Annex 4

PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Functionality of Dengue Virus Specific Memory T Cell Responses in Individuals Who Were Hospitalized or Who Had Mild or Subclinical Dengue Infection

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Abstract

Background

Although antibody responses to dengue virus (DENV) in naturally infected individuals have been extensively studied, the functionality of DENV specific memory T cell responses in relation to clinical disease severity is incompletely understood.

Methodology/Principal findings

Using ex vivo IFNγ ELISpot assays, and by determining cytokines produced in ELISpot supernatants, we investigated the functionality of DENV-specific memory T cell responses in a large cohort of individuals from Sri Lanka (n=338), who were naturally infected and were either hospitalized due to dengue or had mild or sub clinical dengue infection. We found that T cells of individuals with both past mild or sub clinical dengue infection and who were hospitalized produced multiple cytokines when stimulated with DENV-NS3 peptides. However, while DENV-NS3 specific T cells of those with mild/sub clinical dengue infection were more likely to produce only granzyme B (p=0.02), those who were hospitalized were more likely to produce both TNFα and IFNγ (p=0.03) or TNFα alone.

We have also investigated the usefulness of a novel T cell based assay, which can be used to determine the past infecting DENV serotype. 92.4% of DENV seropositive individuals responded to at least one DENV serotype of this assay and none of the seronegatives responded. Individuals who were seronegative, but had received the Japanese encephalitis vaccine too made no responses, suggesting that the peptides used in this assay did not cross react with the Japanese encephalitis virus.

Conclusions/significance

The types of cytokines produced by DENV-specific memory T cells appear to influence the outcome of clinical disease severity. The novel T cell based assay, is likely to be useful in determining the past infecting DENV serotype in immune-epidemiological studies and also in dengue vaccine trials.

Author Summary

Although dengue viral infections cause severe clinical disease, the majority of individuals infected with the dengue virus (DENV) develop asymptomatic infection. The function of DENV specific memory T cells in relation to past clinical disease severity is incompletely understood. In this study, we sought to investigate the function of DENV specific memory T cell responses in a large cohort (n = 338) of individuals who were naturally infected with the DENV but developed varying severity of clinical disease. We found that T cells of individuals who were hospitalized due to dengue and those with mild/sub clinical dengue infection produced multiple cytokines when stimulated with DENV-NS3 peptides. In addition, we have also validated a novel T cell based assay, which can be used to determine the past infecting DENV serotype. We found that 92.4% of DENV seropositive individuals responded to at least one DENV serotype of this assay and none of the seronegatives responded. Moreover, the peptides used in this assay did not cross react with Japanese encephalitis virus. Therefore, this assay is likely to be useful in determining the past infecting DENV serotype in immune-epidemiological studies and also in dengue vaccine trials.