Abstract

One of the key components of the innate immune system are natural killer (NK) cells. The task of these cells is to induce apoptosis in target cells (e.g., cancer or virally infected cells). The target cells are identified by their interaction with surface receptors of the NK cells. On the surface of the NK cells, there are activating and inhibiting receptors. One of the activating receptors is the natural cytotoxicity receptor NKp46. Several ligands of this receptor have been identified, one of them being the epithelial adhesin Epa1 of yeast *Candida glabrata.* The invasive candidiasis caused by this yeast is a feared complication for patients with haematological diseases. The use of the NK cells in immunotherapy includes bispecific fusion proteins which can bind to the NK receptor with one part and to tumour antigen with the other part.

This work focuses on recombinant preparation of the NKp46 protein. To facilitate a study of the effects of O-glycosylation on the binding of the ligands, a mutation of the glycosylation site NKp46 T225A was prepared. A stably transfected HEK293S GnTI$^-$ and HEK293T cells had been prepared and these proteins were then extracellularly secreted.

The Epa1 protein had been produced in *E. coli* bacterial expression system and purified. The binding ability of the Epa1 protein and lactose was verified by two measurements
in solution (thermophoresis and isothermal titration calorimetry). Recombinant bispecific fusion proteins (that consist of the Epa1 protein and a nanobody-targeting tumour antigen HER2) were prepared to allow the study of the interactions of the NKp46 and the Epa1 proteins.

The ability of the Epa1 and bispecific fusion proteins to bind to the surface of cells was verified by flow cytometry. The following lines were used in the process: the HEK293T transfected lines producing NKp46 and NKp46 T225A receptors, the NK92MI cell line, and the SK-BR3 cell line producing the HER2 receptor. Unfortunately, attempts to measure the specific binding of the Epa1 protein variants on the surface of the cells were not successful.

Key words: NK cells, NKp46, Epa1, fusion protein, nanobody, HER2, HEK293