DOI: 10.31357/fmesphd.2015.00118

Defining protective immunity to dengue virus

By

Jeewandara Mudiyanselage Kamal Chandima Jeewandara

Thesis submitted to the University of Sri Jayewardenepura for the award of the Degree of Doctor of Philosophy in Immunology on 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".

J M K Chandima Jeewandara

31 March 2015

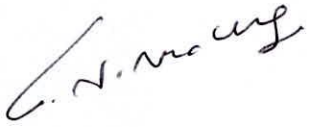
We certify that the candidate has incorporated all corrections, additions and amendments recommended by the examiners to this final version of the PhD thesis

Prof Gathsaurie Neelika Malavige

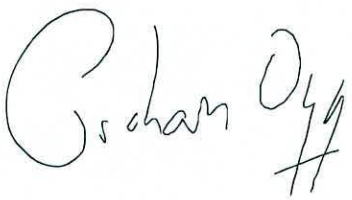
Prof Graham Ogg.

13 June 2016

"We certify that the above statement made by the candidate is true and that this thesis is suitable for submission to the University for the purpose of evaluation".



Prof Gathsaurie Neelika Malavige



Prof Graham Ogg.

Date 17.6.2016

Table of contents

Index of Figures

Index of Tables

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	Flurorescence-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
IFNγ	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 α	Tumor necrosis factor alpha
VEGF	Vascular endothelial growth factor
VZV	*Varicella Zoster* virus
WHO	World health organization
ZO1	Zonula occludens 1
ZONAB	ZO1-associated nucleic-acid binding

Acknowledgements

The final thesis, the culmination of the long hard journey of my PhD would not have been achieved without the support of many individuals who contributed in numerous ways in different stages of my PhD research.

I am deeply indebted to my supervisor Prof Neelika Malavige for her elemental role in my PhD. It is a great honour being her first PhD student. If it had not been her dedication a PhD in immunology, which requires lot of expenditure on research, would have been only a dream. She has been a wonderful teacher, a mentor, who constantly guided and assisted with her expertise in every step of the way. Her well-rounded mentorship helped me to grow as a research scientist. She encouraged me to be an independent thinker while providing valuable feedback. Her generosity with her expertise is remarkable and her enthusiasm for immunology has always been a motivation.

I am extremely grateful to Prof Graham Ogg, my external supervisor who kindly agreed to have me in Oxford as a student. It was a rare privilege to learn from a world-renowned professor in a world-class laboratory. I have been given the exposure to the most sophisticated technology in medical research. The value of experience and knowledge I gained in my training in Oxford is beyond measure. I owe a great debt to Prof Graham Ogg, not just for the opportunity to work in Oxford but for his constant feedback and guidance provided from the beginning to the end.

My studies in Oxford which were an integral part of my research could be achieved only because of two organizations. I wish to express my sincere gratitude to the Association of Commonwealth Universities for granting me a Commonwealth Scholarship to pursue my postgraduate studies in a prestigious university and to the University of Oxford for granting me the studentship and providing excellent facilities to carry out my research work.

I consider myself extremely fortunate to have the University of Sri Jayewardenepura as my employer as I was well supported and encouraged during the course of my PhD. I like to mention Dr N L A Karunathne- former Vice Chancellor of the University, Prof Mohan de Silva, former Dean of the Faculty of Medical Sciences, Prof Sampath Amarathunga, the Vice Chancellor and Prof Surangi Yasawardena, the Dean of the Faculty of Medical Sciences with extreme gratitude.

I have been able to balance the work of a lecturer and a student only because of the great support I received from the Department of Family Medicine of University of Sri Jayewardenepura. I am grateful for the head of the department and my fellow lecturers for being understanding and giving me the time I needed by sharing the duties and responsibilities among them.

The Department of Microbiology and its staff deserve a special mention for the support I received by them. I am grateful for the facilities I was supplied with and the support I received especially from the non-academic staff and the technical officers.

I also thank the staff of the Centre for the Dengue Research for their valuable support, which contributed to the success of my research.

Throughout my PhD, I was greatly supported by some fantastic research assistants who were very committed to their job. I wish to thank Thiruni Adikari, Laksiri Gomez, Dr Samitha Fernando, Dr Randika Fernando and Dr Shiran Paranavithane for being so supportive with such enthusiasm.

My colleagues Achala Kamaladasa, Mariyam Salimi, Danuta Gutowska-Owsiak and Anthony Cheung deserve a special thank for being such great colleagues by sharing knowledge and experience.

I like to thank my parents for all the years of hard work they put in to make the man I am today. I owe them for the good foundation they have given and the positive qualities they instilled

Finally, I thank my wife and son for bearing with me cheerfully during this challenging journey giving me the much needed motivation and encouragement.

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* IFNγ 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 (Spearmans R=0.9, p<0.0001) and in adults (Spearmans R=0.96, p=0.004). Obesity, asthma, allergic rhinitis and a waist circumference of >80cm 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 TNFα and IFNγ (p=0.03) or TNFα 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.