Studies on interactions between NKR-P1D and Clrb membrane receptors

Interaction between murine NKR-P1D and Clrb receptors was originally described as a novel type of „MHC class-I independent missing-self recognition“ and was shown to confer protection from killing by natural killer cells.[1] However, further study brought conflicting results suggesting that NKR-P1D does not binds Clrb strongly if it does at all.[2]

In order to address the issues arising from these conflicting results, we have recombinantly expressed the extracellular domains of both receptors in *E. coli* cells and refolded the proteins *in vitro.* The quality of refolding was confirmed both by determining the disulphide bonding pattern using FTMS and measuring $^{1}H/^{15}N$-HSQC spectra. By means of size exclusion chromatography and analytical ultracentrifuge we were unable to provide convincing results for the interaction itself. However, using SPR technique, a weak, specific, pH-dependent interaction was observed. Interaction between the proteins in solution was immobilized using chemical cross-linking technique. Three cross-linking reagents, EDC, DSG and DSS were used. The reaction mixture was separated by means of SDS-PAGE and protein bands corresponding to dimers were digested in gel. Using FT-MS we were able to find peptides from both proteins connected by the cross-linkers.

Using recently resolved structures of extracellular domains of NKR-P1A and Clrg receptors bearing 86% and 76% sequence identity with NKR-P1D and Clrb respectively, we were able to build homology models of both NKR-P1D and Clrb and a model of the interacting pair. Part of the data obtained from cross-linking experiments fitted nicely into the model of homodimers of both proteins interacting in a face-to-face fashion as would be expected; however significant portion of the observed cross-links could not be explained by this model. In order to allow for these data, we suggest that these receptors do not only interact in the face-to-face fashion but also in a chain-like or cluster-like fashion with each homodimer contacting two homodimers of the second protein at the same time. This would also lead to significant increase in the overall avidity even though the affinity of monomeric units might be very small as indicated by size exclusion chromatography, analytical ultracentrifuge and surface plasmon resonance. (Thesis in Czech)

1. Iizuka, K. et. al. Nat. Immunol., 2003, **4**, 801 – 807

2. Carlyle, J. et. al., Proc. Natl. Acad. Sci, 2004, **101**, 3527 – 3532