ABSTRACT

Natural killer (NK) cells are an intensively studied part of immune system possessing unique ability to recognize and induce death of tumor and virus-infected cells without prior antigen sensitization. Their function is regulated by a fine balance of signals induced by multiple activating and inhibitory cell surface receptors and their interaction with the ligands present on the target cell. This can be illustrated on the homodimeric rat inhibitory receptor NKR-P1B and its ligand Clrb which play, besides other things, crucial role in the immunological response of NK cells to the infection with rat cytomegalovirus (RCMV), one of the most studied NK cell function model in rat model organism.

During RCMV infection the target cell downregulates cell surface expression of Clrb, thus decreasing inhibitory signal transmitted through the NKR-P1B receptor to the NK cell, which would ideally lead to NK cell activation and lysis of the infected cell. However, RCMV carries a gene for "decoy" surface receptor – RCTL that mimics Clrb and thus helps to escape the immunological response of NK cells. Moreover, while this escape strategy was demonstrated in the WAG rat strain, it has been shown that the NKR-P1B homologue from SD rat strain binds only Clrb and does not recognize RCTL. Thus the SD rat strain is less susceptible to the RCMV infection.

This research aims to elucidate the molecular basis of the NKR-P1B:Clrb receptor-ligand recognition and is based on our previous successful results with homologous human NKRP1:LLT1 receptor-ligand pair.

For protein crystallization of NK cell receptors and ligands, it was found out that the best recombinant expression system for production of soluble extracellular domains of these proteins is transiently or stably transfected HEK293S GnTI$^-$ human cell line possessing homogeneous N-glycosylation profile. To increase the yield of recombinant proteins, we have optimized transposon-based doxycycline inducible mammalian cell expression system piggyBac within HEK293S GnTI$^-$ cell line using Clrb soluble expression construct as the target protein.

KEY WORDS

NK cells, Clrb, NKR-P1B, RCTL, HEK293S GnTI$^-$, piggyBac