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## Interplay of CD169+ macrophages and dendritic cells

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## Summary

The immune system exists to protect us from infections. The innate part of the immune system responds quickly to pathogens and helps to activate the adaptive immune system, which responds with high specificity to pathogens and creates immunological memory. Through vaccination, the adaptive immune system can be stimulated to generate protective immunity and prevent disease, without suffering the inherent risks of the disease. Vaccination protects many people around the world against life-threatening consequences of infections and has resulted in the eradication of smallpox. Furthermore, the immune system can be harnessed to induce immunity against cancer and immunotherapies have successfully been applied in cancer patients.

Immune checkpoints are important to maintain the delicate balance between tolerance against self and activation against non-self. A tumor can interfere with this balance by mediating suppression of the immune system. A breakthrough in the treatment of cancer patients was made with the application of immune checkpoint inhibitors, which are antibodies (Abs) that intervene with tumor-induced suppression of the immune system. The treatment of melanoma patients with these checkpoint inhibitors has shown promising results, but, because not all patients respond to this therapy, there is still room for improvement.

There is a growing understanding of why certain patients do not respond to checkpoint inhibition therapy. A negative clinical response after checkpoint inhibitor treatment has been linked to a lower T cell infiltration in the tumor. To improve the treatment of patients with non-responsive tumors, vaccination strategies are being developed to induce strong tumor-specific T cell responses. The combination of vaccines with checkpoint inhibitors will induce T cell responses and simultaneously release the brake on these cells to generate an even more efficient anti-cancer response.

Antigen (Ag) presentation by dendritic cells (DCs) is essential for the induction of Ag-specific cytotoxic- and helper T cells and antigen targeting to DCs has been shown to stimulate strong anti-tumor T cell responses in mice. In Chapter 3 we discuss vaccine strategies in which Ags are directed to C-type lectin receptors (CLR) specifically expressed by DCs and/or macrophages. CLRs are receptors for carbohydrate structures, which are highly expressed by multiple subsets of DCs and macrophages. CLRs are involved in uptake and processing of Ags for presentation and for this reason these receptors are particularly interesting for targeting purposes. We provide an overview of different methods of Ag targeting to different CLRs on DCs

and macrophages in pre-clinical cancer models and describe some clinical studies that have been performed for Ag targeting to CLRs in cancer patients.

An interesting receptor that also binds carbohydrate structures is CD169, also known as sialoadhesin and sialic acid-binding immunoglobulin-type lectin (Siglec-1). CD169 is involved in cell-cell and cell-pathogen interactions and is highly expressed by a specific subset of macrophages residing in the spleen and lymph nodes. The strategic location of CD169<sup>+</sup> macrophages in the marginal zone of the spleen and subcapsular sinus of the lymph nodes enables them to capture antigens from the blood and the lymph, respectively. In Chapter 2 we investigate the requirements for induction of CD8<sup>+</sup> T cell responses by Ags bound by CD169<sup>+</sup> macrophages. Upon immunization with ovalbumin (OVA) conjugated to CD169-specific Abs, we show that a specific subset of DCs, the Batf3-dependent CD8 $\alpha$ <sup>+</sup> dendritic cells (cDC1s), receives OVA from CD169<sup>+</sup> macrophages and activates CD8<sup>+</sup> T cell responses. Induction of CD8<sup>+</sup> T cell responses after CD169 targeting relied on DNGR-1 expression that is selectively expressed by cDC1s. In addition, we demonstrate that DCs and specifically cDC1s express sialylated molecules that mediate CD169 binding. Using a mouse model expressing a mutated CD169 molecule unable to bind sialic acids, we show that Ag transfer to cDC1s is dependent on the sialic acid-binding capacity of CD169 for subsequent CD8<sup>+</sup> T cell activation. Finally, CD8<sup>+</sup> T cell responses to vaccinia virus infection are dependent on functional CD169. Together, these data indicate that the collaboration of CD169<sup>+</sup> macrophages and cDC1s for the initiation of effective CD8<sup>+</sup> T cell responses is facilitated by binding of CD169 to sialic acid-containing ligands on cDC1s.

The strong activation of CD8<sup>+</sup> T cell responses after antigen targeting to CD169 led us to further explore its application as a vaccination strategy for the induction of anti-tumor T cell immunity. To test whether a single linear peptide containing the minimal tumor-specific epitope can be sufficient to induce an anti-tumor immune response, we compared OVA protein and peptide Ab:Ag conjugates that target to CD169 in Chapter 4. We monitored the induction of Ag-specific primary, memory, and recall CD8<sup>+</sup> T cell responses after protein and peptide targeting and observed no significant differences. Moreover, the anti-tumor immune response in mice bearing OVA-expressing melanoma tumors after immunization with either protein or peptide targeted to CD169 showed no significant differences in controlling tumor outgrowth. We conclude that both protein and peptide targeting to CD169 results in strong primary, memory and recall T cell responses and protective immunity against melanoma. This indicates that both forms of antigen, peptide or protein, can be further explored as anti-cancer vaccination strategy.

## Addendum

Important for development of anti-tumor vaccination strategies is the translation from mice to humans and to show the efficacy with tumor-associated or tumor-specific epitopes. In Chapter 5 we continued to investigate the CD169-based vaccination strategy using melanoma-associated peptides for targeting to mouse and human CD169<sup>+</sup> cells. Immunization with gp100 and Trp2-antiCD169 conjugates efficiently induced gp100 and Trp2-specific T cell responses in wild-type mice. Targeting of the HLA-A2.1-restricted human melanoma antigen recognized by T cells 1 (MART-1) peptide in HLA-A2.1 transgenic mice induced strong MART-1-specific T cell responses. These data indicate that Ab-mediated Ag targeting to CD169<sup>+</sup> macrophages is a potential strategy for the induction of melanoma-specific T cell responses in mice. In human spleens we detected CD169<sup>+</sup> cells at an equivalent location as in mice using immunofluorescence microscopy. To study antigen targeting to human CD169<sup>+</sup> cells *in vitro* we used monocyte-derived DCs (MoDCs) that express CD169 upon stimulation with IFN $\alpha$ . Human gp100 peptide conjugated to Abs specific for human CD169 bound to and were taken up by CD169-expressing MoDCs and resulted in activation of gp100-specific CD8<sup>+</sup> T cells. These data support the potential to translate our CD169-based vaccination strategy for clinical application in humans.

In our immunization experiments in mice, we used rat-derived anti-mouse CD169 IgG2a Abs that are immunogenic in mice and that can potentially bind to Fc receptors. To create a better-defined tool for further vaccine-development, we established recombinant CD169-specific mouse Abs, as described in Chapter 6. The variable region of rat-anti-mouseCD169 Ab was cloned in a mouse IgG1 backbone with a mutation to prevent Fc receptor binding and encoding OVA as model Ag. The recombinant hybrid mouse IgG1 Abs induced equivalent OVA-specific CD8<sup>+</sup> T cell responses as the chemically conjugated complexes of the original rat Abs with OVA. We subsequently used these hybrid Abs to determine the most optimal antigen delivery route. Comparison of intravenous and subcutaneous immunization indicated slightly higher T cell responses after intravenous injection compared to subcutaneous injection. Together our data suggest that recombinant Ab:Ag specific for CD169 could provide a delivery strategy of tumor antigens to the immune system for the induction of efficient anti-tumor T cell responses.

In conclusion, our studies demonstrate that antigen targeting to CD169<sup>+</sup> macrophages is effective for both protein as well as peptide Ags and can result in anti-tumor immunity. We show that this immunization route results in effective CD8<sup>+</sup> T cell activation by cDC1 and our experiments with human cells supports the potential to translate our CD169-based vaccination strategy towards clinical application in humans.