Effects of sialic acids in Immunity

The immune system is a complex network of cells with the unique goal of defending the body from the attack of invading pathogens such as bacteria, viruses and fungi. The immune system consists of the innate immune system, which is the first line of defence against the invading organisms, and the acquired immune system, which provides a faster and a stronger response if pathogens are able to escape the first line of defence. The innate immune system comprises natural killer (NK) cells, dendritic cells (DCs), macrophages and granulocytes; while the acquired immune system is made of lymphocytes called CD4$^+$ and CD8$^+$ T cells. DCs are considered the police of the immune system, and they play a crucial role in the interface between the innate and acquired immune branches. Upon encountering the pathogen, DCs arrest, “eat” and process the invading organism in single pieces called antigens, which are exposed on DC surface. During this process, DCs migrate to the lymph nodes where uninstructed CD4$^+$ and CD8$^+$ T cells reside. Here, only T cells that have the receptor specific for the pathogenic antigen are activated. T cells are now instructed and ready to migrate to the site of infection to fight the invader. In this way, the immune response is adapted to the kind of pathogen invading our body, allowing an efficient and specific elimination of the pathogen. However, DCs also eat and process our own cells in antigens (self antigens) that are presented to the T cells. To avoid that T cells are activated against our own cells, the immune system comprises T cells called regulatory T cells (Tregs), because they regulate the activity of the immune system, preventing and avoiding an undesired immune response that could be harmful for the body. Tregs block the activation of T cells recognizing self antigens, preventing the formation of auto-immunity. Moreover, Tregs suppress an unwanted immune activation against exogenous antigens that are harmless for us, avoiding the generation of allergies.

Besides acting against external organisms, the immune system also protect us from our own cells that could damage ourselves such as cells that grow in an aberrant and uncontrollable way, leading thus to the formation of tumor. However, tumors can escape recognition and elimination by the immune system in different ways. One of these include abberantly express sugars on this surface such as sialic acids.

However, a correlation between high expression of sialic acids on tumor and impaired immune system has not been elucidated yet. In chapter 2, we investigated the role of tumor hypersialylation in impairing the immune response against tumor. We found that reduced levels of sialic acids on murine melanoma B16-OVA (Sia$^{low}$) significantly correlated with delayed tumor growth in mice and increased numbers of CD4$^+$ and CD8$^+$ T cells at the tumor site as well as in the tumor draining lymph nodes (TDLNs). In particular, decreasing the expression of hypersialylation in B16-OVA diminished the proportion of intra-tumoral CD4$^+$ and CD8$^+$ Tregs, indicating that reduced hyper-sialylation in B16-OVA switches the function of tumor-specific T cells from tolerogenic to effector. Analysis of intra-tumoral immune composition at early stage of melanoma growth revealed an increased numbers of NK cells together with amounts of IFN-$\gamma$. Moreover, our studies unveiled a crucial role for activated NK cells igniting different pathways that result in the generation of tumor-specific effector T cells. Indeed, Sialow tumors that were injected in mice depleted of NK cells grew at comparable rates of Siahigh tumors, canceling thus the effect seen previously in NK-competent mice. Furthermore, the increased infiltration of IFN-$\gamma$-producing CD4$^+$ and CD8$^+$ T cells as well as the decreased frequency of FoxP3$^+$
CD4+ T cells found within Sia\textsuperscript{low} tumors were absent when NK cells were depleted. The effect of reduced Sia levels on NK activity was also observed in vitro, where NK cells produced more IFN-\(\gamma\) when incubated with Sia\textsuperscript{low} melanoma.

Although in tumors are deleterious to evade and escape the immune recognition and elimination, Tregs are crucial in avoiding and preventing unwarranted and excessive reactions that would be harmful for the body. Therefore, seen the correlation of sialic acids with Treg numbers in tumors, in chapter 3, we analyzed whether sialic acids on antigen can modulate T cell differentiation inducing antigen-specific Tregs. We demonstrated that DC-mediated uptake of Ovalbumin (OVA) modified with sialic acids (Sia-OVA) induced the differentiation of naive OVA-responsive CD4+ T cells into CD4+ FoxP3+ T cells with immunosuppressive functions while preventing induction of IFN-\(\gamma\) CD4+ T cells in vitro and in vivo. Additionally, the de novo generation of CD4+ FoxP3+ was further confirmed by using naive CD4+ T cells from DO11.10 Rag\textsuperscript{-/-} mice, which lack naturally occurring Tregs (nTregs). Here, we also demonstrated that the murine Sialic acid binding receptor Siglec-E prevalently mediated the binding and uptake of Sia-OVA by DCs as the absence of Siglec-E on DCs significant reduced Sia-OVA binding and uptake. The analysis of Sia-OVA-loaded DCs showed a diminution in secretion of anti-inflammatory cytokines IL-10 and TGF-\(\beta\), while the expression of co-stimulatory molecules such as CD80 and CD86 was altered compared to DCs loaded with unmodified OVA. The effects of Sialic acids on DC-mediated T cell differentiation was also seen by modifying other antigens with sialic acids. Indeed, coupling the antigen MOG, which induces the experimental autoimmune Encephalomyelitis (EAE) in mice, with sialic acids remarkably decreases the proliferation and secretion of IFN-\(\gamma\) by MOG-reactive splenocytes form mice with different severity scores of EAE.

As seen for CD4+ T cell differentiation. In chapter 4, we analyzed the effects of sialic acids on antigen in the DC-mediated CD8+ T cell activation. The addition of sialic acids on OVA DCs indeed significantly decreased the generation of DC-mediated effector CD8+ T cells as determined by increased expression of IFN-\(\gamma\) and the enzyme granzyme B, which are released by CD8+ T cells to kill the target cell. Remarkably, the uptake of Sia-modified OVA by DCs promoted the generation of CD8+ FoxP3+ Tregs, although the simultaneous incubation of DCs with Sia-OVA and Lipopolysaccharides (LPS) drastically cancelled this effect, which is in contrast to our findings on CD4+ FoxP3+ T cells. The phenotype analysis of CD8+ Tregs induced by Sia-OVA revealed an overexpression of the exonucleotidase molecule CD39. This highlighted the possible mechanisms through which the de novo generated CD8+ Tregs can explicate their immunosuppressive functions. and dampen unwanted CD8+ T cell responses.

Given the effects of sialic acids in the de novo generation of CD4+ and CD8+ Tregs at the expense of effector T cells, we asked how modification of OVA with sialic acids could lead to this tolerogenic effect. In chapter 5, we showed that DCs taking up sia-modified OVA induced Tregs in a cell-contact dependent manner rather than by soluble factors. Gene expression analysis of DCs upon Sia-modified OVA confirmed the overexpression of tolerogenic molecules such as the ILT-3 receptor, whose expression has been associated with tolerogenic DCs, and mammalian target of rapamycin (mTOR) pathway. Moreover, we also found an overexpression of gene encoding for Siglec-E as well as phosphatases involved in the downstream signal triggered by this Siglec. So, this result further confirms a crucial involvement of Siglec-E in mediating the tolerogenic effects of sialic acids on DCs. The gene expression analysis of Sia-OVA-loaded DCs gave us new insights about the molecules potentially involved in the tolerogenic effects of DCSia-OVA.
In conclusion, in this thesis we showed that manipulation of sialic acids modulate the differentiation of CD4$^+$ and CD8$^+$ T cells in Tregs as well impair the effector functions of NK cells. Therefore, sialic acids can have therapeutical implications in the treatment of tumors and auto-immune disorders. More specifically, if reduction sialic acids can reduce intra-tumoral Tregs, so allowing a more effective immune response against tumors, their addition on antigens can instead induce Tregs that prevent or restrain antigen-related diseases such as multiple sclerosis, Reumathoid Arthritis and Type 1 Diabetes.