In vivo mechanism of antibody-based immunotherapy

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Summary

Antibodies that bind to proteins on the surface of tumors (tumor antigens) or to proteins that have an important regulatory function in the immune system ("immune checkpoints") are successfully used in the clinic to fight cancer. Binding of the antibodies to tumor antigens can not only directly inhibit tumor growth or kill the tumor cells but can also indirectly activate an immune response directed against the tumor, as recent research has shown. Antibodies consist of a variable region that binds to the antigen and a constant region (Fc region) that can bind to Fc receptors (FcγR), molecules on the surface of a number of different types of immune cells. Binding to FcγR of antibodies, bound to a tumor cell with their variable region, activates immune responses that can kill the tumor cell. Antibodies that bind to immune checkpoints block the immune-inhibiting signals that are normally needed to prevent a harmful over-reaction of the immune system. This results in the (re) activation of natural anti-tumor responses. As the response to antibody therapy varies widely in both preclinical and clinical application, the overall goal of the study described in this thesis was to investigate different treatment strategies in a number of pre-clinical mouse models to facilitate the development of improved antibody therapy.

The PD-L1 immune checkpoint inhibits the spontaneous response of the T cells of the immune system against the tumor. Therefore, PD-L1 blocking antibodies increase the immune response against the tumor. However, it is not clear whether the interaction of anti-PD-L1 antibodies with FcR contributes positively or negatively to the anti-PD-L1 antibody therapy. In Chapter 2 four forms of anti-PD-L1 antiserum are used that differed only in the strength of the binding of their Fc part to FcγR. All antibodies were equally effective in suppressing the growth of a colon cancer called MC38 in mice. The therapeutic efficacy of these anti-PD-L1 antibodies was neither reduced nor increased in mice lacking all FcγR, suggesting that FcR did not play a role in anti-PD-L1 therapy in the MC38 tumor model in mice. Interestingly, in another colon cancer model, CT26, the highest therapeutic efficacy was observed in mice receiving anti-PD-L1 with the strongest binding to FcγR. From this it can be concluded that interaction of anti-PD-L1 with FcγR does not negatively influence the therapeutic effect but may increase the therapeutic efficacy of the antibody depending on the tumor model.

Increased production of TGF-β by cells in the microenvironment of the tumor has been shown to reduce the therapeutic efficacy of anti-PD-L1. In Chapter 3 it is demonstrated in the MC38 model that the combination of a TGF-β inhibitor and anti-PD-L1 antibody was more effective than TGF-β or anti-PD-L1 monotherapy. This was accompanied by an increased infiltration of $CD8^+$ T cells into the tumor compared to monotherapy. However, TGF-β was as effective as the combination therapy in suppressing the growth of the aggressive pancreatic tumor KPC1. These results suggest that the cooperative anti-tumor activity of anti-PDL1 and the TGF-β inhibitor is dependent on the tumor model. This emphasizes the importance of evaluating the efficacy of different therapies in different tumor models.

It was previously shown that treatment with an antibody directed against a tumor antigen on an established melanoma has little therapeutic effect in mice. In chapter 4 we show that the

administration of the cytokine IL-2 and an immune-stimulating agent, imiquimod, to mice with an established melanoma, greatly improves the therapeutic effect of a melanoma-specific antibody, resulting in a significantly higher survival of the melanoma bearing mice. By using mice that missed a single type of FcγR or the FcγR on only a particular cell type and by selectively removing specific types of immune cells, we were able to demonstrate that one particular FcγR, FcγRI, on a particular type of immune cell, the macrophage, was essential for the effectiveness of the combination therapy. In the presence of imiquimod, more active macrophages with more FcγR were found to be present on their cell surface in the microenvironment of the melanoma. Moreover, a different type of immune cell, the killer T cell, was indispensable for the therapeutic effect of the combination therapy. These findings show that increasing the presence in the tumor microenvironment of certain immune cells with high FcR on their cell surface by an agent such as imiquimod that stimulates the immune system can serve as a strategy to improve the effectiveness of antibody therapy and also show the importance of initiating T cell responses.

The important role of T cells in the effectiveness of tumor targeting antibodies is further explained in Chapter 5. Tumors deviate from normal healthy tissue and can therefore usually be recognized by the immune system, ie they are immunogenic. However, the degree of immunogenicity varies between tumors. In chapter 5, it is shown that highly immunogenic tumors contain a greater number of infiltrating T cells in their microenvironment than fewer immunogenic tumors. The higher infiltration of T cells was associated with the increased anti-tumor activity of a therapeutic antibody that recognises the tumor antigen rat-neu on mammary tumors. This suggests that classification into groups of cancer patients with $Her2^{+}$ tumors based on the number of T cells already present in the tumor may be useful to predict the response with anti-Her2 antibody therapy. Previous studies suggest that FcγR could contribute to anti-neu antibody therapy. However, most recent research on the role of FcγR was conducted in BALB/c mice that were deficient for the common FcR-γ chain. Although these mice do not have functional activating FcγRs, they also lack other relevant receptor complexes of which the common FcR-γ chain is also part. The role of FcγR in anti-neu antibody therapy has therefore not been clearly established. In addition, the contribution of each type of FcR and type of immune cell that expresses FcγR, to anti-neu antibody therapy has not been established *in vivo* because most FcγR knockout mouse strains are on C57BL/6 background, whereas the mouse MMTV-NeuT mammary tumor model is on BALB/c background. Chapter 6 describes the generation and characterization of a MMTV-NeuT mouse on C57BL/6 background. Importantly, there was no difference in the infiltration of immune cells into the tumors in BALB/c-NeuT and C57BL/6-NeuT mice, suggesting that C57BL/6-NeuT could be an alternative tumor model for preclinical studies. In future studies, with different cell type-specific FcR-deficient C57BL/6-NeuT mice the specific contribution of each FcγR to the therapeutic efficacy of anti-neu mAb therapy can be determined. Such information would help to design new strategies for increasing the therapeutic effectiveness of tumor targeting antibodies.