Visit report on research attachment at Institute of Technology, University of Tartu, Estonia, 2015

Project title:

Cross-neutralisation epitopes study of chikungunya virus (CHIKV) immune sera from Malaysia against Asian and ECSA genotypes of CHIKV

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Summary of research project:

Malaysia has experienced outbreaks of chikungunya (CHIKV) in different geographical areas due to two different genotypes, Asian and East, Central, South African (ECSA). The Asian CHIKV strain was responsible for small, geographically-restricted outbreaks in 1998 and 2006. An imported ECSA outbreak was reported in 2006 prior to an explosive and unprecedented nationwide outbreak which affected over 10,000 people across different states in Malaysia in 2008. After the global outbreaks of ECSA between 2005 and 2010, the Asian genotype has re-emerged to cause large outbreaks in the Americas.

The effect of antigenic variation between different genotypes of CHIKV on immune responses has not been extensively studied. The amino acid differences in the immunogenic viral surface glycoproteins (E1 and E2) could impact the neutralising activity of antibodies elicited in the host, resulting in different immune responses to different genotypes. During my PhD project, I have tested a number of samples from CHIKV seropositive individuals infected with either Asian or ECSA CHIKV with an immunofluorescence-based neutralisation assay using an Asian and an ECSA isolate. All the sera have differential neutralising capacity against Asian and ECSA CHIKV.

Structural proteins of CHIKV, especially E1 and E2 glycoproteins are the target of neutralising antibodies. Comparison of the amino acid sequences of these 2 virus isolates shows 15 amino acid differences in E2 and 10 differences in E1. I had determined the importance of these amino acid differences in cross-genotype protection, by performing site-directed mutagenesis to replace the amino acids of Asian recombinant glycoproteins with ECSA residues. Antibody binding was carried out to identify which of these amino acids are critical for binding. I wanted to further investigate if these are important neutralising epitopes, using Asian and ECSA antisera from CHIKV patients. This further work would require recombinant viruses with mutated amino acids, which our laboratory had limited technology to pursue.

To achieve this aim, we have established collaboration with Professor Andres Merits from University of Tartu, Estonia. He is a well-known alphavirologist, with particular expertise in construction and genetic manipulation of alphaviruses such as Semliki Forest virus and chikungunya virus. During my trip, I learnt how to construct an infectious clone based on the Asian CHIKV isolate, and to perform site-directed mutagenesis of the infectious clone to mutate critical amino acids. These clones were then brought back to my home laboratory to test the effects of the mutations on neutralising capacity.

The work to understand cross-protection between genotypes is particularly important and timely as the recent widespread outbreaks in the Americas was unexpectedly due to the Asian genotype rather than the previously epidemic ECSA strains. This has implications for both continued outbreaks and vaccine development, as most of the recently reported vaccines have been based on the epidemic ECSA strains. My work will give insights into the importance of

viral glycoproteins from different genotypes in conferring immunity and designing vaccine formulation for preclinical studies.

Outcome of research attachment:

The research attachment was arranged from December 2014 to Mid-February 2015. Prof. Andres Merits kindly funded the consumables and reagents required for this project. The full-length cDNA clone was engineered by gene synthesis and assembled by the restriction enzymes approach based on the DNA consensus sequences available on GenBank. He and I worked together to assemble all the fragments into a plasmid and performed clone selections based on control restriction and sequencing analysis. Most of the work involved was molecular cloning, and we engineered the constructs with the insertion of reporter genes such as zsGreen, mCherry, and Renilla luciferase, which can be used in different platforms with similar constructs. I also learnt how to perform site-directed mutagenesis using conventional PCR-based methods which can be done in a simple and efficient way.

During the later stages of my lab attachment, I moved on to learn how to rescue the virus from infectious clones. The plasmid was linearised, viral RNA was *in-vitro* transcribed and electroporated into BHK-21 cells following the protocol established by the Merits lab. The infectivity of the rescued viruses was verified by infectious centre assay and plaque assay to ensure the infectivity of mutant clones similar to parental clones. All the constructs worked well as I expected and were sent back to Malaysia for further characterisation.

This research attachment was an unforgettable experience as I needed to travel far to Estonia and stayed there during the winter season for the first time. I had the opportunity to escape the hustle and bustle from when I came from and stayed in Tartu, which is a little, tranquil city during my visit. I also had the chance to join the activities organised by the host institute, including two winter conferences (at Võru and Narva) where students shared their research with each other. I am happy to have the opportunity to work with Prof. Andres, with his impressive research skills and mentoring. This research attachment and collaboration had benefited me greatly, as I have acquired cutting-edge knowledge in molecular virology, which will contribute to my PhD. This technology transfer will also improve virus research in Malaysia, as I am able to teach this to other students in my laboratory.

Acknowledgements:

I would like to thank my supervisors for their effort in establishing the collaboration and my international supervisor for giving me a golden opportunity to pursue my research attachment in his lab. Besides that, I would like to acknowledge the support of the staff, post-docs, and students from Institute of Technology, especially Age Utt for her excellent technical guidance. Last, but not least, a sincere thank you to the judging panel of the James S Porterfield Prize in International Virology, for awarding me this grant and supporting my travel and living costs to advance my research skills.

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The containment room in which I worked on infectious agents.