SUMMARY

Th cells regulate the immune response in part by the secretion of cytokines. Upon stimulation with antigen naive Th cells differentiate. During this differentiation they receive an imprinting for a certain cytokine profile. It depends on this imprint whether the immune response is adequate or pathologic i.e. autoimmune. Therefore the manipulation of this differentiation is a possibility to treat autoimmunity. This manipulation can be achieved through DNA immunisation. In DNA immunisation simple expression vectors are injected, resulting in an immune response against the *in vivo* produced protein. By co-injecting of DNA coding for immune modulating proteins this immune response can be modified.

In this study several DNA co-immunisations were tested. Different cytokines and co-stimulating proteins were tested for their capacity to induce antigen specific Th cells capable of suppressing the development and effect of pro-inflammatory Th1 cells. Since the number of Th cells specific for a certain antigen in a not immunised animal is to low for direct analysis, we used a transfer system. That allowed the early analysis of the specific Th cell response to DNA immunisation on a single cell level. Among several tested immunisation routes the Gene Gun was the most reliable. It induced a reproducible Th cell reaction in strength comparable to that of immunisation using protein and adjuvans. Using this transfer system we showed for the first time, that the Gene Gun induced a Th1 profile with more IFNγ than IL-4 producing Th cells. The transfer system also allowed a detailed analysis of the Th cell response to different DNA co-immunisations. The major Th1 cell development inducing cytokine IL-12 shifted the immune response to Th1. The major Th2 inducing cytokine IL-4, that had been used in several DNA immunisation studies to induce Th2 development, surprisingly also induced a Th1 shift. In a Th1 dependent arthritis animal model IL-4 DNA co-immunisation worsened the disease. This Th1 shift was most likely mediated by dendritic cells (DCs). Supporting that we showed an increased TNFα production of the DCs after IL-4 DNA immunisation. A determination of the number and phenotype of these transfected DCs showed that around 30 DCs per draining lymph node produced a detectable amount of protein. We think that the effect of this low number of direct transfected DCs might be negligible if the majority of DCs takes up antigen produced by resident cells. DCs that take up antigen present the antigen without the co-stimulus. This might be a reason for the unexpected outcome of many DNA co-immunisations.

To safely develop a DNA immunisation based therapy the effects of the immune modulating substances on the different cell types have to be considered. This could be done by limiting the expression of the antigen and the co-stimulating substances to the cells that actually present the antigen.