ImmunoTools *special* Award 2015

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Study of proteins involved in mechanism of signal transduction and pathogenesis of dengue virus infection in human monocytes

Dengue virus (DENV) infection remains a major public health burden worldwide. DENV belongs to the family *Flaviviridae*, genus Flavivirus and currently is identified to four antigenically different serotypes of DENV (DENV-1 - DENV-4). Clinical manifestation of dengue infection is ranged from asymptomatic cases of dengue fever (DF) to severe form of diseases called dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) **(Flipse J et al, Traffic 2013;14:25-35)**.

Antibody-dependent enhancement (ADE) of dengue virus (DENV) infection is an important process of secondary infection that resulted in the pathogenesis of severe dengue in human. Enhancement is facilitated through efficient interaction of the virus-antibody complex with Fc receptors **(Martina BE *et al*, Clin Microbiol Rev 2009;22:564-81)**. It has been postulated that subneutralizing antibody concentrations, remnants of previous infection, facilitate viral infection of macrophages and other Fc receptor-bearing cells, promoting virus replication. U937 cell line, human monocytic cells that present Fc receptors can be used as model of *in vitro* ADE conditions **(Puerta-Guardo H *et al*, J Gen Virol 2010;9:394-403)**.

Signal transduction occurs when an extracellular signaling molecule activates a specific receptor located on the cell surface or inside the cell such as ADE **(Shresta S *et al*, J Virol 2004;78:2701-10)**. In turn, this receptor triggers a biochemical chain of events inside the cell, creating a response. Signal transduction controls and regulates many biological processes of function such as cell proliferation, cell death and cell division. Abnormality in signaling pathway may lead to serious complication and diseases.

The present proposal is aimed to observe the role of signal transduction in human monocytes (U937) during dengue virus infection in the presence of immune serum or under ADE condition. The phosphoproteins enrichment will be used to enrich the proteins involved in signal transduction and the differential phosphoproteins will be analyzed with one- or two-dimensional gel electrophoresis and further subjected to the analysis of proteins with mass spectrometry. Finally the interested proteins will be studied to observe whether pharmacological agents or other biomolecules may affect on the function of proteins.

MATERIALS AND METHODS

Cultivation and infection of dengue virus in human monocytes using antibody-dependent enhancement condition (ADE)

Human monocytes (U937) will be used as a model for the study of dengue virus (DENV) infection in the presence of ADE. Monoclonal against dengue virus will be mixed with DENV to form immune complex for the experiment group of culture, while the control cells will be infected alone with DENV.

Enrichment of phosphoproteins

Phosphoproteins will be isolated from infected cells with phosphoprotein enrichment kit for further study of protein analysis.

Analysis of proteins using one dimension or two dimension polyacrylamide gel electrophoresis (1D or 2D – PAGE)

1D or 2D – PAGE will be used for protein analysis and the phosphoproteins on gel will be stained with fluorescent dye.

Differential protein expression analysis and protein identification

The differential expression of phosphoprotiens will be analyzed with software and the significance difference of altered proteins will be determined using statistical analysis. The altered phosphoproteins will be analyzed with mass spectrometry and protein identification will be compared to protein database using software analysis.

Functional category of altered proteins and functional analysis

The altered phosphoproteins will be categorized in their functional group or pathway and some of these proteins will be explored for the functional significance in dengue virus infection and pathogenesis using either siRNA knockdown or treated with specific inhibitors.

EXPECTED RESULTS

The human monocytes (U937) infected with DENV in the presence of ADE condition may activate some signal transduction pathway different from the absence of ADE condition. The signal transduction proteins and cascade mechanism has been proposed as key factors in alteration of molecular and biological functions of proteins which may relate to pathogenesis. Usually the secondary DENV infection in human contribute more severe dengue cases than primary infection which the secondary infection will be resembled to the model of ADE of DENV infection in U937 cells in the present proposal.