6. CONCLUSION

The aim of the thesis was to establish, in the national conditions, technology of preparation of antibody recombinant fragments and to verify the complete procedure using several model monoclonal antibodies with a potential diagnostic and therapeutic use.

For three antibodies, mAb TU-20, M75 and MEM97, recombinant fragments in various formats (scFv fragments monovalent and bivalent, i.e. diabody, and intrabody for intracellular expression) were constructed and for their heterologous expression, vectors allowing expression in *E. coli* (as cytoplasmic inclusions, periplasmic inclusions and in soluble form) and in *Drosophila* S2 cells (expression of glycosylated forms of scFv fragments into the medium) were used. In case of proteins expressed in insoluble form, especially scFv F11.2.32, renaturation procedures to obtain active scFv fragments were developed and optimized.

The effect of the length of the linker -$(Gly_4Ser)_x$- (where x is 1 to 4) connecting the variable domains of the light and heavy chain on the formation of different multimeric forms of scFv was studied. For obtaining solely monomeric scFv fragment, the length of 20 amino acid residues turned out optimal. Fragments with a linker 15 residues long, formed a mixture of monomers, dimers and trimers, the proportion of which was dependent on scFv concentration in the solution (higher concentration supports dimerization). With the linker of 10 and 5 amino acid residues, the equilibrium is markedly shifted towards dimeric form. It was shown that the sequence of variable domains also plays imporatnt role.

Besides the „common" methods for characterization of scFv fragments, such as ELISA, Western blot analysis or flow cytometry (FACS), more advanced physicochemical methods ITC and DSC were also employed.

Among the studied constructs was also the so-called intrabody format of the scFv fragment, which allows targeted intracellular expression and binding to the antigen inside the cell, with aim to decrease its concentration on the cell surface.

The effect of V_H and V_L domain orientation on stability and binding activity was studied in scFv fragment derived from mAb M75. It was found that V_H-V_L orientation showed higher stability, especially manifested in the course of purification. However, the binding activity as determined by ITC under optimal conditions was very similar for both orientations.