UNIVERSITI PUTRA MALAYSIA

IN VITRO EXPRESSION OF FILARIAL SXPI GENE FOR THE DEVELOPMENT OF A NUCLEIC ACID BASED VACCINE

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By

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Chairman: **Professor Dr. Wan Omar Abdullah, Ph,D**

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The objectives of this study were to clone gene that encode filarial SXP1 protein followed by *in vitro* expression of the protein. The Special Programme for Research and Training in Tropical Diseases (TDR) WHO has advocated SXP1 as one of the vaccine candidate to curb filarial infection. SXP1 antigen has been reported to confer protective immunity, causing reduction of microfilaraemia levels in jirds (*Meriones unguiculatus*) blocking subsequent *Brugia malayi* infection. In this study, the gene that encode SXP1 antigen was 517 bp in length and was extracted and amplified from the infective stage (L_3) of subperiodic *Brugia malayi.* The gene was successfully cloned into replication vector pCR®2.1 (Invitrogen) followed by subcloning into mammalian expression vector pVAX1 (Invitrogen). The presence of *SXP1* gene in both vectors were validated by polymerase chain reaction (PCR), restriction enzymes analysis (RE) and finally by automated sequencing. The

cloned *SXP1* in pVAX was designated as pVAX/*SXP1*. The plasmid bearing *SXP1* gene was transfected into two types of animal cell lines (COS-7 and CHO) using Polyfect Transfection Reagent (Qiagen). The successful expression of targeted gene in the mammalian cell lines were determined by RT-PCR and Western Blotting. The PCR product of the transfected cells was 517 bp on the agarose gel. In addition, the ~20 kDa of expressed SXP1 protein was detected on nitrocellulose membrane by rabbit polyclonal antibody against the SXP1 protein. This study has successfully established the ground work for future deliberations towards the development of anti-brugia transmission blocking genetic vaccine.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

EKSPRESI *IN VITRO* KE ATAS GEN *SXP1* FILARIA UNTUK PEMBANGUNAN VAKSIN ASID NUKLEIK

Oleh

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February 2005

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Objektif kajian ini ialah pengklonan gen yang mengkodkan protein SXP1 cacing filaria dan seterusnya mengekspresinya secara *in vitro*. *SXPI* telah dipilih sebagai calun di dalam kajian ini berdasarkan kajian-kajian lepas yang menunjukkan keupayaan antigen SXP1 memberi perlindungan di dalam mengurangkan tahap mikrofilaremia di dalam gerbil (*Meriones unguiculatus*); yang dijangkiti dengan *Brugia malayi*. Gen *SXP1* juga dicadangkan oleh TDR sebagai calon vaksin bagi mengatasi masalah jangkitan cacing filaria. Gen *SXP1* berberat molekul 517 bp telah dicerakinkan daripada peringkat L_3 *Brugia malayi* subperiodik dan seterusnya diamplifikasikan dengan kaedah tindakbalas rantai polimerase (PCR). Gen *SXP1* kemudiannya diklonkan di dalam vektor replikasi pCR®2.1 (Invitrogen) dan seterusnya gene *SXP1* di subklonkan di dalam vektor eukariot pVAX1 (Invitrogen) untuk proses ekspresi protein SXP1. Gen *SXP1* yang telah diklonkan tadi telah dibuktikan kehadirannya dan pada kedudukan yang betul melalui kaedah tindakbalas rantai polimerase (PCR), kaedah pencernaan enzim pembatas (RE) dan juga

melalui kaedah penjujukan gen secara automasi. Bagi membuktikan kebolehan gen *SXP1* di ekspresikan secara *in vitro*, pVAX/SXP1 telah di transfeksikan dengan menggunakan "Polyfect Transfection Reagent" (Qiagen) ke atas dua sel haiwan COS-7 dan sel CHO. Kejayaan ekspresi protein SXP1 telah dibuktikan melalui kaedah tindakbalas rantai polimerase berbalik (RT-PCR) dan ini diikuti dengan proses Western Blot. Keputusan ujian tindakbalas rantai polimerase menunjukkan gen *SXP1* telah ditranskripsikan dan ini diikuti dengan keputusan Western Blot yang menunjukkan protein SXP1 yang mempunyai berberat molekul ~ 20 kDa telah berjaya diekspresikan secara *in vitro* apabila di probekan menggunakan antibodi poliklon terhadap protein SXP1 yang dihasilkan di dalam arnab. SXPI secara zahirnya tidak lagi diketahui akan sifat kimiawi dan fungsinya, tetapi kami percaya dengan kejayaan mengekspresikan protein ini secara *in vitro* merupakan langkah awal di dalam pembangunan vaksin jangkitan yang berpunca daripada cacing filaria brugia.

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LIST OF ABBREVIATIONS

Ag	antigen
Ab	antibody
ADL	adenolymphangitis
AFL	acute filarial lymphangitis
AP	alkaline phosphatase
APCs	antigen presenting cell
BM	*Brugia malayi*
Bp	base pair
BSA	bovine serum albumin
cDNA	complementary dseoxyribonucleic acid
CMI	cell mediated immune
CMV	cytomegalovirus
CO_2	carbon dioxide
CTL	cytotoxic T lymphocyte
Da	Daltons
DEC	diethylcarbamazine citrate
DEPC	diethyl pyrocarbonate
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetaacetic acid
EST	express sequenced taq
EtBr	ethidium bromide
FCS	foetal calf serum
FGP	filarial genome project

GM-CSF	granulocyte-macrophage colony stimulating factor
GST	glutathione-S-transferase
HCL	hydrochloric acid
ID	intradermal
IP	intraperitoneal
IFN	interferon
Ig	immunoglobulin
IL	interleukin
IPTG	isopropyl-β-D thiogalactoside
IV	intravenous
Kb	kilobase
kDa	kilodalton
KCL	potassium chloride
LB	Luria Brutani
LPS	lipopolysaccharides
M	molarity
MCS	multiple cloning sites
Mf	microfilria
$MgCl_2$	magnesium chloride
MHC	Major histocompatibility complex
M	mole
mRNA	messenger ribonucleic acid
MW	molecular weight
NaCl	sodium Chloride
NaOH	sodium hydroxide

OD	optical density
ORF	Open Reading Frame
PBS	phosphate buffer saline
PC	phosphorylcholine
PCR	polymerase chain reaction
Pcmv	cytomegalovirus promoter
RE	restrion enzyme
RNA	ribonucleic acid
RSV	Raos Sarcoma Virus
SC	subcutaneous
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SV40	simian virus 40
TBE	Tris-boric-EDTA buffer
TBST	Tris-buffered saline-tween20
TEMED	N,N,N',N'-tetramethylethylenediamine
Th	helper T cells
tRNA	total ribonucleic acid
Tris-HCl	Tris hydrochloride
TPE	Tropical Pulmonary Eosinophilia
UM	University Malaya
UV	Ultra violet
WHO	World Health Organization
X-gal	5-bromo-4-chloro-3-indolyl-β-D-Galactoside

CHAPTER I

INTRODUCTION

Lymphatic filariasis has existed as a recognizable disorder and it has also been recorded since the beginning of human history. Ancient Chinese and Indians writings have described this disease as swellings of extremities and the genitalia that were highly reminiscent of filarial lesions. Sushruta, the Indian physician/surgeon in his book, called this disease as *slipada* (*sli* elephant; *pada* leg) and also described the prevalence rate was higher in individuals living close to stagnant water. Ar Rhazes and Avecenna the two famous Persian physicians described this disease in Arabic and Avecenna, had reported that the disease was endemic in Alexandria, Egypt. Lymphatic filariasis was wrongly diagnosed as leprosy by the Greek physicians. The dominant figure in the early history of lymphatic filariasis was Sir Patrick Manson, a Scottish physician stationed in China during the second half of the nineteenth century. He correctly attributed the profound, deforming swelling of the extremities to the infection with filarial parasites. He also demonstrated the numerous microfilariae in the blood of a Chinese patient, and described that if all the microfilariae were to grow into adult worms, there would be no space for any other structure within the human body. He also correctly surmised that in order to develop and grow the parasites had to leave the human body

Lymphatic filariasis is a major cause of clinical morbidity and an impediment to socio-economic development (Evans *et al.,* 1993). The disease

is mosquito-borne and very common in the tropics. The worms that caused the infection are *Wuchereria bancrofti, Brugia malayi* and *Brugia timori. W. bancrofti,* the most common filarial parasite, is found in Africa, India, Pacific Islands, the Caribbean, South America and South East Asia. Infection due to *W. bancrofti* contributed to 90% of total infections in the tropics and in some sub-tropical areas world-wide. In the South East Asia, particularly in Malaysia, *B. malayi* is a the main species that caused lymphatic filariasis, and *B. timori* is limited to Timor Island and islands adjacent to it. In Malaysia, *W. bancrofti* is mainly found in Sabah and Sarawak. More than 1.2 billion people, i.e. 20% of the world's population live in areas where they are at risk of infection, of which 90% of the infections are with *W. bancrofti* and 10% with *B. malayi* (WHO, 2000). It is currently estimated that some 512 million people are at risk of infection in the sub-Saharan Africa.

Lymphatic filariasis causes the most debilitating and disfiguring of all disease. Lymphatic filariasis has been recognised as one the most prevalent of tropical diseases, and the most neglected disease. It afflicts poor people in both urban and rural areas. Rarely fatal, it causes extensive disability, gross disfigurement and untold suffering for millions: young and old; men, women and children. In every community where it occurs, this disease remains a strong impediment to socioeconomic development. Lymphatic filariasis has been identified as among the world's six potentially 'eradicable' infectious disease by the International Task Force for Disease Eradication (WHO, 1992) and was designated as the world's second leading cause of permanent and long term disability by WHO, The two main strategies are through drug

therapy and vector control which are going to be implemented toward the complete elimination of the disease by the year 2020.

The development of vaccines for lymphatic filariasis is still in the state of relative infancy in comparison to other parasitic diseases such as schistosomiasis and malaria. This is due to the complexity of the filarial parasite itself and also due to complex host immune responses, which are poorly understood. With the advancement in the field of molecular biology, the development of vaccines for lymphatic filariasis has undergone a new dimension.

Prior studies have shown that a degree of protective immunity to filariasis can be induced in animals by vaccination with irradiated L_3 (Yate *et al.,* 1985, Weil .G., *et al.,* 1992). The potential of using live anti-filarial vaccines in humans is limited because of safety issues and limited availability of larvae. Several laboratories are working to develop effective recombinant antigen-based vaccines that would be more practical and effective than live parasite vaccines.

DNA vaccination is a promising approach that may have several advantages over vaccination with live parasites or protein antigens. DNA vaccines have been shown to be an effective means of generating cellular and humoral immune responses, and they have conferred protection against a wide range of infectious agents including viruses, parasites, and bacteria in animal models (Montgomery, *et al.,* 1997).

Objectives

The general objective of this study is to identify gene that encode filarial antigen toward the development of a DNA based vaccine against *B. malayi* .

The following are the specific objectives:

1. To amplify the *SXP1* sequence from *B. malayi*.
2. To clone the amplified *SXP1* gene into an appropriate vector.
3. To express the SXP1 protein *in vitro* after gene transfection in mammalian cell lines.

CHAPTER II

LITERATURE REVIEW

Lymphatic Filariasis in Malaysia

Lymphatic filariasis constitutes the principal mosquito-borne nematode infection due to three types of filarial worms namely *W. bancrofti*, *B. malayi* and *B. timori*. *W. bancrofti* caused bancroftian filariasis and *B. malayi* and *B. timori* caused brugian filariasis. Bancroftian filariasis is the more prevalent of the two (contribute to 90% of total infections), occurring throughout the tropics and subtropics countries; Africa, India, Pacific Islands, the Caribbean, South America and Southeast Asia, except Middle East region where infection appears to be endemic only in Egypt (Figure 1). In Malaysia, urban bancroftian filariasis is unheard of these days, while cases of rural bancroftian filariasis have been reported only from Sabah and Sarawak. By contrast, bugian filariasis is restricted to South East Asia, including Southern China (Figure 2), whereby the *B. malayi* is the major caused of lymphatic filariasis. *B. timori* is found in Timor Island, Flores and the adjacent islands of Indonesia.

Mak in 1985 was estimated two billion peoples are at risk of infection in Malaysia. The predominant species of filarial parasites is subperiodic *B. malayi* which contribute 80.2% of all cases followed by periodic *B. malayi* 12.9%, *W. bancrofti* 5.7% and mixed infection accounts for 1.3%