of HEV71 shows that the strains from all states in Peninsular Malaysia had several genetic lineages namely genogroups B3, B4, B5, C1, C2, C3 and C4. Although they were closely related to the strains that have been circulating and associated with outbreaks in the Asia Pacific region since 1997, these strains were not associated with huge outbreak in Peninsular Malaysia. The nucleotides and amino acids substitutions were also analyzed. There was about 18–25% differences in nucleotide sequences between the peninsular isolates and the prototype BrCr-CA-70. Nevertheless, all the changes in VP4 region of the isolated strains were synonymous substitutions.

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46.015
Pharmacological C-Abl Kinase Inhibitors as Potential Anti-Viral Molecules for Dengue Virus
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Background: Dengue virus is a mosquito-borne flavivirus that represents an important emerging infectious disease and is an international health concern. Currently, there is no vaccine or effective antiviral drug to prevent or to treat dengue virus infection. The development of specific anti-dengue molecules would be facilitated by the availability of efficient anti-dengue screening assays, adaptable to high-throughput format. In this study, we have developed an immunofluorescence imaging-based platform that detects dengue virus replication. We used this assay to screen a structurally diverse collection of pharmacological kinase inhibitors.

Methods & Results: Small molecule inhibitors of c-Abl and c-Src kinases exhibited significant anti-dengue activity, suggesting that these kinases play critical roles in dengue virus biology. Here, we uncovered a unique role of c-Abl tyrosine kinase harnessed by dengue virus to mediate virus entry via clathrin endocytosis. The infectivity of dengue virus was severely reduced in cells pretreated with GNF2 (specific inhibitor for c-Abl) in a dosage-dependent manner. Moreover, dengue virus infection also triggered the activation of c-Abl by inducing phosphorylation of c-Abl Tyr412. Using immunofluorescence assay and transmission electron microscopy, dengue virus particles were observed binding to the surface of the GNF2 pretreated cells but failed to enter into the cells. These observations were substantiated in cells transfected with small interfering RNA designed to inhibit clathrin and c-Abl expression. Virus infection was significantly reduced in knockdown cells relative to controls cells. Furthermore, dengue virus infection was also aborted in c-Abl−/−, c-Arg−/− and double c-Abl−/−, c-Arg−/− deficient cell line.

Conclusions: These findings reveal a novel role for c-Abl in facilitating the clathrin-mediated endocytosis of dengue virus into cells and may serve as a drug target for the development of effective anti-viral strategies against dengue virus infection.

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46.016
Identifying Host Factors Involved in Mediating Vascular Permeability During Dengue Virus Infection
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Background: Dengue virus (DV) is a mosquito-borne virus, belonging to the family Flaviviridae. This virus causes the mild form, Dengue fever (DF) or in severe cases, Dengue Hemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS) characterized by increased capillary permeability. Currently, there is no specific antiviral treatment available to treat patients with DV infection. Furthermore, the pathogenesis of DHF is poorly understood. Hence the aim of this study is to identify the host factors that may play a role in the progression of DHF/DSS during DV infection.

Methods: Human Umbilical Vein Endothelial Cells (HUVEC) was infected with DV2 at a multiplicity of infection (M. O. I.) 10. At 72 h post infection (h. p. i.), the expression of different genes specific to endothelial cell biology was analyzed using real-time PCR.

Results: Comparing to non-infected controls, host proteins associated with apoptosis such as caspases 1 and 8 were up-regulated in the infected HUVEC. In addition, pro-inflammatory cytokines such as IL-3, IL-7 and IFN- β1 were also up-regulated in the DV-infected cells. These molecules may in turn promote the expression of pro-inflammatory adhesion molecules like ICAM-1, Selectin E and P, as observed in our results. All these cellular molecules are associated with cell migration which could play a role in vascular permeability. In addition, this group of cells also presented with down-regulation of platelet derived factor family genes such as PF4 which may play a role in the aggregation of platelets.

Conclusion: Identification of these host proteins allowed us to investigate further their roles in the pathogenesis of DHF/DSS which in turn enable us to identify potential therapeutic leads and better clinical management of patients suffering from DHF/DSS.

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46.017
B- and T-cell Epitope of the Envelope Glycoprotein E of Dengue Virus Defined by Bioinformatics, ELISA and Enzyme-linked Immunospot
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Background: None of the multivalent dengue vaccines is close to licensure and commercially available even after several decades of dedicated effort. Researchers applied the conventional approaches to vaccine development, which is not successful in all serotypes of dengue virus, thus in our research, we adopted the reverse vaccinology approach to design B- and T-cell epitope based vaccine in silico using the genomic and proteomic information. B- and T-cell responses to dengue viruses are of vital importance in both protective immunity and pathogenesis and the peptide vaccine includ-
ing both the B-cell and T-cell epitope is the most potential vaccine candidate.

Methods: Databases located in http://bio.dfci.harvard.edu/Tools & http://www.imtech.res.in gave us a new way to define the B-and T-cell epitope on the envelope glycoprotein of dengue virus type 2 NGC strain, and the peptide RHVL-GRILTVNPIVT (345-359 amino acid) was predicted as the more prevalent epitope, then enzyme-linked immunosorbent assay (ELISA) and enzyme-linked immunospot (ELISPOT) were performed to verify the probability whether the peptide could become a peptide vaccine candidate.

Results: In indirect ELISA assay, the OD_{50nm} values of antibody in experimental group were higher than that in the control and blank group, which indicated that the peptide could strongly react with the serum both from convalescent patient and mice infected with the dengue virus type 2 NGC strain. Moreover, the high values of IFN-γ production in the spleen cell of mice were observed in the ELISPOT assay after inducing the second infection by the peptide inoculation in the C57/BL6 mice infected with DEN-2 virus.

Conclusion: The ELISA and ELISPOT results suggest that the peptide defined is possible to be a peptide vaccine candidate which could induce the humoral and cellular immune response to the dengue virus infection.

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46.018
Role of IL-10 in Dengue Infection: Pathogenic or Protective?

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Background: Several previous studies associate the illness severity in dengue-infected patients with high levels of IL-10. However, Treg cells have been recently proposed as a critical role in the control of pro-inflammatory phenomenon associated to the severe disease. The aim of this work was to elucidate the role of IL-10 in the dengue disease pathogenesis.

Methods: The serum levels of IL-10 in acute kinetic samples from dengue infected individuals with different clinical pictures were measured using commercial ELISA kit. Total RNA was isolated from dengue virus stimulated PBMC from healthy dengue immune and control individuals, cDNA synthesized from mRNA and quantified by real-time PCR analysis using the ABI Prism 7700 sequence detection system for the expression of IL-10 gene. Genomic DNA was purified from blood of individuals who had suffered DHF/DSS and controls and polymorphism of IL-10 gene was studied by polymerase chain reaction-sequence specific primer (PCR-SSP) by the -1082IL-10, -819IL10, -592IL-10 gene SNPs exploration.

Results: Higher levels of serum IL-10 were observed in Dengue Hemorrhagic Fever patients when compared to Dengue Fever patients (P = 0.04) and controls (P = 0.003). IL-10 gene expression showed a higher response to homologous dengue 1 virus than heterotypic dengue 2 virus after ex vivo re-challenge of PBMC from dengue 1 immune individuals. Differences in the frequency of C allele in the -592 locus of IL-10 gene promoter between DHF patients and controls could suggests a relationship between IL-10 production and the severe infection.

Conclusion: The results patients’ kinetic study and genetic polymorphism analysis suggest a pathogenic role of IL-10. However, a higher expression of IL-10 gene in ex vivo PBMC cultures was associated to homologous serotype response. Further studies will contribute to the better definition of the role of this soluble mediator in the outcome of dengue infection.

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46.019
Antiviral Activity of Avicennia Marina Leaf Extract on HSV-1 and Vaccine Strain of Polio Virus in Vero Cells

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Background: Nowadays finding the new natural compound with antiviral effects is very interesting. Avicennia marina is the most current species among the mangrove plants in Iran. Regarding to their applications in traditional medicine and some well known biological constituents of this plant an attempt was made to evaluate the in vitro antiviral effects of its leaf extract.

Methods: The hot glycerine extract of the leaf was evaluated against HSV-1 (KOS strain) and vaccine strain of the polio virus (Sabin strain) in cell culture by calculating IC50 criteria. Also antiviral activity of the extract was evaluated against HSV-1 (KOS strain) and vaccine strain of the polio virus (Sabin strain) in cell culture by calculating IC50 criteria.

Results: The CC50 of the leaf extract was 5750.96 μg/ml. The IC50 of the extract against HSV-1 were 66 μg/ml and 137.24 μg/ml for the before attachment and post attachment stages of virus replication cycle respectively. The IC50 of the extract against Sabin strain of polio virus were 145.7 μg/ml and 314.3 μg/ml for the before attachment and post attachment stages of virus replication cycle respectively.

Conclusion: The selectivity index (SI) values of the extract before and after virus attachment to the cells were 87.1 and 41.9 for HSV-1 and 39.5 and 18.3 for Sabin strain of polio virus respectively. According to the obtained SI values it could be concluded that the hot glycerine extract of Avicennia marina leaf showed the significant in vitro antiviral activity against HSV-1 as an enveloped virus and Sabin strain of polio virus as a naked virus. Therefore, it could be a good candidate for further research in order to finding new natural antiviral compound.

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