Magapu Solomon Sudhakar

The Lymphatic Fluid and T-Cell Receptor Vβ expression in Peripheral Blood Mononuclear Cells (PBMCs) of patients with Wuchereria Bancrofti Infection

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ANTIGENIC ANALYSIS OF LYMPHATIC FLUID AND T-CELL RECEPTOR Vβ (1-24) REPERTOIRE GENE EXPRESSION IN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCs) OF PATIENTS WITH CHRONIC PATHOLOGY HARBOURING *WUCHERERIA BANCROFTI* INFECTION

A THESIS

Submitted by

M. SOLOMON SUDHAKAR

in the fulfilment for the award of the degree

of

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BONAFIDE CERTIFICATE

Certified that this thesis titled **"ANTIGENIC ANALYSIS OF LYMPHATIC FLUID AND T-CELL RECEPTOR Vβ (1-24) REPERTOIRE GENE EXPRESSION IN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCs) OF PATIENTS WITH CHRONIC PATHOLOGY HARBOURING *WUCHERERIA BANCROFTI* INFECTION"** is the bonafide work of **Mr. M. SOLOMON SUDHAKAR** who carried out the research under my supervision. Certified further that to the best of my knowledge the work reported herein does not form part of any other thesis or dissertation on the basis of which a degree or award was conferred on an earlier occasion of this or any other candidate.

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ABSTRACT

Lymphatic filariasis (LF) is a disease that is currently the target of a major global initiative for elimination. During the past decade, both the treatment and the control strategies for LF have undergone major paradigm shifts – due to rapid increase in knowledge and understanding of LF, that is derived directly from a series of commendable progress made by scientific and medical research communities. As a result, a public health dimension with a focus on affected populations, now supplements the earlier, predominantly patient-oriented clinical approach to LF.

The Indian government launched a nationwide LF control programme with the components of transmission interruption by annual mass-drug therapy, using diethylcarbamazine with/without albendazole, and the alleviation of disability and suffering among affected people.

In pursuit of understanding such a disease to which a social stigma is attached, the chronic pathology of LF was taken as a model to understand the immunopathological mechanisms that lead to acute lymphangitis and lymphadenitis, in filarial infections, harboring *Wuchereria bancrofti.* Hence, an attempt was made in this thesis to identify parasite specific antigens and antibodies in lymphatic fluid, which accumulates in CP conditions. At the same time, the corresponding serum from the same patient who was suffering from chronic pathology of filariasis, was also taken for analysis. It is a well

established fact that elephantiasis is a consequence of immune reactivity to adult worm antigens. Therefore, it was thought that T-cells infiltrating the lesions in chronic pathology disease, could augment for elevated inflammation seen in CP. An attempt was also made to examine T cell by TCR Vβ analysis using RT PCR and antigen stimulated PBMC's. Therefore, 24 Vβ gene families in the given repertoire was the choice for experimental analysis. Along with all CP cases for these studies in both the objectives, appropriate controls were also included in this study as EN, who are normal healthy individuals and MF, who are carriers of this disease. At the onset, it was thought that bacterial infections could predominate the sequence of infections in CP. As such, an attempt was also made to study the effect of lymphatic fluid on the bacterial growth *in vitro*, and the presence of antibodies against bacteria that cause secondary infections, was assessed.

Lymphatic fluid and corresponding serum contains parasite specific antigens and antibodies when analyzed by SDS-PAGE and Western blot. At the sametime, LF promoted the growth of *beta –hemolytic streptococci* bacteria which usually predominates the secondary infections. Specific T Cell Receptors which get overrepresented in the CP subjects when the PBMCs of these subjects come in contact with crude antigen of the parasite BmA but MF's, do not show any overrepresentation for BmA under similar conditions.

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µg	-	Microgram
µL	-	Microliter
ADL	-	Acute Adenolymphangitis
Ag	-	Antigen
ALP	-	Alkaline Phosphatase
ANOVA	-	Analysis Of Variance
Anti-BmA	-	Polyclonal antibodies raised against *Brugia malayi* adult antigen crude extract
Anti-ES	-	Polyclonal Antibodies Raised Against Excretory And Secretory Antigens
BCIP	-	Bromochloro Indole Phosphate
BmA	-	*Brugia malayi* Adult Antigen Crude Extract
bp	-	Base Pairs
BSA PBS	-	Bovine Serum Albumin In Phosphate Buffer Saline
BSA	-	Bovine Serum Albumin
cDNA	-	Complementary DNA
CDR3	-	Complementary Determining Region 3
CHISAM	-	Chloroform Isoamyl Alcohol Mix
CIC	-	Circulating Immune Complex
CP	-	Chronic Pathology
cRPM1640	-	Complete Roswell Park Memorial Institute 1640 Medium
DEC	-	Diethyl Carbamazine
DEPC	-	Diethylpyrocabonate
dNTP	-	Deoxy Nucleotide Triphosphate
DTT	-	Dithiotheritol

EDTA	-	Ethylene Diamine Tetra Acetic Acid
EGTA	-	Ethylene Glycol Tetra Acetic Acid
ELISA	-	Enzyme Linked Immunosorbo Assay
EN	-	Endemic Normal
FCS	-	Fetal Calf Serum
g	-	Gravity
IDV	-	Integrated Density Value
kD	-	Kilo Dalton
LF	-	Lymphatic filariasis
LSM	-	Lympho Separation Medium
MF	-	Microfilaremics
mf	-	Microfilariae
MOPS	-	3-(N-Morpholino) Propanesulfonic Acid
NBT	-	Nitro Blue Tetrazolium
NCP	-	Nitrocellulose Membrane
NEN	-	Non Endemic Normal
ng	-	Nanogram
nM	-	Nanomoles
NMS	-	Normal Mouse Serum
NRS	-	Normal Rabbit Serum
OD	-	Optical Density
PBMCs	-	Peripheral Blood Mononuclear Cells
PBST	-	Phosphate Buffer Saline Tween 20 (0.05%)
PEG	-	Polyethylene Glycol
PHA	-	Phytohaemagglutinin
pM	-	Pico mole
PMSF	-	Phenyl Methyl Sulphonyl Fluoride
pNPP	-	Para Nitro Phenyl Phosphate
PPD	-	Purified Protein Derivative of Mycobacterium tuberculin
RPMI1640	-	Roswell Park Memorial Institute 1640 Medium