Abstract of Master's Dissertation

No.1

Course	Health Innovation Course	Name	Choo Ee Mei
Thesis Title	Study of the role of Dengue virus enhancing activity by the development of a simple and high-throughput ELISA-based ADE assay.		

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Objective :

Dengue causes a significant disease burden in tropical and subtropical regions, mainly concentrated in impoverished populations in the low-income countries. Currently, there is no vaccine or antiviral therapy available. Antibody plays distinct roles in controlling DENV infections. Neutralizing antibodies are protective against DENV infection, whereas sub-neutralizing antibodies are capable of enhancing DENV infection, termed antibody-dependent enhancement (ADE). Plaque-based assay represents the most widely accepted method for measuring immune-enhancing antibodies, but this conventional method is time-consuming, laborious, and low-throughput. Hence, there is a great need of new platforms that can characterize DENV-enhancing antibodies in a simple, rapid, and high-throughput manner. In this study, a simple and high-throughput enzyme-linked immunosorbent assay (ELISA)-based ADE assay for DENV using FcγR-expressing BHK-21 cells was developed.

Method :

This study was carried out by determining the levels of E-antigen produced in in culture supernatants derived from BHK-21 cells and FcγRIIA-expressing BHK-21 cells in ELISA-based ADE assay, respectively. Levels of infection-enhancing anitbodies were quantified by determining the levels of viral envelope (E) antigen. Serial dilutions of monoclonal antibodies were mixed with viruses (DENV 1-4 and ZIKV) at a 1:1 ratio (amount of virus: 1000-5000 PFU per well) and were incubated at 37°C in 5% CO2 for 1 h. Each mixture was inoculated onto plates with cells and incubated at 37°C in 5% CO2 for 1 h. Fresh EMEM medium was added and the plates were further incubated at 37°C in 5% CO2 for 3 days. Each plate included both a virus control (no antibody) and a cell control (no virus, no antibody). Three days after inoculation, culture supernatant was collected, and an in-house antigen-detection ELISA was performed. The observable dose-response profiles of enhancing activities against all DENV serotypes and ZIKV were produced with flaviviral and DENV envelope cross-reactive mouse-derived monoclonal antibodies like 12D11/7E8, 3H5 and 2H2.

* The abstract, containing the objective, method, result and conclusion should not exceed 300-500words and printed double sided on A4 paper)

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Result :

The results showed that the range of optimal PFU of 1000 to 5000 was used in subsequent ADE tests for DENV1-4 and ZIKV for both cell lines. The corresponding PFUs were approximately the highest dilution of virus that produced an OD of 1.0-3.0 in the antigen-detection ELISA at third day of incubation. The ADE activity was showed in DENV-4 in the presence of 12D11/7E8 and 2H2 antibodies, whereas for ZIKV, only showed ADE activity in the presence of 12D11/7E8 antibody. No ADE activity was observed using DENV-1, DENV-2 and DENV-3 in the presence of the antibodies.

Conclusion :

This simple and high-throughput ELISA-based ADE assay offers advantages for quantitative measurement of infection-enhancement that can potentially be applied to large-scale sero-epidemiological studies of DENV and ZIKV infection and vaccination. In this present study, ELISA-based ADE assay based on FcγRIIA-expressing BHK-21 cells was able to show ADE activity in DENV-4 and ZIKV. Additionally, different strains of DENV-1, DENV-2 and DENV-3 is needed to be performed to measure antibody-dependent enhancement. The major limitation of this study was that no clinical samples were enrolled, hence for future study, clinical samples are needed to validate the ELISA-based ADE assay.

This abstract contains 496 words.

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