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Superagonist peptide variants of hMBP115-126 tolerize antigen-specific T cells in HLA-DR4 transgenic mice

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Multiple sclerosis (MS) is a heterogeneous demyelinating disease of the central nervous system affecting approximately one million patients world wide. As current therapeutic strategies are far from being effective and carry many unacceptable side effects, novel and more efficient therapies are needed. Since the pathogenesis of MS is far from being completely understood, animal models of the disease remain an important tool in providing new insights into disease-concepts as well as developing new therapies. Experimental autoimmune encephalomyelitis (EAE) in mice is the most widely used model for MS research. The current concept is that autoreactive $CD4^+$ T cells are critically involved in the pathogenesis of the disease and susceptibility to MS appears to be strongly linked to HLA-DR molecules, particularly to HLA-DR4 (HLA-DRB1*0401) in a certain subgroup. Thus, the introduction of human HLA genes into the murine EAE model emulates more closely human conditions and facilitates the development and translation of therapeutic approaches for human disases. We applied a humanized EAE model using HLA-DR4 (HLA-DRB1*0401) transgenic mice. Historically, most of the attention was focused on T cells specific for high-affinity MHC-binding myelin peptides in MS patients, such as MBP85-99, but clinical trials aimed at therapeutically targeting these T cells showed only limited success. Recently, high avidity T cells specific for low affinity MHC binding myelin peptides have been shown to be involved in EAE as well as MS, therefore we studied whether high-avidity autoreactive T cells specific for the immunodominant low-affinity peptide MBP115-126 can be tolerized as a possible treatment for MS. Studies in a conventional EAE mouse model suggested that high-avidity T cells specific for the low-affinity MHC binding immunodominant peptide can be deleted by superagonist, i.e. analogues of this peptide with greatly enhanced binding-affinity for MHC. Accordingly, we translated this approach to a “humanized” HLA-DR4 transgenic mouse model.
Our results show that we successfully devised variant peptides with higher binding affinity to HLA DR4 as compared to the immunodominant WT peptide MBP115-126, whilst preserving TCR contact sites. Peptides were engineered by application of a computer based algorithm which allowed for the prediction of the peptide-binding affinity to the HLA-DR4 molecule. Of several newly generated variant peptides we identified peptide PV550 as a candidate superagonist peptide. The peptides were tested *in vitro* using several MBP115-126 specific TCC and TCL generated in our laboratory as well as *in vivo* in HLA-DR4 tg mice. T cell stimulation and tolerization was measured by ELISPOT and proliferation assay Peptide PV550 was found to deliver an unphysiologically strong TCR mediated signal thus tolerizing MBP115-126-specific T cells *in vitro* as well as *in vivo*. We also showed that loss of T cell reactivity after supraoptimal stimulation with the peptide PV550 was mainly due to apoptosis as one possible mechanism of T cell tolerance induction.

Conclusion: The aim of this study was to engineer variant superagonist peptides of MBP115-12 with highly increased binding affinity for HLA-DR4 to induce tolerance of MBP115-126-reactive T cells *in vitro* as well as in HLA-DR4 transgenic mice. Our studies demonstrate for

the first time that superagonist peptides of the immunodominant epitope hMBP115-126 can successfully be designed and that these peptides inherit highly immunogenic properties but still successfully induce tolerance *in vitro* as well as *in vivo*. We identified peptide PV550 as a superagonist peptide and a promising candidate to silence encephalitogenic T cells in $MBP^{+/+}$ HLA-DR4 transgenic mice. It remains to be elucidated if EAE can be effectively prevented in HLA-DR4 transgenic mice and thus provide a promising approach for MS therapy in humans.