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T cells- friend or foe in dengue infection?

Dengue viral infections is a major public health problem affecting more than 100 countries and 390 million individuals annually[1]. Since the year 1990, the global incidence of dengue has doubled every decade and apart from the morbidity due to acute illness, disability due to post dengue related chronic fatigue was estimated to be 186,000 to 1,415,000 years lived with disability in 2013 [2]. Although currently there is a dengue vaccinewhich has been registered in 4 countries so far, the efficacy of this vaccine failed to reach expectations. For instance, a phase 3 clinical trial of this dengue vaccine demonstrated an overall efficacy rate of 56.5%, with the efficacy rates being a mere 14.4% in dengue seronegative children less than 9 years of age[3].Although the vaccine did reduce rates of hospitalization in dengue seropositive older children, the relative risk of hospitalization in dengue naïve children, aged 2-5 years who received the vaccine, when compared to the placebo was 7.45[4]. Therefore, although this vaccine does appear to be effective in older children, it appears to have very low efficacy rates and safety issues in dengue naïve, children less than 9 years of age. The main hurdle in the development of a safe and effective vaccine, is the lack of knowledge regarding correlates of protection [5].

The occurrence of severe clinical disease is thought to be a result of the complex interplay between the virulence of the infecting virus [6, 7], host genetic factors [8, 9] and the host immune response [10, 11]. Currently the pathogenesis of severe clinical disease is poorly understood and especially the role of T cells in the pathogenesis of dengue infections is not clear [10, 12, 13]. It has been speculated that highly cross reactive T cells for the previous

infecting heterologous DV serotype, which produce pro-inflammatory cytokines, leading to a 'cytokine storm' contribute to disease pathogenesis[14, 15]. These cross reactive T cells are believed to be suboptimal in clearing the infection with the current DV-serotype[12, 16]. While some debate that highly cross reactive DENV specific T cells contribute to severe dengue and are instrumental in the 'cytokine storm', more recent data show that DENV-specific T cells might be protective.

T cell responses in acute dengue infection

In order to understand the role of T cells in acute dengue infection, we initially proceeded to determine the cytokine and T cell profiles in patients with varying severity of dengue infection.

We found that patients who developed DSS, had significantly lower T cell numbers when compared to those who did not develop shock and that serum IL-10 and IP-10 levels positively and significantly correlated with T cell apoptosis and with T cell numbers.

Cell population	Shock N=8 Median (SD) and range Cells/mm^3	No shock N=27 Median (SD) and range Cells/mm^3	P value
CD3+ T cells	269.8 (131.2)75.97 to 468.4	336.6 (428.5)141.5 to 2379	0.04
CD4+ T cells	182.9 (76.88)103.6 to 305.1	224.3 (262.1)116.5 to 1410	0.07
CD8+ T cells	120.4 (35.48)40.34 to 134.8	136.0 (166.5)20.42 to 903.8	0.07
B cells	36.0 (64.31)17.05 to 205.2	54.8 (46.44)10.15 to 202.6	0.16
Natural killer cells	47.25 (32.23)9.470 to 104.0	41.5 (58.33)1.610 to 233.2	0.42

Table 1: Lymphocyte subpopulations in patients with DHF who developed shock and those who did not progress to shock.

Although not significant, there was a trend towards higher IFNγ DENV-3 NS3 specific responses in patients who developed shock (mean 639.3, SD± 820.1) when compared to those who did not develop shock (mean 313, SD± 513.1). Most importantly, we did not observe significant production of TNFα, IL-10, IL-4, IL-13, IL-17 by dengue NS3-specific T cells, suggesting a different source for these cytokines in the serum.

As in our earlier studies, we had only used DENV-NS3 protein to determine DENV-specific T cell responses, one of the criticisms have been that responses to DENV-NS3 does not reflect responses to the whole virus. Therefore, in our most recent studies, we have used

other immunodominant proteins such as DENV-NS5 and DENV-NS1 along with DENV-NS3 and have used these pooled peptides to study the virus specific T cell responses. Again, we did not find any differences in ex vivo IFNγ production in patients with DHF when compared to those with DF.

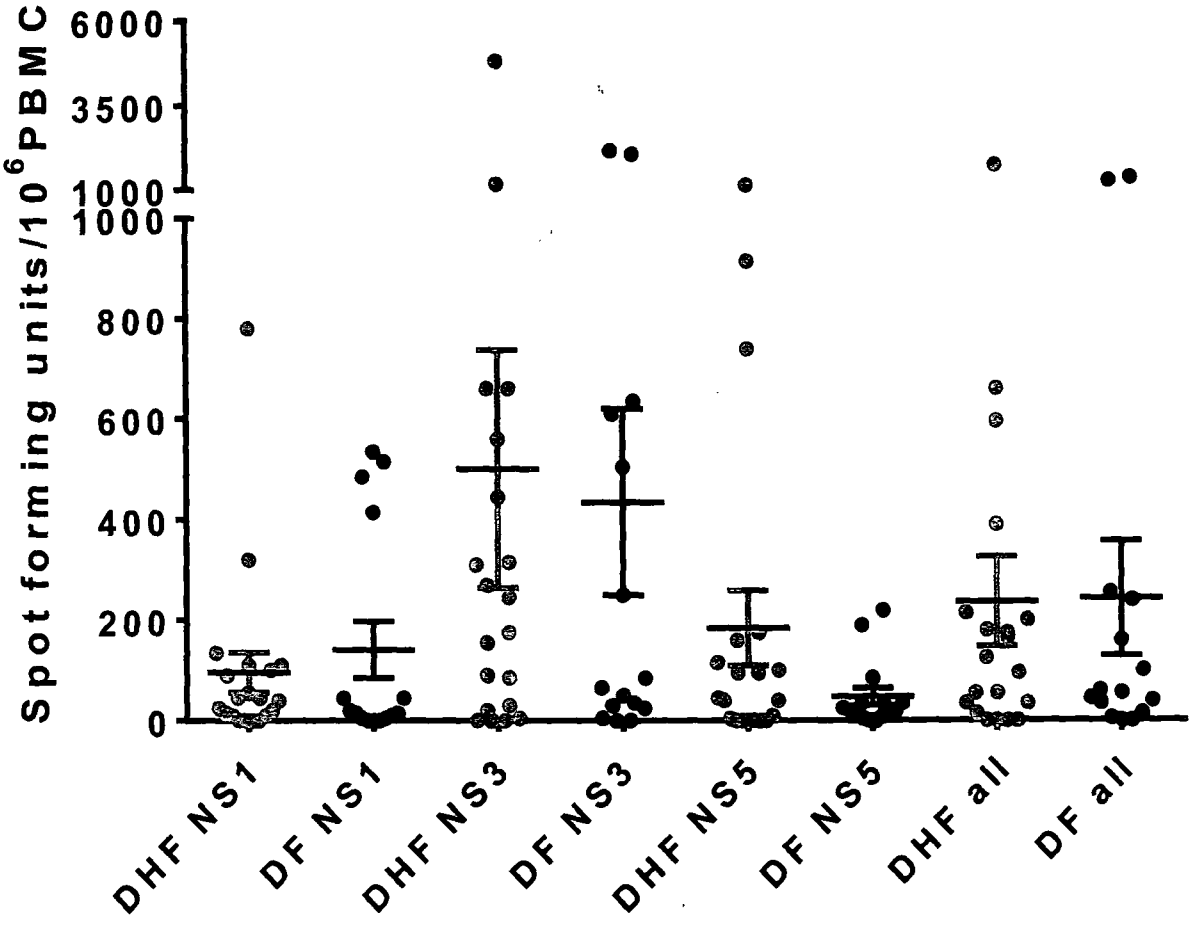


Fig 1: Ex vivo IFNγ ELISpot responses in patients with DHF (n=20) and DF (n=15)

We determine TNFα and IL-2 production by DENV-specific T cells in patients with acute dengue and except for one patient with DHF and one with DF, none of the DENV-specific T cells produced TNFα and none produced IL-2.

IL-10 and DENV specific T cell responses

It has been previously reported that DENV-specific T cells lacked proliferative capacity to both the DENV and T cell mitogens [17]. It has also been shown that DEN-2 in vitro resulted in downregulation of CD25, which is the IL-2 receptor[18]. These reports contradict the

hypothesis of the DENV-specific highly cross reactive T cells contributing to disease pathogenesis. Our data has also shown IL-10, which is a potent immunosuppressive cytokine, was significantly higher in patients with severe dengue[19] and that IL-10 was associated with suppression of DENV specific T cell responses.

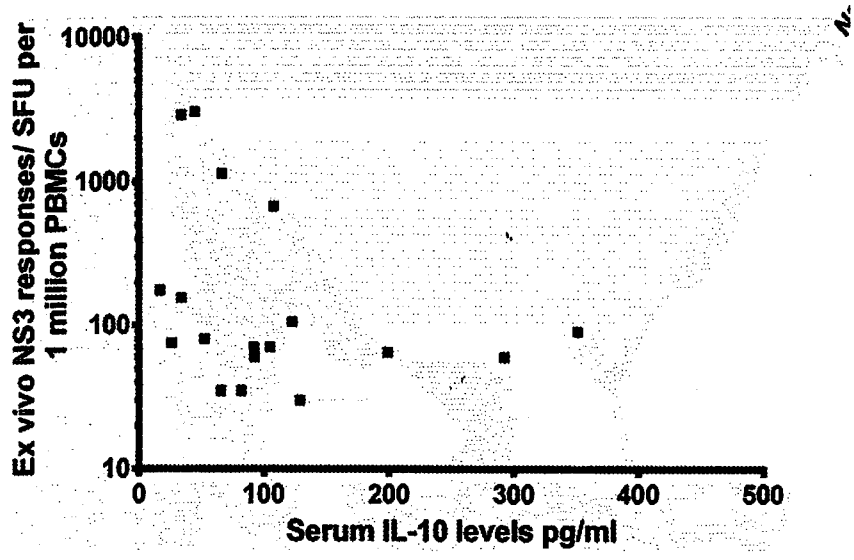


Fig 2: Association of serum IL-10 levels with DENV-NS3 specific T cell responses.

We found that IL-10 blockade led to a significant recovery of DENV-specific T cell responses, but not other virus specific T cell responses in acute dengue infection (fig 3)[20].

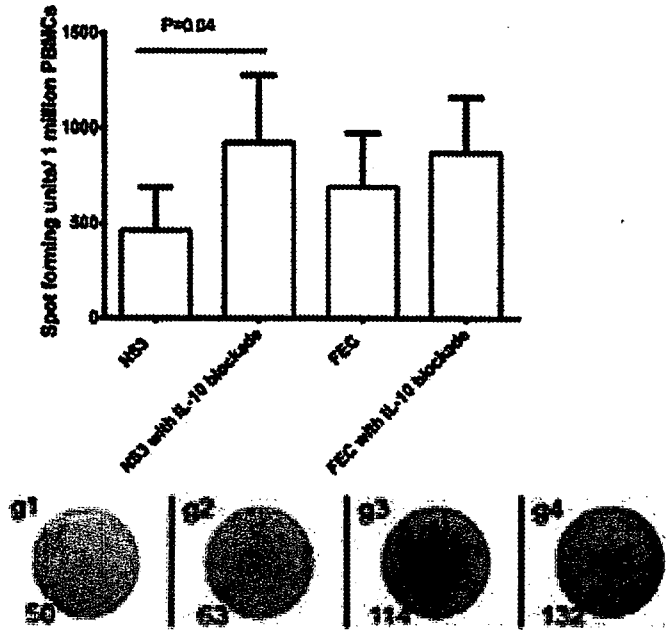


Fig 3: DENV NS3 specific T cell responses and FEC (Flu, Epstein Barr and Cytomegalovirus) specific T cell responses in the presence and absence of IL-10 blockade

Dengue NS1 and IL-10

Since our experiments showed that DENV-specific T cell responses were impaired in acute dengue, due to IL-10, we further investigated the source of IL-10 and the factors that stimulate its production. After determining IL-10, dengue NS1, dengue antibodies and other cytokine levels, twice a day in 40 patients from the time of admission to the time of discharge, we found that patterns of dengue NS1 closely followed that of IL-10 (Fig 4)[21].

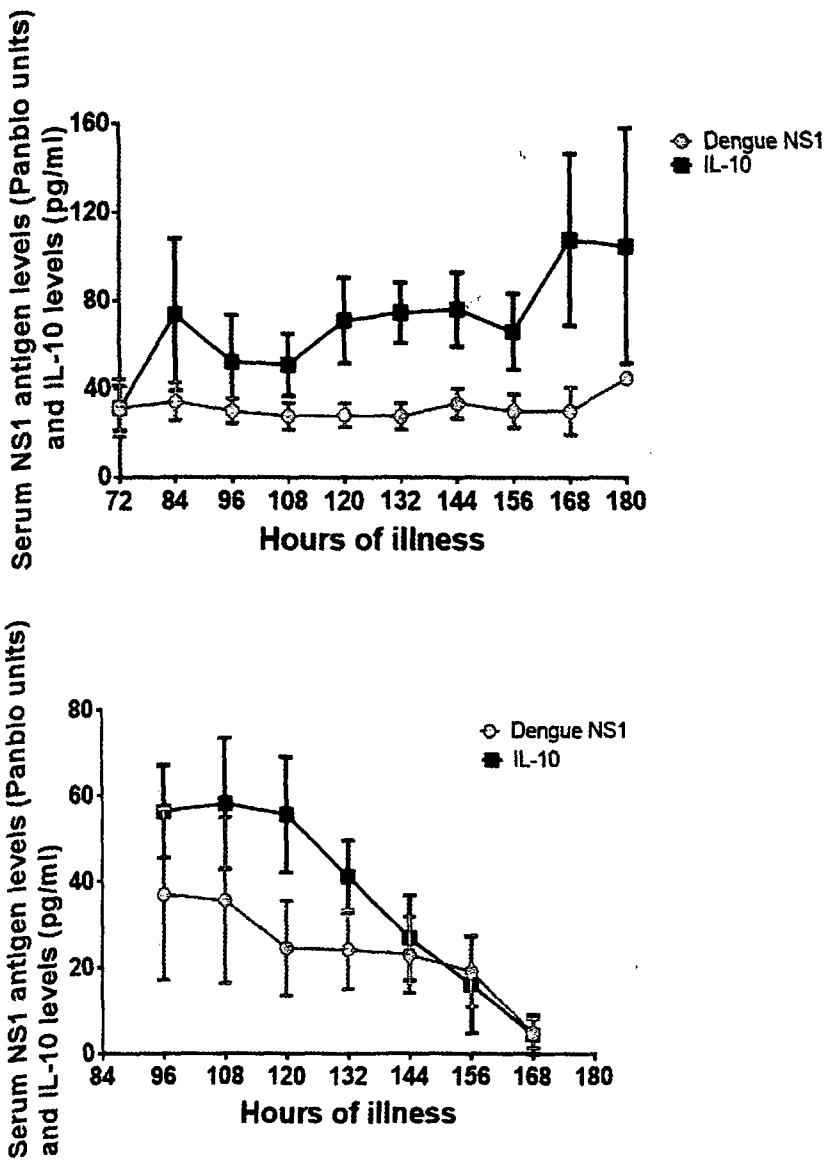


Fig 4: Relationship between serum IL-10 levels and dengue NS1 antigen levels in patients with DHF (top) and those with DF (bottom)

Since we had found that monocytes were the main source of IL-10 and our subsequent experiments showed that NS1 stimulated production of IL-10 from monocytes. We used both recombinant E.coli derived dengue NS1 (certified as LPS free) and mammalian derived NS1 for these experiments (Fig 5) [21].

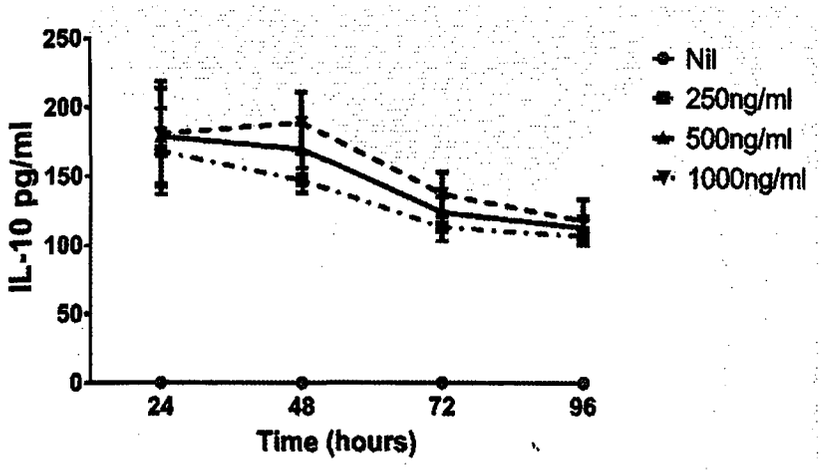
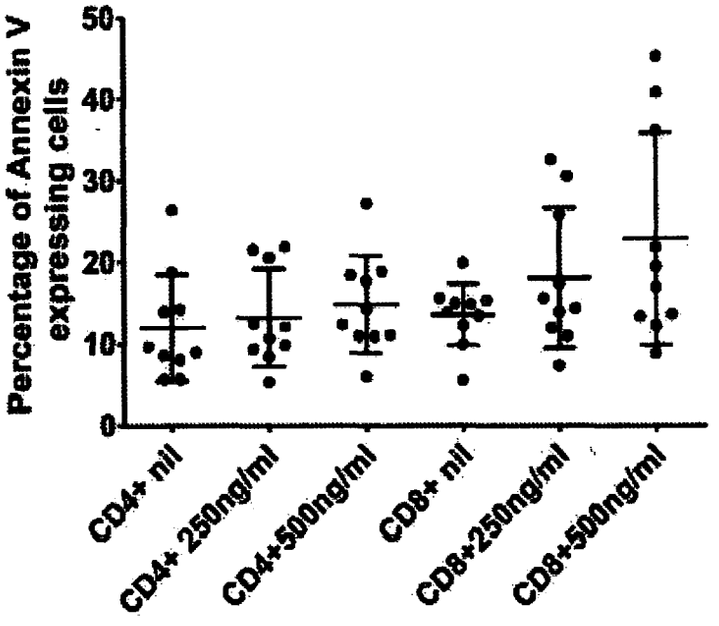


Fig 5: IL-10 production of primary human monocytes co-cultured with various concentrations of human recombinant NS1

Many studies have reported the occurrence of massive T cell apoptosis in acute dengue [12, 22] and many genes involved in the apoptotic pathways were shown to be up regulated in the early phase of dengue infection [23]. However, whether all T cells equally undergo apoptosis or if DV-specific T cells preferentially undergo apoptosis is not known. We found that serum IL-10 levels were associated with T cell apoptosis in acute dengue infection and serum IL-10 has been shown to be elevated in patients with more severe forms of dengue [19, 22, 24, 25]. However, in vitro studies carried out by us, using varying concentrations of human recombinant IL-10 on PBMCs of healthy individuals failed to show that IL-10 itself caused T cell apoptosis in healthy PBMCs[22].

As we found that NS1 induced production of IL-10 from monocytes, we investigated if NS1 itself caused apoptosis of T cells. We found that dengue NS1 was associated with apoptosis of T cells. In these experiments, varying concentrations of dengue NS1 was co-cultured with PBMCs and apoptosis was measured by determining annexin V expression. Although NS1 was associated with both CD4+ and CD8+ T cell apoptosis, the degree of apoptosis varied widely among individuals (Fig 6)[21].

(a)



'ig 6: Apoptosis of CD4+ and CD8+ T cells co-cultures with varying concentrations pf iS1 for 24 hours

ince the functionality of T cell responses and the expression of co-stimulatory markers have ot been studied in detail before, we also investigated the expression of PD-1, CTLA-4, D28 and also TIM-3 in 24 patients (second cohort of patients) with acute dengue and 13 ealthy individuals. In order to assess the functionality of T cell responses intracellular ytokine assays were performed for IFNγ and CD107a expression following stimulation by engue NS3 overlapping peptides. We found that dengue NS1 antigen levels (Panbio units) ad no correlation with the frequency of NS3 specific IFNγ producing T cells, or NS3 pecific CD107a expressing T cells.

As CD28 is essential for antiviral responses we proceeded to investigate its role in acute engue [26]. We found that in patients with acute dengue, CD28 expression by DENV-NS3 pecific T cells positively correlated with CD107a expressing DENV-NS3 specific T cells Spearmans R=0.59, p=0.007). Although, CD28 has been shown to be required for levelopment of early antiviral responses, we found that CD28 expression was significantly ower (p=0.01) in T cells of patients with acute dengue (mean 56.8, SD±19.04 percentage of D3+ T cells) when compared to healthy individuals (mean 73.48, SD±10.94 percentage of D3+ T cells) (Fig 7)[21].

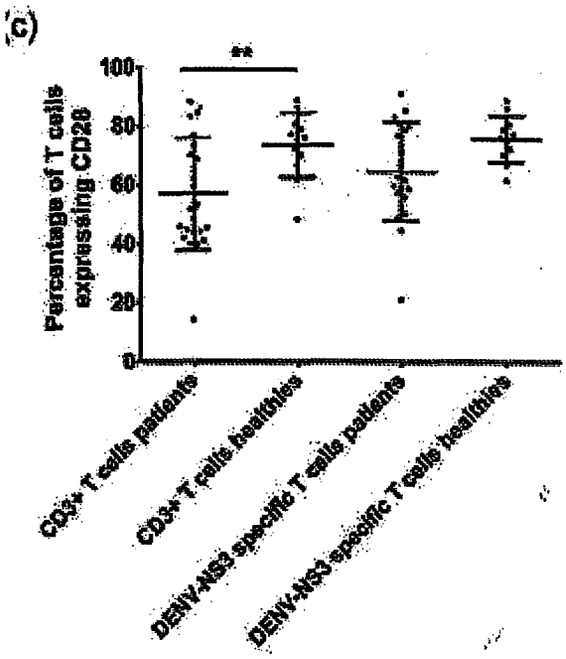


Fig 7: Expression of CD28 by DENV specific T cells and the whole T cell population

In summary, the above studies showed that DENV specific T cells were not responsible for the cytokine storm and also that the DENV-T cells activity was suppressed during acute infection, predominantly due to the effects of IL-10. Others have also shown that DENV-specific T cells were first detectable one day after progression to the critical phase and in the majority of patients the T cell responses were detectable 2 days after deferverscence[27]. Therefore, although highly cross reactive DENV specific T cells do appear to be present in acute dengue infection, their role in causing severe clinical disease is questionable.

Although the emerging data support a protective role of DENV-specific T cells in acute infection; in order to find definitive answers to the type of T cell responses that are associated with protection; T cell responses in relation to frequency, magnitude, breadth and functionality and clinical disease severity should be investigated. Such data is crucial for safe and effective vaccine development.

Reference:

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3. Capeding MR, Tran NH, Hadinegoro SR, Ismail HI, Chotpitayasunondh T, Chua MN, Luong CQ, Rusmil K, Wirawan DN, Nallusamy R *et al*: **Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial.** *Lancet* 2014, **384**(9951):1358-1365.