Abstract

Aim of this diploma thesis was to prepare two protein antigens and two monoclonal antibodies, all based on VP2 minor protein of human polyomaviruses BK virus and Merkel Cell Polyomavirus. One monoclonal antibody was being prepared against unique part of VP2 protein (N-terminal epitope, not present in VP3 protein). A cell line producing such monoclonal antibody has never been established before due to low immunogenicity of the epitope. Our approach was successful in terms of mouse immunization, however, serious problems with hybridoma line stability appeared later during the preparation process. Preparation of antibody targeted to the sequence of VP2 protein of Merkel Cell Polyomavirus was another aim of this thesis. Mouse immunization and hybridoma fusion were performed successfully. After four rounds of cloning in order to purify an established clone, nine clones were cultivated in larger scale. This cultivation probably led to diminished antibody specificity and loss of production ability in most of the hybridoma cells. One more cloning should give rise to an established clone with sufficient production. Two preparations of protein antigens were performed in two expression systems. DNA encoding C-terminally truncated protein VP2 of BK virus fused with His-tag was cloned into a vector suitable for expression in *E.coli*. This approach has been both successful and unsuccessful earlier in our laboratory depending on the length of truncation. Truncation performed in this diploma thesis, together with His-tag fusion did not prove suitable for purification from a bacterial expression system. The second antigen, VP2 protein of Merkel Cell Polyomavirus, was coexpressed with VP1 major structural protein in a baculoviral expression system. A recombinant baculovirus producing proteins VP1 and VP2 was prepared. Both proteins were expressed in the system and assembled into virus-like particles. This system was used as a source of recombinant protein in this thesis, but will find further applications in our laboratory research.