Summary

Every day the human body is exposed to various different threats. These can come along in the form of pathogens, like viruses and bacteria, however, also processes in the body can cause damage, for instance in the case of cancer. The function of the immune system is to protect the human body against these dangers. So called antigen presenting cells are present in different tissues and in the blood stream. These cells are specialized in the detection of dangerous components. They recognize danger signals via receptors, present on the surface of these cells. In addition, they can take up pathogens and travel to the lymph nodes where they activate the effector arm of the immune system: the T- and B-cells. An over-activated immune system can lead to the development of allergy and auto-immune diseases. In these cases the immune system responds to harmless particles, like nuts or components naturally present in the human body, such as the joints in rheumatoid arthritis.

The dendritic cell is one of the professional antigen presenting cells present in the human body. Different receptors, present on the surface of dendritic cells, participate in the detection of pathogens and other dangerous components, including the C-type lectin receptors (CLRs). CLRs are involved in the recognition of different sugar or glycan structures. Glycan structures are present on the surface of virtually all cells, including pathogens. During the synthesis of membrane proteins, these proteins are covered with glycans. The attachment of glycan structures to the protein depends on a specific amino acids sequence, which are the building blocks of proteins. Glycan structures themselves are built up from a variety of monosaccharides, like the well-known sugar glucose, but also other monosaccharides, like mannose, fucose and N-acetylgalactosamine (GalNAc) are present in the human body. The configuration of the glycan structure is dependent on the cell type and the protein it is attached to. Usually, glycan structures present on pathogens are different from those present on human cells. In this way dendritic cells can distinguish pathogens from healthy self-antigens. Glycan structures on human cells can be altered during cellular activation or cell aging. Furthermore, cancer cells possess different glycan structures compared to normal cells, which can dampen the immune response instead of the desirable immune activation needed for the eradication of the cancer cells.

The different members of the CLR family each recognize specific glycan structures, which vary between group members. Extensive research has been performed to elucidate the function and glycan specificity of the CLR DC-SIGN. DC-SIGN binds the sugars mannose and fucose, and therefore interacts with both pathogens, like the HIV-1 virus, and self-glycoproteins, in both healthy situation and during disease. As a result DC-SIGN can internalize the virus and present it to T-cells that can become infected. In addition, it can also facilitate the cell diapedesis by binding firmly to proteins expressed on endothelial cells. Another CLR present on the surface of dendritic cells is DCIR. This
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receptor is thought to give inhibitory signals to the immune system and is therefore an important target for the development of therapeutic strategies against allergy and auto-immune diseases. However, one must first know the exact function and glycan specificity of the CLRs, before they can be utilized as targets for immune therapies.

Because the primary glycan binding site, involved in the interaction between CLRs and glycan structures, is quite similar for DCIR and DC-SIGN, it is believed that DCIR has a comparable glycan specificity as DC-SIGN. We have investigated the glycan specificity of DCIR and found that DCIR indeed interacts with mannose and fucose-containing glycans as well (Chapter 2). However, the glycan specificity of DCIR is much more restricted, since only a small variety of mannose and fucose-containing glycans can bind to DCIR, whereas almost all these glycan structures interact with DC-SIGN (Chapter 3).

Since DCIR and DC-SIGN are expressed on the cell surface, they are glycosylated by the glycosylation machinery of dendritic cells. The glycan structures on DC-SIGN are located far away from the glycan binding site, while the glycan structures on DCIR are situated close by the glycan binding site. This glycan on DCIR could potentially hinder the interaction between DCIR and glycan structures on other particles. We indeed observed that by removing the glycan structure on DCIR or by reducing the size of the glycan structure present, the potency of DCIR to bind glycans was effected. Our results revealed that the glycan structure in DCIR indeed prohibits the potential of DCIR to bind glycan structures on other particles (Chapter 2). In addition, we also discovered that binding of DCIR to a glycan structure induces a signal inside the cell.

We next investigated which ligands contain glycan structures that can interact with DCIR. Binding of DCIR to different primary human cells, tumor cells and pathogens was investigated. DCIR specifically bound to keratinocytes, cells present in the upper layers of the skin, but also to different kinds of tumor cells. In addition, DCIR could bind to HIV-1 and some helminth species, but not to yeast particles tested. This indicates that DCIR, like DC-SIGN can interact with different self-proteins and pathogens. Indeed most compounds that bound DCIR could also interact with DC-SIGN. Nevertheless, keratinocytes selectively bound to DCIR and not to DC-SIGN, while DC-SIGN binds specifically to yeast and DCIR does not (Chapter 3).

Besides its signaling function, DCIR could also be involved in the uptake of glycosylated particles. Once taken up, these particles can be degraded inside the cell into smaller fragments that are subsequently presented to T cells to initiate particle-specific T cell activation. Peptides from ingested ligands activate CD4⁺ T helper cells that facilitate antibody production by B cells in order to eliminate extracellular pathogens and help macrophages to actively destroy internalized pathogens, while CD8⁺ T cells are activated by virally infected cells which give a signal for their eradication. Dendritic cells can also present peptides derived from ingested antigens to CD8⁺ T cells via a process called cross-presentation. Prior research has already revealed that glycosylated particles taken up by DC-SIGN can be presented to CD8⁺ T-cells, however the specific
intracellular routing of DC-SIGN ingested particles is not exactly known. We addressed this by employing a new technique that allows high throughput analysis of both the morphology of a pool of individual cells and the visualization of fluorescently labeled ingested proteins in time in combination with fluorescently labeled marker proteins for intracellular compartments. Using this technique we characterized a new intracellular route for particles taken up by DC-SIGN that end up in proteins present on the cell membrane involved in the activation of CD8+ T cells (Chapter 4). In addition, we compared the intracellular routing of particles taken up by DC-SIGN with that taken up by DCIR and two other CLRs, MGL and MR. We observed a differential intracellular routing of particles that were taken up via DCIR and an effect of DCIR stimulation on the routing of DC-SIGN ingested particles (Chapter 5).

Finally, we investigated an unique glycan structure, displayed on a commensal bacterium present in the human intestinal flora. This glycan structure can be taken up by antigen presenting cells, leading to its direct presentation to T-cells, while this normally only occurs for protein antigens. The exact mode of uptake of this glycan structure was not known and we hypothesized that a CLR might be involved. Indeed, we found a profound role for DC-SIGN in the uptake and presentation of this specific glycan structure, while, for example DCIR, was not involved (Chapter 6).

In conclusion, we began to unravel the specific function of DCIR, by discovering glycosylated compounds that can interact with DCIR. More research is needed to elucidate when DCIR is occupied and when it is free to bind glycans on other proteins. In addition, it is important to elucidate under which circumstances DCIR is stimulated by its own glycan structures and if DCIR signaling could dampen the immune response. When these factors are known DCIR could be used as target for immune therapies.
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