University of Glasgow

23.05.12

Dear Doc. RNDr. Jan Kopecky, CSc.

Kristyna Kvardova undertook a research project in my group between 01.09.2009 to 30.06.2010 at the University of Glasgow in Microbiology, in the Research Institute of Infection, Immunity and Inflammation supervised in the laboratory by Dr. Andrea Mitchell.

The topic of the project was: "Molecular genetic analysis of virulence factors from *Streptococcus pneumoniae*". Ms Kvardova received training in basic microbiological techniques, including culturing and handling of the pathogen *Streptococcus pneumoniae*, basic bioinformatics instruction for handling of gene, genomic and protein sequences, nucleic acid and protein gel electrophoresis, toxin assays and PCR techniques, including qPCR for genotyping.

The project was designed to study the effect of genomic variation on expression of virulence factors, in particular of the toxin pneumolysin, and consequent disease causing potential of the pneumococcus. A number of serotype 1 clinical isolates were selected for the study to investigate the difference between genomes and virulence across one Serogroup. The results obtained when comparing the presence of toxin and other virulence factors with the disease phenotypes observed in the *in vivo* model illustrate the ability of the pneumococcus to employ a number of factors in concert cause disease. The serotype one strains differed markedly in animal models of infection. Kristyna's work showed that these differences were not solely due to expression of different allelic variants of the toxin. Interestingly, she showed that one strain did not express the toxin protein *in vitro* even though the gene was present. Preliminary experiments suggested that the toxin may be expressed *in vivo*. This is an important finding as it may allow the identification of *in vivo* signals that modulate virulence gene expression. Kristyna also developed an RT-PCR based test for single-nucleotide polymorphisms (SNP) in virulence genes. This was used to screen strains for SNP in the gene for hyaluronidase. We already know that there are SNPs in hyaluronidase that lead to production of truncated protein. The test

developed allowed rapid screening of large numbers of clinical strains for the presence of this SNP. The technology worked well and will be developed for other genes to allow us to do 'virulence sequence types' of large collections of clinical strains

Ms Kvardova was a very diligent and conscientious student. She produced very good quality data that was of great value to our ongoing research programme. She is a good communicator and came up with some good original ideas and questions during the course of her project. She was a pleasure to have in my laboratory and I recommend her thesis most highly.

Yours sincerely,

Tim Mitchell

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