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The role of glycosylation as a modulator for signalling strength in T cells

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Adoptive immunotherapy has proven efficiency in patients suffering from malignancies. However, the quality of *in vitro* generated tumor antigen-specific T cells which can be used for such therapies is limited. Therefore, methods have to be developed in order to increase potency of these tumor antigen-specific T lymphocytes. One possibility arises from the modification of glycoproteins such as the T cell receptor (TCR) or CD8. By their size and charge, sugar chains of these molecules play a role in the interaction of a T cell with the major histocompatibility complex (MHC). It is also reported that glycosylation decreases mobility of cell surface molecules by cross linking to carbohydrate binding proteins (so called lectins, e.g. Galectin-3). The resulting molecular lattice constrains TCR and CD8 clustering. Deglycosylation of the TCR and CD8 might be a possibility in order to increase mobility of the TCR or CD8 and to augment signalling strength. Unspecific global O- and N-deglycosylation were compared as well as a specific removal of selective glycosylation motives in defined proteins as the TCR.

For exogenous deglycosylation, T cells were treated with neuraminidase, which removes sialic acids at the end of sugar chains. This resulted in a better communication of the O-glycosylated CD8 with the MHC I molecule which was demonstrated by an increased specific tetramer-binding. Surprisingly, the ability to kill tumor cells was not increased. To inhibit the interaction of N-linked sugars of the TCR with Galectin-3 and so to increase mobility of the TCR, T cells were treated with lactose, which competes with the Galectin-binding sugars for Galectin-3. However, lactose-treatment did also not ameliorate target cell lysis. Thus, T cell deglycosylation could increase tetramer-binding, but this was not translated into a better T cell activity. We assumed, that other surface molecules which are important for signalling were negatively affected by these treatments. As consequence, we silenced N-glycosylation motives in the TCR molecule itself by point mutation. Wild-type and mutant TCRs were transduced into human T cells or the murine T cell hybridoma 58a-b- by retroviral gene transfer. Efficacy of TCR deglycosylation was ascertained by western blot analysis. Removing one or two defined sugar chains increased antigen-specific cytokine secretion. Deletion of three or more glycosylation sites impaired T cell function.

In summary, T cell deglycosylation is a sensitive mechanism. Deletion of defined glycosylation motives within a TCR is an efficient tool to increase signalling strength of a T cell.