

## **Defining protective immunity to dengue virus**

**By**

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Thesis submitted to the University of Sri Jayewardenepura for the  
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Defining protective immunity to dengue virus.

"The work described in this thesis was carried out by me under the supervision of Prof Gathsaurie Neelika Malavige and Prof Graham Ogg. A report on this has not been submitted in whole or in part to any university or any other institution for another Degree/Diploma".



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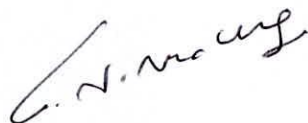
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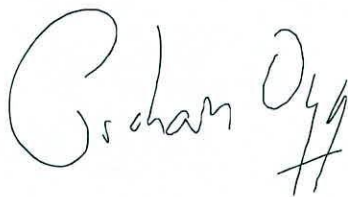
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## List of abbreviations

ADE	Antibody-dependent enhancement
CMV	Cytomegalovirus
CFR	Case fatality rate
DENV	Dengue virus
DHF	Dengue haemorrhagic fever
DNA	Deoxyribonucleic acid
DSS	Dengue shock syndrome
ELISpot	Enzyme-linked immunospot assay
ELISA	Enzyme linked immunosorbent assay
EBV	Epstein Barr virus
FACS	Fluorescence-activated cell sorter
FCS	Foetal calf serum
FEC	Influenza virus, Epstein-Barr virus, and cytomegalovirus peptides
HCV	Hepatitis C virus
HIV	Human Immuno deficiency virus

HLA	Human leukocyte antigen
HUVEC	Human umbilical vein endothelial cells
IFN $\gamma$	Interferon gamma
IL	Interleukin
IgG	Immunoglobulin G
IgM	Immunoglobulin M
JEV	Japanese encephalitis virus
MHC	Major Histocompatibility Complex
NK	Natural killer
NS	Non-structural
NKT	Natural killer T cells
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PHA	Phytohaemagglutinin
PAF	Platelet activating factor
PAFRA	Platelet activating factor receptor antagonist

PRNT	Plaque reduction neutralization test
RNA	Ribonucleic acid
RANTES	Regulated on activation normal T expressed and secreted
SEM	Standard error of mean
SD	Standard deviation
SFU	Spot forming units
SS	Serotype specific
TEER	Trans endothelial electrical resistance
TNF $\alpha$	Tumor necrosis factor alpha
VEGF	Vascular endothelial growth factor
VZV	<i>Varicella Zoster virus</i>
WHO	World health organization
ZO1	Zonula occludens 1
ZONAB	ZO1-associated nucleic-acid binding



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## **Defining protective immune responses to the dengue virus**

**J M Kamal Chandima Jeewandara**

### **ABSTRACT**

**Background:** Although dengue infections can lead to severe clinical disease sometimes resulting in fatalities, the majority of both primary and secondary dengue infections result in mild/asymptomatic disease that is usually not diagnosed as dengue. Therefore, I proceeded to investigate epidemiological and co-morbid risk factors associated with severe dengue and the functionality of DENV specific memory T cell responses in relation to clinical disease severity. Furthermore, as platelet activating factor (PAF) is associated with an increase in vascular permeability in other diseases, I investigated its role in acute dengue infection.

**Methods:** 1689 healthy individuals were recruited. Information regarding their co-morbid illnesses, anthropometric measurements and Japanese Encephalitis vaccination status was recorded. The dengue and Japanese Encephalitis virus (JEV) antibody status was determined in all individuals. Using *ex vivo* IFN $\gamma$  ELISpot assays and by determining cytokines produced in ELISpot supernatants, I investigated the functionality of DENV-specific memory T cell responses in 338 individuals, who were naturally infected and either had severe dengue or had sub clinical dengue infection. PAF levels were assessed in 25 patients with acute dengue infection. The effect of dengue serum on tight junction

protein ZO-1 was determined by using human endothelial cell lines (HUVECs). The effect of dengue serum on trans-endothelial resistance (TEER) and its reversibility in the presence of PAF receptor blocker was also measured on HUVECs.

**Results:** 1152/1689 (68.2%) individuals were seropositive for dengue however only 133/1152 (9.8%) of them had been hospitalized due to dengue. A significant and positive correlation was observed for dengue antibody seropositivity and age in children (Spearman's  $R=0.9$ ,  $p<0.0001$ ) and in adults (Spearman's  $R=0.96$ ,  $p=0.004$ ). Obesity, asthma, allergic rhinitis and a waist circumference of  $>80$ cm in women was significantly associated with increased risk of hospitalization for dengue. JEV antibody positivity was significantly associated with an increased risk of hospitalization in both adults ( $p<0.001$ ) and in children ( $p=0.03$ ). T cells of individuals with both past sub clinical dengue infection and severe dengue produced multiple cytokines when stimulated with DENV-NS3 peptides. DENV-NS3 specific T cells of those with sub clinical dengue infection were more likely to produce only granzyme B ( $p=0.02$ ), while those who had severe dengue were more likely to produce both  $TNF\alpha$  and  $IFN\gamma$  ( $p=0.03$ ) or  $TNF\alpha$  alone. PAF levels were significantly higher in patients with acute dengue ( $n=25$ ;  $p=0.001$ ). Serum from patients with dengue significantly down-regulated expression of tight junction protein, ZO-1 ( $p=0.004$ ) in HUVECs. This was significantly inhibited ( $p=0.004$ ) by the use of a PAF receptor antagonist (PAFRA). Serum from dengue patients also significantly reduced TEER and this reduction was significantly ( $p=0.02$ ) inhibited by prior incubation with the PAFRA.

**Conclusions:** Obesity, asthma and JEV antibody positivity appear to be associated with a higher risk of hospitalization due to dengue virus. The types of cytokines produced by DENV-specific memory T cells appear to influence the outcome of clinical disease severity. The PAF is likely to be playing a significant role in inducing vascular leak in acute dengue infection, which offers a potential target for therapeutic intervention.