Abstract

Author: Bc. Simona Frydrychová

Title: The development of an eukaryotic expression system for the analysis of structure, expression and function of NK cell receptors

Diploma Thesis

Charles University in Prague

Faculty of Pharmacy in Hradec králové

Field of study: Specialist in Laboratory Methods

The theoretical part of the diploma thesis aims to introduce in detail human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV). The SIV infection of certain monkey species represents currently the only available model of human AIDS. In addition, readers will learn about the characteristics of NK cells. This part also focuses on receptors of NK cells, especially KIRs. The results of previous studies have shown that some variants of KIR alleles are associated with a favourable course of HIV infection. On the basis of this key information the attention is concentrated on the development of monoclonal antibodies that uniquely determine individual variants of KIR receptors. Such monoclonal antibodies will then allow for more detailed studies of the role of certain KIRs in the disease progression and enable to deepen our knowledge of mechanisms of the disease.

The experimental part of the diploma thesis focuses on the development of the expression system of individual variants of KIR alleles. It represents the necessary preceding step before the actual development of the antibodies, because the individual antibody-hybridoma samples need to be tested on a valid system. There were samples of 15 individual variants of KIR3DL alleles obtained from rhesus monkeys infected by SIV. To create an expression system it was necessary to amplify KIR3DL alleles by PCR, insert restriction sites and insert the amplification product into the expression plasmid p-Display, in which it was subsequently transformed into competent bacteria for amplification. After the purification of plasmid it was crucial to verify a correct orientation and sequence of the allele. The transfection of p-Display into eukaryotic cells Jurkat was then performed and positive clones of the cells containing the plasmid were selected. Expression of the plasmid was verified by detecting plasmid-coded hemagglutinin A peptide

by Western blot. Protein expression on the cell surface was verified by the detection of haemagglutinin A using flow cytometer. Positive clones were subsequently used for screening of newly developed monoclonal antibodies against KIR3DL with Western blot.

At the beginning 48 newly designed mAb were tested in the first screening, after which four antibodies were selected and examined in the second screening. From these, three KIR3DL antibodies were chosen. Two KIR3DL antibodies were designed against all alleles (DMY +RCR 1.2, DMY+RCR 2.2) and the other one against 13 mm and 14 mm alleles (NYS + TFK 1.2). The results of the detection of HA on the flow cytometer have shown that the expression of proteins on the cell surface was fairly low. It remains to be analyzed whether this expression is sufficient for testing of the suitability of newly developed antibodies for flow cytometry.

Key words

KIR, NK cells, HIV - Human Immunodeficiency Virus, AIDS - Acquired Immune Deficiency Syndrome, SIV - Simian Immunodeficiency Virus