DEVELOPMENT OF A DEOXYRIBONUCLEIC ACID VACCINEAGAINST ENTEROVIRUS 71

By

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DEVELOPMENT OF A DEOXYRIBONUCLEIC ACID VACCINE

AGAINST ENTEROVIRUS 71

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Decemeber 2005

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Enterovirus 71 (EV71) is a major causative viral agent responsible for large outbreaks of hand, foot and mouth disease (HFMD), a common rash illness in children and infants. There is no effective antiviral treatment for severe EV71 infections and no vaccine is available. The objectives of this study were to design and construct a DNA vaccine against Enterovirus 71 using the viral capsid protein (VP1) gene of EV71 and to verify the functionality of the DNA vaccine *in vitro* and *in vivo*. The VP1 gene of EV71 isolate S2/86/1 and isolate 410/4 obtained from Prof. Mary Jane Cardosa, Universiti Malaysia Sarawak (UNIMAS) were amplified using PCR and then inserted into a eukaryotic expression vector, pVAX1. The 3.9 kb recombinant constructs were transformed into competent *E. coli* cells and the positive clones were screened and selected using PCR analysis, restriction digestion analysis and DNA sequencing. The pVAX1 vector that was successfully cloned with the VP1 gene from each of the isolate (S2/86/1

and 410/4) in the correct orientation and in-frame, were designated as pVAX1/VP1-S and pVAX1/VP1-4, respectively. The DNA vaccine constructs with the VP1 gene were shown to be expressed in a cell-free *in vitro* expression system. The constructs were then tested for protein expression in Vero cells. The VP1 protein was successfully expressed in the mammalian cell line and was detected using RT-PCR, Indirect Immunofluorescence Assay (IFA) and western blotting. Subsequently, in the *in vivo* studies, female Balb/c mice were immunized with the DNA vaccine constructs. Enzyme Linked Immunosorbent Assay (ELISA) was performed to detect the presence of anti-VP1 IgG in mice. The anti-VP1 IgG levels in mice immunized with the DNA vaccine constructs increased after the first booster but declined following the second booster. The anti-VP1 IgG in the mice immunized with the DNA vaccine constructs exhibited neutralising activity against EV71. The promising results obtained in the present study have prompted further testing to improve the expression and immunogenicity of this potential EV71 DNA vaccine.

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

PEMBANGUNAN SATU VAKSIN ASID DEOKSIRIBONUKLEIK TERHADAP ENTEROVIRUS 71

Oleh

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Enterovirus 71 (EV71) merupakan satu agen penyebab yang bertanggungjawab ke atas wabak penyakit tangan, kaki dan mulut, satu penyakit kudis yang biasa dijumpai di kalangan kanak-kanak dan bayi. Setakat ini, tiada rawatan yang berkesan terhadap jangkitan EV71 dan tiada vaksin. Objektif kajian ini adalah untuk mereka bentuk dan membangun satu vaksin DNA terhadap Enterovirus 71 dengan menggunakan gen kapsul protein (VP1) EV71 dan menentusahkan keberkesanan vaksin DNA secara *in vitro* dan *in vivo*. Gen VP1 isolat S2/86/1 dan 410/4 dari EV71 yang didapatkan dari Prof. Mary Jane Cardosa, Universiti Malaysia Sarawak (UNIMAS) digandakan dengan PCR dan kemudian dimasukkan ke dalam satu vektor ekspresi eukariotik, pVAX1. Binaan rekombinasi yang bersaiz 3.9 kb ini ditransformasikan ke dalam sel *E. coli* yang kompeten dan klon positif disaring dan dipilih dengan menggunakan analisis PCR, analisis penghadaman terhad dan penjujukan DNA automasi. Vektor pVAX1 yang berjaya diklonkan dengan gen VP1 dari setiap isolasi (S2/86/1 dan

410/4) dalam orientasi yang betul, telah masing-masing dinamakan sebagai pVAX1/VP1-S dan pVAX1/VP1-4. Binaan vaksin DNA dengan gen VP1 telah ditunjukkan dapat diekspres dalam satu sistem ekspresi *in vitro* bebas sel. Binaan ini kemudiannya diuji untuk ekspresi protein dalam sel Vero. Protein VP1 telah diekspreskan dengan berjayanya dalam sel selanjar mamalia dan telah ditentukan dengan menggunakan RT-PCR, asai immunofloresen tidak langsung (IFA) dan blot western. Seterusnya, dalam kajian *in vitro*, mencit Balb/c betina telah diimunisasikan dengan binaan vaksin DNA. Enzyme Linked Immunosorbent Assay (ELISA) telah dijalankan untuk menentukan kehadiran IgG anti-VP1 dalam mencit. Paras IgG anti-VP1 dalam mencit yang diimunisasikan dengan vaksin DNA meningkat selepas suntikan penggalak pertama tetapi merosot berikutan suntikan penggalak yang kedua. IgG anti-VP1 dalam mencit yang diimunisasikan dengan vaksin DNA menunjukkan aktiviti neutralisasi terhadap EV71. Hasil yang menggalakkan yang didapati dalam kajian ini telah mendorong ujian selanjutnya bagi memperbaiki ekspresi dan immunogenisiti vaksin DNA EV71 yang berpotensi ini.

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I certify that an Examination Committee met on 13th December 2005 to conduct the final examination of Wong Siew Tung on his Master of Science thesis entitled "Development of a Deoxyribonucleic Acid Vaccine Against Enterovirus 71" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for equations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

_____

(WONG SIEW TUNG)

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TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	iv
ACKNOWLEDGEMENT	vi
APPROVAL	viii
DECLARATION	x
LIST OF TABLE	xv
LIST OF FIGURE	xvi
LIST OF ABBREVIATION	xviii
CHAPTER	
1. INTRODUCTION	1
2. LITERATURE REVIEW	
2.1 Enterovirus 71	5
2.1.1 Taxonomy	5
2.1.2 Virus Structure and Genetics	6
2.1.3 VP1 Gene	8
2.1.4 History of EV71: Endemicity and Epidemic Activity	9
2.2 Hand, Foot and Mouth Disease	12
2.2.1 Introduction	12
2.2.2 Symptoms	13
2.2.3 Contagious Period and Spread	14
2.2.4 Diagnosis	15
2.2.5 Treatment and Prevention	15
2.3 Neurological Disease and Other EV71-Associated Diseases	16
2.4 Antiviral Agents	17
2.5 Vaccine Development for EV71	18
2.6 Vaccination	20
2.6.1 Introduction	20
2.6.2 Types of Vaccine	21
2.7 DNA Vaccination	26
2.7.1 Introduction	26
2.7.2 Early History of DNA Vaccination	28
2.7.3 Plasmid DNA Design	29
2.7.4 DNA Vaccine Delivery	31
2.7.5 Advantages and Disadvantages of DNA vaccines	33
2.8 Immune Responses to DNA Vaccines	39
2.8.1 Mechanism of Antigen Presentation	39
2.8.1.1 Direct Priming of Somatic Cells	40
2.8.1.2 Direct Transfection of Professional	43

APCs
2.8.1.3 Cross-Priming 45

2.8.2 Cellular Immunity 47
2.8.2.1 Cytotoxic T cell responses 48
2.8.2.2 T-helper cell responses 49
2.8.3 Humoral Responses 51
2.9 Enhancement of Antigen Expression and Immunostimulation 52
2.10 Safety Issues of DNA Vaccines 56

3. MATERIALS AND METHODS 59
3.1 Stock Preparation of *E. coli* Clones Carrying VP1 Gene in pCR® Blunt Vector 60
3.2 Extraction of Plasmid DNA 61
3.3 Amplification of VP1 Gene 63
3.3.1 Primers Design 63
3.3.2 PCR Condition for VP1 Gene Amplification 65
3.4 Detection and Confirmation of PCR Products 66
3.4.1 Agarose Gel Electrophoresis 66
3.4.2 Purification of PCR Products 67
3.4.3 DNA Sequencing Analysis 68
3.5 Preparation of Insert (VP1 Gene) for Cloning 69
3.5.1 Restriction Digestion of Insert (VP1 Gene) 69
3.5.2 Purification of Digested Insert 70
3.6 Preparation of pVax1 Vector for Cloning 70
3.6.1 pVAX1 Vector 70
3.6.2 Restriction Digestion of pVAX1 Vector 72
3.6.3 Purification of Digested Vector 72
3.7 Ligation of VP1 Gene and pVax1 Vector 72
3.8 Transformation Reaction 73
3.9 Screening and Selection of Positive Recombinant Clones 74
3.9.1 PCR Analysis 74
3.9.2 Restriction Digestion Analysis 75
3.9.3 DNA Sequencing Analysis 75
3.10 Verification of pVAX1/VP1-S and pVAX1/VP1-4 Constructs 76
3.10.1 Eukaryotic Cell-free *In Vitro* Expression System 76
3.10.2 Detection of VP1 Protein Synthesized *In Vitro* 77
3.11 Preparation of pVAX1/VP1 for *In Vitro* Transfection 79
3.12 Propagation and Maintenance of Vero Cells 81
3.13 *In Vitro* Transfection 83
3.13.1 Optimisation of Transfection using 83

pVAX1/*Lac*Z
3.13.2 B-Gal Staining 85
3.13.3 Transfection of pVAX1/VP1-S and pVAX1/VP1-4 87

3.14 Detection of VP1 Gene Expression 88
3.14.1 Detection of VP1 mRNA 88
3.14.1.1 Total RNA Isolation 88
3.14.1.2 Reverse Transcription of RNA 90
3.14.1.3 Amplification of cDNA 91
3.14.2 Indirect Immunofluorescence Assay (IFA) 91
3.14.3 Western Blotting 93
3.15 DNA Vaccination of Mice 94
3.15.1 pVAX1/VP1 Purification for DNA vaccination 95
3.15.2 Delivery of DNA Vaccine Using Intramuscular Injection 97
3.15.3 Blood Samples Collection 98
3.16 Detection of Anti-VP1 IgG in Mice Sera 98
3.16.1 Coating of VP1 Antigen on 96-well Microtiter Plates 99
3.16.2 Enzyme Linked Immunosorbent Assay (ELISA) 100
3.16.3 Statistical Analysis 102
3.17 Virus Neutralization Test 102

4. RESULTS
4.1 Amplification of VP1 Gene by PCR 104
4.2 Confirmation of VP1 Gene by Sequencing Analysis 106
4.3 Screening and Selection of Positive Recombinant Clones 107
4.3.1 PCR Analysis 107
4.3.2 Restriction Analysis 110
4.3.3 DNA Sequencing Analysis 112
4.4 Eukaryotic Cell-free *In Vitro* Expression of VP1 Protein 113
4.5 Optimization of Transfection using pVAX1/*LacZ* 117
4.6 Detection of VP1 Gene Expression 120
4.6.1 Detection of VP1 mRNA in Transfected Cells Using RT-PCR 120
4.6.2 Indirect Immunofluorescence Assay (IFA) 122

4.6.3 Detection of VP1 Protein in Transfected Vero Cells by Western Blotting 124

4.7 Detection of Anti-VP1 IgG in Sera of Immunized Mice by ELISA 126
4.8 Virus Neutralization Test 133

5. DISCUSSION
5.1 Designing and construction of VP1 DNA Vaccine 138
5.2 *In Vitro* Expression of VP1 Protein 144
5.3 *In Vivo* Testing of VP1 DNA Vaccine 150

6. FUTURE DIRECTIONS 157

7. CONCLUSION 159

REFERENCES 161
APPENDIXES 181
BIODATA OF THE AUTHOR 189