ImmunoTools *special* Award 2015

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Effect of cytoskeleton proteins in membrane permeability of endothelial cells upon dengue virus infection and TNF-α induction

Dengue Virus (DENV) belongs to the *Flaviviridae* family and transmits to human by biting of Aedes mosquitoes. Dengue disease is an endemic disease in tropical and subtropical countries and causing tremendous health problems because of unavailable vaccines and potential antiviral drugs. DENV causes an acute febrile illness which clinical manifestation will be varied from asymptomatic of dengue fever (DF) to severe disease of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Severe DENV infection can be identified by thrombocytopenia, shock and hemorrhage. Likewise, the vascular leakage is the hallmark of severe DENV infection (**Amorim JH *et al*, Virus Res 2014;181:53-60**). The endothelium is the primary fluid barrier of the vasculature and ultimately the effects of DENV infection that cause capillary leakage affect endothelial cell (EC) barrier functions **(Dalrymple NA and Mackow ER, Adv Virol 2012;2012:840654)**. Moreover, secreted pro-inflammatory cytokines and chemokines, cytoskeleton reorganization, T cell apoptosis, complement and lymphocyte activation also affect on ECs during DENV infection **(Yen YT *et al*, J Virol 2008;82:12312-24**). The ERM (ezrin-radixin-moesin) family of proteins is comprised of three members having high sequence similarity. Interestingly, moesin acts as signal transduction and linking between membrane-associated protein and the actin cytoskeleton. DENV infection deteriorates cell structure which includes moesin, may involve in endothelial permeability or vascular leakage **(Kanlaya R *et al*, J Proteome Res 2009;8:2551-62)**.

In addition, TNF-α plays roles in controlling expression of adhesion and tight junction molecules which lead to vascular leakage, inducing remodeling of cell morphology and regulating vascular permeability. Thus, TNF-α can affect to endothelial cells due to TNF-α has ability to stimulate inflammation and recruit macrophages and neutrophils.

The proposed study is aimed to investigate the function of moesin which interacts with other cytoskeleton proteins for retaining human endothelial cells membrane and the important activity of moesin upon the association effect of DENV infection and TNF-α activation.

Materials and Methods

Dengue virus infection and TNF-α induction of human endothelial cells

Human endothelial cells (EA.hy926) will be cultivated in culture medium containing dengue virus serotype 2 (DENV2) and *TNF-α* and the expression of cytoskeleton proteins (i.e., moesin) will be determined by western blot analysis using specific antibody against moesin and/or p-moesin.

In vitro vascular permeability assay

The *In vitro* vascular permeability assay will be used to evaluate the effect of both DENV2 infection and TNF-α induction on human endothelial cells permeability. Human endothelial cells will be seeded onto inserts and performed a monolayer. Then those cells will be treated with DENV2 and TNF-α. After treatment, FITC-Dextran will be added in each well and allowing it to permeate through the cell monolayer. The extent of permeability will be determined by measuring the fluorescence of the plate well solution.

Functional study of the effect of altered protein expression upon DENV infection and TNF-α induction

The functional study will be explored either using small interfering RNAs (siRNAs) targeting interested mRNA in naïve human endothelial cells to simulate the similar effect occurs in DENV2-infected EA.hy926 cells and TNF-α induction or overexpression of interested protein in DENV2-infected EA.hy926 cells and TNF-α induction to gain the normal function of interested protein.

Expected results

The altered expression of cytoskeleton proteins will be observed upon DENV2 and TNF-α induction in human endothelial cells. The changes in the level of cytoskeleton proteins will affect on maintaining the structure and membrane permeability of endothelial cells which the effect will be demonstrated with various functional studies (i.e., in vitro vascular permeability assay, siRNA knock down or overexpression of protein). The information from the study will provide better understanding of the pathogenesis of vascular leakage of the DHF and DSS in dengue patients.

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includes 17 reagents

FITC - conjugated anti-human control-IgG1, IgG2a, IgG2b, Annexin V,

human ELISA-set for 96 wells, human IL-6, human IL-8, human IL-12p40 total, TNF-alpha,

recombinant human cytokines: rh TNF-alpha

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