Abstract

The Natural Killer (NK) cells have an important role in the nonspecific immunity of the organism. They have the ability to identify and to kill tumor cells and cells infected by a virus without preceding sensitization by antigen. Their function is directed by the amount of stimulation and inhibition receptors interacting with ligands on the tumor or infected cell. This thesis focuses on the preparation and the study of the complex of rat NK cellular inhibition receptor NKR-P1B („natural killer cell receptor - protein 1B“) and its ligand Clrb („C-type lectin-related ligand b“). The Clrb initiates the inhibition of NKR-P1B, meaning that if the cell express Clrb, it won't be destroyed. If the cell gets infected by the rat cytomegalovirus, it loses Clrb from its surface and its destruction is therefore no longer prevented. Cells infected with this virus defend themselves from destruction by expression of the viral gene of C-type lectin RCTL, which is a homolog of Clrb.

Transient transfection of human embryonic kidney 293 cell line with simple glycosylation (HEK293S GnTI$^-$) was used for the recombinant preparation of the soluble form of these two receptors of the rat NK cells. The native forms of the receptors - disulfidic homodimers - were prepared as the fusion construct with IgG Fc (using vector pYD5), which after the cleavage using TEV protease provided purely dimeric form of the extracelular part of the receptors without any affinity tag. The complex of NKR-P1B with Clrb was characterized using fluorescence anisotropy and surface plasmon resonance. The ligand Clrb itself was used for the crystallization with the aim to determine its structure and the crystals of Clrb were obtained.

(In Czech)

Keywords

NK cell, receptor, NKR-P1, Clrb, HEK293, SPR, protein crystallography