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Evidence of plasticity in the dengue virus: Host cell interaction

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ABSTRACT

Dengue virus (DENV) is the most important mosquito transmitted human viral pathogen. There are four different dengue viruses (DENV 1 to DENV 4) with multiple genotypes and strains. Whether there are significant differences in how these DENVs interact with and modulate the host cell proteome remains unclear. Using a panel of 12 DENVs representative of one isolate for each DENV from three different origins (lab adapted, low passage isolates from dengue fever patients, low passage isolates from dengue hemorrhagic fever patients) LLC-MK2 cells were equally infected and proteomic alterations compared by MALDI-TOF and principal component analysis and a sub-10 kDa peptidome analysis. There was no clear segregation of data with respect to either virus origin or serotype in either the MALDI-TOF or the peptidome analysis. The two isolates with the greatest variation from the other isolates in the MALDI-TOF analysis were a low passage DENV 3 dengue fever isolate and a low passage DENV 4 dengue hemorrhagic fever isolate. Analysis of the sub-10kda protein fraction by LC-MS/MS identified 128 proteins of which only 28 (20%) were constantly expressed in all infections, while 80% showed variable expression, with no clear relationship with either serotype or virus origin. These results suggest that the interaction between DENV and the host cell is characterized by a degree of plasticity, whereby the end biological processes are not rigorously determined by specific proteome alterations, and that virus strain plays a role in determining the specific proteome changes.

1. Introduction

Dengue virus (DENV) is the most important mosquito transmitted virus worldwide. Each year there are believed to be close to 400 million human infections, of which some 100 million are symptomatic [1]. The virus is transmitted to humans by the bite of an infected *Aedes* mosquito during a blood meal, and symptomatic infections range from a mild undifferentiated fever, to overt dengue fever and in some cases the more severe presentation of dengue hemorrhagic fever and dengue shock syndrome [2]. Although fatalities are rare in areas where adequate hospitalization facilities exist, the disease causes a significant number of hospitalizations each year, particularly amongst children [3].

DENV belongs to the family *Flaviviridae*, genus *Flavivirus*, species *Dengue virus* [4], and four distinct dengue viruses are recognized, DENV 1 to DENV 4 [5], which are frequently referred to as DENV serotypes. The virus is enveloped and consists of a nucleocapsid and an outer protein shell consisting of 180 copies of the viral envelope (E) and cleaved membrane (prM) proteins [6]. The genetic material is a positive sense, single stranded RNA with one open reading frame that encodes three structural and seven non structural proteins that mediate viral replication [7]. Each of the four DENVs comprises of a number of genotypes which consist of a large number of characterized strain [8]. The relative contribution of virus (or serotype), genotype or strain type to human pathogenicity remains contentious [8]. While some evidence suggests for example that Asian genotype DENV 2 is more pathogenic than American genotype DENV 2 [9], the basis for this remains to be fully elucidated [8].

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While it is clearly established that the four DENVs can elicit distinct immune responses [10], whether the four DENVs have overt differences in how they interact with and modulate the host cell proteome remains unclear. While some studies seem to have identified clear serotype or strain type specific difference, for example in receptor usage [11], NS5 localization [12], changes in the level of metabolites [13], NS3 protease-cofactor complex formation [14], regulation of type 1 interferon signaling [15], other studies are divided as to whether serotype specific differences exist, such as in the activation of the unfolded protein response [16,17]. These examples highlight two significant problems in attempting to determine whether there is serotype as opposed to genotype or strain specific variation. Firstly, studies may depend upon only a single strain as a representative of the serotype, and secondly, where live viruses are used the infections must be comparable. As we showed when examining the activation of the unfolded protein response, unequal infection condition can result in apparent serotype specific differences, which are not evident when care is taken to ensure that the infected cell populations are comparable [16].

To begin to dissect out the contribution of serotype and strain type in mediating changes in the host cell proteome, we compiled a panel of 12 different DENVs. Four of the DENVs were laboratory adapted strains (one isolate for each serotype), four DENVs were low passage DENVs isolated originally from dengue fever patients (one isolate for each serotype), and four were low passage DENVs originally isolated from dengue hemorrhagic fever patients (one isolate for each serotype). MALDI-TOF and principal component analysis (PCA) were used to determine the differences in proteomic changes between the various infections, which were further explored by a peptidome analysis. The results showed little grouping by either virus origin or serotype, arguing that proteome changes are largely mediated by the specific viral strain, and that the dengue virus: host cell interaction is largely characterized by a great deal of plasticity.

2. Materials and methods

2.1. Ethics statement

The low passage DENV isolates described in this study were originally collected in 2006 after written individual informed consent in a study approved by the Ethical Review Committee for Research in Human Subjects, Ministry of Public Health, Thailand [18,19].

2.2. Cells and viruses

The African Green monkey kidney cell line Vero (ATCC Cat No. CCL-81) and the Rhesus macaque kidney cell line LLC-MK2 (ATCC Cat No. CCL-7) were cultured at 37 °C, 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM; Gibco, Invitrogen, Carlsbad, CA) supplemented with 5% heat-inactivated fetal bovine serum (FBS; Gibco) and 100 units of penicillin and 100 μg streptomycin/ml. The human embryonic kidney cell line HEK293T/17 (ATCC Cat No. CRL-11268) was cultured at 37 °C, 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco) and 100 units of penicillin and 100 μg streptomycin/ml. C6/36 cells [20] were cultured at 28 °C in MEM (Gibco) supplemented with 10% FBS and 100 units of penicillin and 100 μg streptomycin/ml. Both the human monocytic cell line U937 (ATCC CRL-1593.2) and the human myeloid leukemia cell line K562 [21] were cultured in RPMI-10% consisting of RPMI-1640 (Gibco) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco) and 100 units of penicillin/streptomycin mL−1 (PAA Laboratories GmbH, Coelbe, Germany) at 37 °C and 5% CO_2.

Viruses were four laboratory adapted high passage strains (one for each DENV serotype), four low passage isolates originally from dengue fever patients (one for each DENV serotype) and four low passage isolates originally from dengue hemorrhagic fever patients (one for each DENV serotype). Details of the viruses are given in Table 1. Viruses were passaged three times in C6/36 before use in this study and were stored at −80 °C until required. The viral titer was determined by standard plaque assay on LLC-MK2 cells, essentially as described elsewhere [22].

2.3. DNA sequence analysis

RNA was extracted from stock virus using TRI Reagent (Molecular Research Center, Inc., Cincinnati, OH). cDNA was reverse transcribed from 1 μg of total RNA with D2-R primer [23] and Improme-II™ reverse transcriptase enzyme (Promega, Madison, WI) and amplified using D2-R and D2-F primers. The PCR products were separated by electrophoresis on 1.5% agarose gel and after staining with ethidium bromide the amplification products were excised and sent for commercial sequencing (Macrogen, Seoul, Korea). The sequence data was compared against nucleotide sequences in all databases using the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). A sequence alignment analysis was undertaking using the on line Clustal Omega alignment tool [24] using the UPGMA clustering method. All sequences were deposited with the GenBank database, except for DENV 3 DF as the readable sequence was below the database inclusion limit (200 nucleotides). The sequence of DENV-3 DF is given in Supplemental File S1.

2.4. Infection and determination of infection

2.4.1. ADE mediated infection

Antibody dependent enhancement of infection of either U937 cells or K562 cells was undertaken essentially as previously described [16]. Briefly a known titer of the requisite DENV was incubated with monoclonal antibody HB114 [25] in RPMI-1640 medium for 1 h at 4 °C and the virus antibody complex was incubated with either U937 or K562 cells for 2 h at 37 °C in a 5% CO_2 humidified atmosphere. After incubation, RPMI medium supplemented with 10% FBS was added to a final concentration of 3×10^5 cells/ml. Mock infections were undertaken in parallel, and cells were incubated under standard conditions and infected cells were incubated at 37 °C, 5% CO_2 for 2 days. All experiments were undertaken as independent triplicates.

2.4.2. Direct infection

LLC-MK2 or HEK293T/17 cells were seeded into 6-well plates and cultured until confluent at which point cells were incubated with DENV at the required multiplicity of infection (m.o.i.) in DMEM media for 2 h with constant agitation at 37 °C, 5% CO_2. After incubation, DMEM medium with 5% FBS was added into each well and cells incubated under standard conditions until required. All infections were undertaken independently in triplicate. Mock infected cells were treated exactly as infected cells, but with no virus in the incubation.

2.5. Antibody neutralization assay

Antibody neutralization assays using HB114 [25], HB112 [25] or HB48 [25] were undertaken essentially as described elsewhere [16]. Briefly, known amounts of DENV were mixed with appropriate amounts of the respective antibody in RPMI-1640 medium for 1 h at 4 °C and the virus antibody complex was assayed for plaque titer by standard plaque assay on LLC-MK2 cells.