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High CTLA-4 expression in T cells in patients with acute dengue infection

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Background: Patients with severe dengue infection have shown to have impaired dengue virus (DV) specific T cell responses and higher viral loads. Our previous data have shown that DV-specific T cell responses are suppressed by IL-10. Therefore, we set out to investigate the possible mechanisms of inhibition of DV-specific T cell responses in patients with acute dengue infection.

Methods & Materials: 18 adult patients with acute confirmed dengue infection were recruited following informed written consent. The first blood sample was collected during day 3-5 of illness and the second sample was collected 2 days later. Clinical disease severity was classified according to the WHO 2011 dengue guidelines. Flow-cytometry was done on freshly extracted peripheral blood mononucleocytes (PBMCs). Intracellular cytokine staining was done by stimulating PBMCs with DV- NS3 overlapping peptides. Expression of CTLA-4, TIM-3, PD-1, CD27 and CD28 were determined in T cells in general and DV-specific T cells. IL-10 quantitative ELISA was done in the patients and in 10 healthy DV seropositive individuals.

Results: Expression of IL-10R was significantly higher in both CD4+ T cells ($p = 0.007$) and CD8+ T cells ($p = 0.005$) in patients with acute dengue when compared to healthy individuals. Expression of CTLA-4 was also significantly higher ($p < 0.0001$) among T cells in patients with acute dengue (mean 411.3, SD ± 100.2 MFI) when compared to healthy individuals (mean 256.4, SD ± 84.4 MFI). Expression of CTLA-4 was also significantly higher ($p = 0.009$) in DV-NS3 specific T cells (mean 419.5; SD ± 69.5) when compared to CD3+ T cells in general (mean 386.6; SD ± 86.81 MFI) in the same patient. Although not significant ($p = 0.11$) serum IL-10 levels correlated with CTLA-4 expression in T cells (spearman's $r = 0.48$). CTLA-4 expression also significantly ($p = 0.04$) correlated with serum alanine transaminase levels (spearman's $r = 0.59$). PD-1 expression was higher in T cells in patients with acute dengue (mean 106.8, SD ± 65.43 MFI) when compared to healthy individuals (mean 88.71, SD ± 22.77MFI), although not significant ($p = 0.85$).

Conclusion: CTLA-4 expression appears to be higher in T cells in patients with acute dengue and significantly higher in DV-specific T cells, suggesting a role in inhibition of DV-specific immune responses.

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The dynamics of influenza isolates in Uganda: Their implications and way forward

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Background: The isolation of Influenza viruses is important in detection of circulating strains in surveillance programs and development of vaccines. The fact that Influenza B viruses are rare in animals and there is no known animal reservoir and thus poses negligible pandemic threat has caused a decline in information concerning Influenza B in comparison to Influenza A viruses. We analyzed the changes that have occurred in Influenza A and B virus isolates in Uganda from November 2011 to May 2013 to determine extent of changes that have occurred in the viruses.

Methods & Materials: Clinical ILI and SARI samples positive for Influenza viruses by Polymerase Chain Reaction (PCR) were inoculated and propagated on Madin-Derby Canine Kidney (MDCK) cell line. Hemmaglutination and Hemmaglutination inhibition (HA/HAI) test was carried out using guinea pig and turkey erythrocytes. All isolates that did not show HA at passage 1 were taken through a second passage. The isolates were tested for drug resistance, sequenced and phylogenetically characterized.

Results: Out of 3455 samples collected, 439 samples were positive for Influenza A and B, with 324 Influenza A and 115 Influenza B. Of the 324 Influenza A, only 41 (12.6%) isolates were recovered with 33 isolates (80.5%) being recovered at passage two. Of the 115 Influenza B, 57 (49.6%) isolates were recovered, 36 isolates (63.2%) being recovered at passage one. Influenza B viruses were classified as group 1 B/Brisbane/60/2008-like with amino acids changes at positions N75K, N165K and S172P of the HA gene. AH3N2 HA gene was classified in subgroup 3B and 3C, with subgroup 3B having amino acid changes at N145S and subgroup 3C at S45N (glycosylation) and T48I. Influenza A viruses showed decreased HA titre at 16HA units but had constant HAI titre at 1280, B viruses showed higher HA titre at 128HA and a decreased HAI titre at 640. Influenza B and A/H3N2 viruses were sensitive to the antiviral drugs Oseltamivir and Zanamivir.

Conclusion: Influenza B viruses are easier to isolate using MDCK cells than Influenza A viruses. There are no significant changes in current circulating strains to cause a pandemic threat.

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