1.2. Summary

Generation and in vitro-Characterization of a CD30-TNF-Immunocytokine for Targeting Hodgkins Lymphoma

Hodgkin's Lymphoma (Hodgkin's disease) is a malignant disease of the lymphatic system. A diagnostic hallmark are CD30 positive Reed-Sternberg- and Hodgkin-Cells detected by histology. Regarding therapy, there has been a great success in the last years by combination of chemo- and radiotherapy, so that Hodgkin's Lymphoma has become a curable disease for most patients. A disadvantage of the therapy is toxicity with a significant proportion of therapy related secondary neoplasias. Besides, therapeutic options and the rate of success are limited in case of relapse. Immunotherapy could be a solution to this problem, as this mode of therapy has a much lower long time toxicity and it can be used in addition to already established therapeutic regimens.

In this thesis, the generation and in vitro-characterization of a fusion protein consisting of a chimeric anti-CD30-antibody and human tumor-necrosis-factor (TNF) replacing the IgG1 CH2/CH3 Fc domain is described. The construct was generated by recombinant DNA technology and preserved its IgG1-derived structure with the TNF molecule linked as a dimer. Transient transfection of HEK293-cells was used to produce sufficient amounts of protein for preclinical in vitro-assays. CD30-antigen-recognition of this new construct was analyzed by flow cytometry and revealed slightly lower values than for the parental CD30-IgG-antibody. Construct-integrity and correct protein expression was shown by flow cytometry as well by using a secondary anti-TNF-antibody. The function of this immunocytokine was tested using TNF sensitive WEHI-S cells. Bound to CD30 positive cells apoptosis in WEHI-S cells could be induced, i.e. in spite of its dimeric structure binding and activation of TNF-receptors is possible. This work shows in vitro functionality of this construct and proves a potential therapeutic approach for chimeric immunocytokines in cancer therapy. The results presented in this work lay the basis for further studies with this construct possibly leading to clinical trials.