MOLECULAR EVOLUTION AND EPIDEMIOLOGY OF DENGUE-4 VIRUSES IN BANGKOK, THAILAND

Klungthong C, Holmes EC, Mammen MP Jr, Nimmannitya S, Kalayanarooj S, Ubol S and Zhang C

Fifty-three dengue-4 (DEN-4) virus isolates from children in Bangkok, Thailand, admitted with varying severity of dengue [dengue fever/dengue hemorrhagic fever/dengue shock syndrome (DF/DHF/DSS)] from 1976 to 2002 were selected to sequence the envelope (E) gene for identifying specific sequence patterns which may correlate with disease severity and for assessing potential trends in molecular evolution and epidemiology of circulating DEN-4 viruses within Bangkok. Phylogenetic analysis of these isolates revealed that the majority collected in the past 27 years comprised genotype I (47 cases), 5 of the 6 more recent isolates comprised a genotype never previously described. This newly discovered genotype was associated with DF (2 cases) and DHF/DSS (3 cases). No specific sequence differences were identified between the DF and DHF/DSS isolates, suggesting that the E gene alone did not determine disease severity. The phylogenetic tree revealed that genotype I of DEN-4 virus circulating in Bangkok appears to have become extinct beyond 1999 and has been replaced by new lineages that emerged in 1998 and evolved locally, rather than having been introduced. However, it remains unclear and requires further study as to whether this replacement represents a selection event, so that strains differ in fitness, or a random population bottleneck. One case of the genotype IIA appears to have been introduced in 2000 from neighboring Malaysia or Indonesia where this genotype is known to circulate. However, it appears that this genotype could not be sustained. Sequencing of the entire virus genome of selected specimens is underway to ascertain the molecular basis to any differences in fitness among strains.

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NITRIC OXIDE RADICAL: FROM AN *IN VITRO* EXPERIMENT TO DENGUE PATIENTS

Takumpanya R, Chareonsirisuthigul T, Kalayanarooj S, Charnsilpa V, Mammen MP Jr, Padmanabhan R, and Ubol S

Nitric oxide, NO, is well accepted as one of the defense which inhibit viral dissemination. Macrophage and cell in macrophage linage are professional nitric oxide producer which sub-serves as target for dengue virus, DV. The interaction between NO and DV during natural infection is unknown. Therefore, the effect of NO on DV replication was investigated in vitro using s-nitroso-L-acetylpenicillamine, SNAP, as an exogenous nitric oxide donor. NO inhibited DV replication in a dose and MOI dependent manner that was NO from 50 and 75 μM SNAP delayed and suppressed replication while higher concentration of NO, 100 μM SNAP, completely

inhibited production of infectious particles up to 36 h study. Using RT-PCR and immunoblotting, the mechanism of inhibition was found to be at the level of RNA replication rather than at the level of protein synthesis. To prove whether inhibitory effect of NO targets at the viral RNA-dependent-RNA polymerase, an *in vitro* RdRp assay of purified NS5 was performed in the present and absent of NO. Results demon-strated that NO suppressed NS5 activity which was detected by significant reduction in viral RNA synthesis. To elaborate the in vitro effect of NO into natural dengue infection, the relationship between plasma NO and viral load in dengue patients was investigated. Plasma was obtained from DF and DHF patients on fever day and convalescence phase. From our preliminary data, during the fever day, plasma from secondary DHF patients contained low level of NO when compared to DF patients, 3.5 ± 2.42 and 8.49 ± 3.45 μM/ml, respectively. During convalescence phase, these groups of patients generated a similar level of plasma NO which were 8.24 ± 3.12, and 10.33 ± 5.61 μM/ml, respectively. The secondary DHF plasma samples were selected for viral RNA copy number quantitation by Real Time RT-PCR. Results indicated that copy numbers of dengue viral RNA in plasma reversely correlated to the level of plasma NO. In conclusion, nitric oxide radical exerted an inhibitory effect to DV replication. The inhibitory target of NO was NS5, viral RNA polymerase. The profound effect of NO on viral load during natural dengue virus infection was detected in secondary DHF patients.

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A PHASE I/II TRIAL OF A TETRAVALENT LIVE-ATTENUATED DENGUE VACCINE IN THAI CHILDREN

Simasathien S, Thomas SJ, Watanaveeradej V, Nisalak A, Mammen MP Jr, Putnak R, Gibbons RV, Eckels KH and Innis BL

Prevention of dengue through widespread vaccination is an important objective of the WHO and governments of dengue endemic regions. The Walter Reed Army Institute of Research developed live-attenuated monovalent DEN-1, -2, -3, and -4 vaccine candidates and mixed promising candidates to produce tetravalent dengue vaccine (TDV) formulations. Over 174 human subjects have safely received various formulations of the TDV. Formulation 17 (F17) has emerged as a promising candidate. Two doses, 6 months apart, of F17 was administered to 6 and 7 year-old children (6 flavivirus-naive children and 1 flavi-immune child). There were no serious or unexpected adverse events and no alert laboratory values. A measured temperature ≥37.5ºC occurred in 4/7 (57.1%) recipients following dose #1 and 3/7 (42.9%) following dose #2. The highest temperature after either dose was 38.6ºC and was observed in one child. Mild headache, muscle and joint aches, and fatigue were also experienced. Percent seroconversion ($PRNT_{50} \geq 1:10$) following dose #2 in 7 recipients against DEN-1, -2, -3, and -4 was 85.7%, 100%, 100%, and 100%, respectively. Post dose #1 and #2 GMTs for DEN-1, -2, -3, and -4 were 5, 22, 6, 23 and 39, 583, 290, and 163, respectively. Authors will provide detailed reactogenicity and immunogenicity data.

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