functional consequences of glycosylation on dendritic cell biology

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The immune system

Our bodies are constantly exposed to pathogens present in our environment, including bacteria, viruses and fungus. The immune system is directed to protect our body against these pathogens. An important cell of our immune system is the dendritic cell. Dendritic cells are located at sides of our body where pathogens can invade, such as skin, lung and mucosa. Dendritic cells are specialized in trapping pathogens with their long cellular extrensions (dendrites). When dendritics cell sense for pathogen in these tissues, they are in an immature state. After recognition of pathogens, dendritic cells become activated and a cellular maturation process is initiated. Subsequently, mature dendritic cells migrate from the tissues via the lymphatic vessels to the lymph nodes. T cells are the soldiers of the immune system and these cells are largely present in the lymph node. The dendritic cell informs the T cell in the lymph node at which site of the body the pathogen was found and what specific T cell response is neccessarry to clear the infection. This information transfer is followed by migration of the T cell to the site of infection to kill the pathogen.

Glycosylation

Pathogens can be recognized by our immune system by their specific glycan structures. However, not only pathogens are covered with glycans, also our own cells highly express glycosylated structures. The diverse and abundant repertoire of glycans found on surfaces of cells is produced by a processs termed glycosylation. The glycosylation process produces linkages of monosaccharides to other saccharides, proteins and lipids. This is mediated by a set of enzymes, chaperones, regulatory molecules, co-factors, sugar donors, and other molecules, that constitute the “glycosylation machinery”. Amongst the enzymes of the glycosyolation machinery are the glycosyltransferases, which catalyze the transfer of sugar moieties from activated donor molecules to specific acceptor molecules. The step-wise addition of carbohydrates to other saccharides, proteins, or lipids is initiated in the Endoplasmic Reticulum and the glycan biosynthesis continues in the Golgi apparatus. Finally, the glycoproteins or glycolipids carrying these synthesized glycans are transferred to the cell membrane or to secretory vesicles. Glycans can be classified in four general classes: N-glycans, O-glycans, Glycosaminoglycans, and glycolipids.

Glycan binding receptors expressed by dendritic cells

As discussed before, pathogens as well as our own cells highly express glycosylated structures. Pathogens and tumour cells should be attacked by the immune system. In contrast, our own healthy cells should not be attacked by the immune system, as occurs in autoimmune diseases. In addition, different classes of pathogen require specific immune responses to clear the infection elicited by these pathogens. The first line of defence in our body is the dendritic cell, which bears special glycan-recognizing sensors. These sensors, or lectins, are specialized to discriminate between self- and foreign antigen, and

between different subclasses of pathogens. After glycan recognition by its lectins, the dendritic cell is able to initiate an appropriate immune response in case of pathogens, or will induce tolerance if a self-antigen is addressed. Dendritic cells express a broad range of glycan recognizing lectins.
A well-characterized group of lectins expressed by dendritic cells are C-type lectins. DC-SIGN is a member of the family of C-type lectins. In **chapter 2** we have investigated binding of the tumour antigen gp100 to DC-SIGN on dendritic cells. Gp100 binds low to DC-SIGN, but when the glycans expressed by gp100 were modified special glycan structures that are ligands for DC-SIGN, the binding of gp100 to dendritic cells was increased. Interaction of DC-SIGN with the modified form of gp100 resulted in an enhanced antigen presentation to T cells. This shows that lectins expressed by dendritic cells can be targeted with specific glycan structures to improve the immune response.
We next investigated in **chapter 3** if glycan structures on pathogens which target different DC-expressed lectins can modify the immune response. Herefor, we compared two strains of the bacteria *C. jejuni*, the causative agent in the Guillain-Barré syndrome. The only difference between thsese two strains was the linkage of the terminal monosaccharide. For this purpose we examined another member of the family of DC-expressed lectins, the Siglecs. We observed that Siglec-1 only bound to one of the two strains of *C. jejuni*, whereas Siglec-7 bound with high affinitiy to the other *C. jejuni* strain. Interestingly, targeting these different DC-expressed Siglecs with strains of *C. jejuni* with only a difference in the terminal monosaccharide resulted in opposite T helper cell responses (Th1 versus Th2). This shows that targeting specific lectins expressed by dendritic cells not only improve T cell responses (chapter 2), but also modify T cell responses.
Another important property of dendritic cells is to discriminate between self and foreign antigens. C-type lectins and Siglecs bind, besides glycosylated pathogens, to glycosylated self-proteins as well. Siglecs expressed on dendritic cells bear inhibitory motifs in their cytoplasmic tail. By binding to self-antigens the dendritic cell should induce tolerance. In **chapter 4** we investigated if glycans that are found on human self-antigens induce tolerance by dendritic cells. Herefore, we coupled the glycan ligand for Siglec-E, which is under homeostatic conditions expressed on gangliosides in the human brain, to an antigen. Indeed, coupling a self-glycan that is found under normal conditions to an antigen resulted in increased binding to dendritic cells compared to the binding of unmodified antigen to dendritic cells. The antigen with modified glycans bound to Siglec-E, a Siglec with an inhibitory motif. Binding of the glycosylated antigen to Siglec-E resulted in reduced antigen presentation of dendritic cells to T cells. This showed us that targeting lectins of dendritic cells can improve (chapter 2), modify (chapter 3) and dampen (chapter 4) the immune response depending on the nature of the glycan ligand.

Glycosylation of immune cells

Glycosylation of our own cells plays an essential role in the maintenance of homeostasis. Only recently it has been shown that glycans expressed on our cells are implicated in multiple aspects of the immune system. Every cell of the immune system expresses its own set of glycans that can be changed during cellular differentiation, activation and apoptosis. Furthermore, other important immune cell functions, including cell trafficking,

cellular interactions, antigen- and cytokine-receptor activation, autoimmunity and pathogen recognition are linked to glycan alterations.
Immature dendritic cells reside in the tissue where they capture pathogens, whereas mature dendritic cells are able to activate T cells in the lymph node. This dramatic functional change of immature dendritic cells to mature dendritic cells is mediated by an important genetic reprogramming. To investigate the involvement of glycosylation in the changes that occur during dendritic cell maturation, we have studied the differences in the expression of glycans on immature dendritic cells and mature dendritic cells as well as their glycosylation machinery in **chapter 5**. We found a profound maturation-induced upregulation of glycan structures that serve as ligands for Siglecs and Galectins. We demonstated that Sialoadhesin (Siglec-1) only bound to mature dendritic cells and not to immature dendritic cells, which was further investigated in **chapter 6**. Sialoadhesin is expressed on macrophages, which is an antigen presenting cell, similar to dendritic cells. Sialoadhesin expressed on macrophages bind to glycan structures expressed on mature dendritic cells to induce cellular interactions between dendritic cells and macrophages to transfer antigens. Through antigen transfer, the immune response is improved by the ability to present more antigens to T cells in the lymph node.
Glycan structures that are upregulated on the cell surface of dendritic cells during maturation are not only involved in lectin binding, but they improve the migratory capacities of mature dendritic cells as well. In **chapter 7** we describe that the glycan structure Polysialic acid, a long homopolymer of linked sialic acids, captures chemokines expressed by the lymph node. Hereby the migration of mature dendritic cells to T cells present in the lymph nodes is improved.

Conclusion

It becomes more and more evident that glycosylation plays an essential role in the immune system. In this thesis we show that glycosylation of pathogens, tumour cells and self-antigens modulates dendritic cell-mediated immune responses. Our results indicate that glycan modification of tumour antigens to target lectins of dendritic cells is a new way to develop dendritic cell vaccination strategies that enhance the induction of tumour-specific T cell responses. In addition, we show that targeting Siglecs expressed on dendritic cells *in vivo* with specific glycans is a promising tool to dampen unwanted T cell responses, as occur in auto-immune diseases. Beside the role of glycans expressed on pathogens and tumour cells for dendritic cell modulation, we investigated the glycosylation of the dendritic cell itself as well. We unraveled the functional consequences of glycan structures that are upregulated during cellular activation. Glycan structures expressed on the cell surface of mature dendritic cells are involved in cellular interactions of dendritic cells with other cells of the immune system. Beside cellular interactions, glycans exclusively expressed by mature dendritic cells regulate the migration of dendritic cells towards T cells in the lymph node. Based on the results of this study, it should be taken into account to analyse glycan expression levels on dendritic cells in vaccination based therapies in which efficient migration of *ex vivo* generated dendritic cells is required. In general we conclude that that glycosylation has important functional consequenses on dendritic cell biology.