



**Multiple epitope immunogens (MEI) mimic the variability of the V3 loop of HIV-1 subtype C**

Raymond Hewer

# **Multiple epitope immunogens (MEI) mimic the variability of the V3 loop of HIV-1 subtype C.**

by

RAYMOND HEWER

B.Sc. (Rand Afrikaans University, Johannesburg, South Africa) 1999

B.Sc. Hons. (Rand Afrikaans University, Johannesburg, South Africa) 2000

## **Dissertation**

Submitted in fulfillment of the requirements for the degree



in the

**FACULTY OF SCIENCE**

at

**RAU UNIVERSITY**

Auckland Park

South Africa

**Supervisor: Dr. Debra Meyer**

December 2002

## Acknowledgements

My utmost gratitude to Dr. Debra Meyer who is not only an excellent supervisor but also a remarkable and inspiring person.

I would like to thank the MRC for financial support for the duration of this project and the laboratory of Professor J. Torres at the Medical Microbiology and Immunology Department, University of California, Davis for the kind donation of plasma samples.

Mostly, I would like to acknowledge my parents for too many things to list. Your never-ending altruism never ceases to amaze me.

I thank my sister Jackie for genuine support and belief in me. I thank Keith who will always do more than expected and for the excellent motivation and advice. I thank Pamela, who is a true little sister and is always there for me- especially when I reach the 13<sup>th</sup> hour.

I thank Megan for everything, especially patience and understanding.

To my lab friends – thanks for the memories, thanks for the laughs.

*You'll remember me when the west wind moves*

*Upon the fields of barley*

*You'll forget the sun in his jealous sky*

*As we walk in fields of gold*

**Forever In Memory**



**Jaederic I. Modoo**

01/02/80 – 20/07/01

# Preface

Contents of this thesis have been compiled in two manuscripts:

- 1) Hewer R., Meyer D. (2002). Producing a highly immunogenic synthetic construct active against HIV-1 subtype C. *Vaccine* 20: 2680 – 2683.
- 2) Hewer R., Meyer D. (2003) Peptide immunogens based on the envelope region of HIV-1 are recognized by HIV / AIDS patient polyclonal antibodies and induce strong humoral immune responses in mice and rabbits. Submitted to *Molecular Immunology*.

A copy of manuscript 1 is included in the Appendix



# Contents

Abbreviations	I
List of Figures	V
List of Tables	VII
Abstract	VIII
Samevatting	IX
<b>Chapter 1</b>	<b>1</b>
<i>Literature survey</i>	
<b>1. Introduction</b>	<b>1</b>
<b>1.1. HIV / AIDS</b>	<b>3</b>
1.1.1. Early history of HIV	3
1.1.2. Epidemiology of HIV in South Africa	4
1.1.3. Taxonomy	6
1.1.4. Nomenclature and phylogeny	8
1.1.5. Properties of the virion.	10
1.1.6. Genomic organization	11
1.1.7. Life cycle of HIV	14
<b>1.2. AIDS pathogenesis</b>	<b>16</b>
1.2.1. Overview of the function and components of the immune system	16
1.2.2. Disease progression	18



<b>1.3. Viral strategies to avoid host immune responses</b>	<b>21</b>
1.3.1. Genetic variation	21
1.3.2. Viral latency	23
<b>1.4. HIV Vaccines</b>	<b>24</b>
1.4.1. Current HIV vaccine approaches	25
1.4.2. Neutralizing epitopes of HIV-1	27
1.4.3. Problems hindering HIV vaccine development	28
1.4.4. Animal models	29
<b>1.5. Synthetic peptides</b>	<b>32</b>
1.5.1. Synthetic peptide-based vaccines	32
1.5.2. Design of synthetic peptides	33
1.5.2.1. Peptide design methodology and considerations	33
1.5.2.2. Synthesis of synthetic peptides	35
1.5.3. Novel peptides designed to target hypervariability	36
1.5.4. Enhancement of peptide immunogenicity	37
1.5.4.1. Carriers	37
1.5.4.2. Multiple antigenic peptide (MAP)	38
1.5.4.3. Adjuvants	39
1.5.5. Characterization of synthetic peptides	41
<b>1.6. Objectives</b>	<b>42</b>

<b>Chapter 2</b>	<b>46</b>
<i>Materials and Methods</i>	
<b>2.1. Design, synthesis and characterization of synthetic peptide constructs</b>	<b>46</b>
2.1.1. Design and synthesis	46
2.1.2. Analysis and characterization	53
<b>2.2. MEIV3b4-induced humoral immunity</b>	<b>55</b>
2.2.1. Immunization and sera collection of experimental and control animals	55
2.2.2. Standard ELISA assays	57
2.2.3. Cellular proliferation	57
2.2.4. Assessment of virus neutralizing ability of antibodies.	59
<b>2.3. Additional antigens and further modified MEIs</b>	<b>60</b>
2.3.1. Synthetic peptides	60
2.3.2. Acetylated MEIV3b4	60
2.3.3. Pelleted virus	61
2.3.4. Envelope glycoproteins	61
2.3.5. Poly-L-lysine	62
<b>2.4. Whole protein and peptide induced antibodies</b>	<b>62</b>
2.4.1. Polyclonal antibodies to comparison peptides and proteins	62
2.4.2. Anti-gp120 monoclonal antibodies	63



<b>2.5. <i>Galanthus nivalis</i> ELISA</b>	<b>64</b>
<b>2.6. <i>In vivo</i> functionality of synthetic peptide / peptide constructs</b>	<b>65</b>
<b>Chapter 3</b>	<b>67</b>
<b><i>Results</i></b>	
<b>3.1. Design, synthesis and characterization of synthetic peptide constructs</b>	<b>67</b>
3.1.1. Design and synthesis	67
3.1.2. Analysis and characterization	69
<b>3.2. MEIV3b4-induced humoral immunity</b>	<b>88</b>
3.2.1. Immunization and sera collection of experimental and control animals	88
3.2.2. The effect of Freund's adjuvant on types of immunizations employed	88
3.2.3. Cellular proliferation	93
3.2.4. Assessment of virus neutralizing ability of antibodies.	94
<b>3.3. Additional antigens and further modified MEIs</b>	<b>95</b>
3.3.1. Anti-MEIV3b4 antibody detection	95

<b>3.4. Whole protein and peptide induced antibodies</b>	<b>96</b>
3.4.1. Polyclonal antibodies to comparison peptides and proteins	96
3.4.2. Anti-gp120 monoclonal antibodies	96
3.4.3. Solid phase MEIV3b4 construct-based ELISA	98
<b>3.5. <i>Galanthus nivalis</i> ELISA</b>	<b>98</b>
<b>3.6. <i>In vivo</i> functionality of synthetic peptide     / peptide constructs</b>	<b>100</b>
<b>Chapter 4</b>	<b>106</b>
<i>Discussion</i>	
4.1. Design, synthesis and characterization of synthetic peptide constructs	107
4.2. MEIV3b4-induced humoral immunity	112
4.3. Additional antigens and further modified MEIs	114
4.4. Whole protein induced antibodies	116
4.5. <i>Galanthus nivalis</i>	117
4.6. <i>In vivo</i> functionality of synthetic peptide/peptide constructs	117
4.7. Shortcomings and future prospects	122
<b>Chapter 5</b>	<b>124</b>
<i>References</i>	
<b>Appendix</b>	



## Abbreviations

Å	Amstrong
ACN	acetonitrile
AGM	African green monkey
Ahx	aminohexanoic acid
AIDS	acquired immunodeficiency syndrome
ANC	antenatal clinic
APC	antigen presenting cell
BSA	bovine serum albumin
CDC	centers for disease control
CE	capillary electrophoresis
CFA	complete Freund's adjuvant
CHO	Chinese hamster ovary
CMV	cytomegalovirus
ConA	concanavalin A
DCCD	dicyclohexylcarbodiimide
DNA	deoxyribonucleic acid
ELISA	enzyme linked immunosorbent assay
<i>env</i>	<i>envelope</i>
FBS	fetal bovine serum
FA	Freund's adjuvant
FCS	fetal calf serum
FIV	feline immunodeficiency virus

FMDV	foot and mouth disease
Fmoc	9-fluorenylmethoxycarbonyl
<i>gag</i>	group specific antigen
gp	glycoprotein
HAART	highly active anti-retroviral therapy
HCl	hydrochloric acid
HEC	hypervariable epitope construct
HF	hydrogen fluoride
HIV	human immunodeficiency syndrome
HPLC	high performance liquid chromatography
HSP	heat shock protein
HTLVIII	human T-cell lymphotropic virus type 3
IFA	incomplete Freund's adjuvant
IFN	interferon
Ig	immunoglobulin
IM	intramuscular
IP	intraperitoneal
KLH	keyhole limpet hemacyanin
KS	Kaposi's sarcoma
LAV	lymphadenopathy-associated virus
LC-ESMS	liquid chromatography electrospray mass spectrometry
LTR	long terminal repeats

mA	milliAmps
MAP	multiple antigenic peptide
MAPS	multiple antigen peptide systems
MHC	major histocompatibility complex
MS	mass spectrometry
MTCT	mother-to-child transmission
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
MVA	modified vaccinia Ankara
NAIDS	National Institute of Allergy and Infectious Diseases
NCI	National Cancer Institute
NIH	National Institute of Health
NK	natural killer (cells)
nm	nanometers
nt	nucleotides
NZW	New Zealand White (rabbit)
O.D.	optical density
OI	opportunistic infection
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PBMC	peripheral blood mononucleocytes
PBS	phosphate buffered saline

PCP	Pneumocystis carinii pneumonia
PDB	Protein data bank
PHD	Profile fed neural network systems from HeiDelberg
PML	progressive multifocal leukoencephalopathy
PND	principle neutralizing determinant
<i>pol</i>	polymerase
PVDF	polyvinylidene difluoride
RAU	Rand Afrikaans University
RNA	ribonucleic acid
RP-HPLC	reversed-phase HPLC
RT	reverse transcriptase
SC	subcutaneous
SCID	severe combined immune deficiency
SISA	Simple Interactive Statistical Analysis
SDS	sodium dodecyl sulfate
SHIV	simian/human immunodeficiency virus
SIV	simian immunodeficiency virus
TB	tuberculosis
<i>tBoc</i>	<i>tert</i> -butyloxycarbonyl
TFA	trifluoroacetic acid
TLC	thin layer chromatography
TMB	3,3',5,5'-Tetramethylbenzidine

TN	Tris NaCl
U.S.A	United States of America
VEE	Venezuelan equine encephalitis
WHO	World Health Organization

## **List of Figures**

### **Chapter 1**

Figure 1.1. Primate lentivirus phylogenetic relationships	<b>9</b>
Figure 1.2. Schematic representation of the HIV structure illustrating major viral components.	<b>11</b>
Figure 1.3. HIV-1 genome	<b>12</b>
Figure 1.4. HIV life cycle	<b>16</b>



### **Chapter 2**

Figure 2.1. Schematic representation of the derivation of the MEIV3b4 sequence	<b>47</b>
Figure 2.2. Schematic representation of the complete structure of MEIV3b4	<b>49</b>
Figure 2.3. Schematic representation of the complete structure of poly-L-MEI	<b>50</b>
Figure 2.4. Theoretical representation of b-MEI-s	<b>52</b>
Figure 2.5. Peptide sequence of the CCD4 peptide	<b>53</b>

### **Chapter 3**

Figure 3.1. Hydrophobicity plot of the most hydrophilic sequence represented by the MEIV3b4 construct	<b>72</b>
---	-----------

Figure 3.2. Hydrophobicity plot of the least hydrophilic sequence represented by the MEIV3b4 construct	<b>73</b>
Figure 3.3. Helical wheel of the most hydrophilic sequence represented by the MEIV3b4 construct	<b>74</b>
Figure 3.4. Helical wheel of the least hydrophilic sequence represented by the MEIV3b4 construct	<b>75</b>
Figure 3.5. Percentage amino acid composition of the MEIV3b4	<b>76</b>
Figure 3.6. The theoretical charge of the MEIV3b4 construct as a function of pH	<b>77</b>
Figure 3.7. LC-ESMS spectrum of the 108 sequences presented by the linear non-conjugated MEIV3.	<b>80</b>
Figure 3.8. The LC-ESMS spectrum of the MEIV3b4	<b>81</b>
Figure 3.9. HPLC chromatogram of the MEIV3 construct	<b>82</b>
Figure 3.10. Hydrophobicity plot of the consensus sequence of the b-MEI-s construct	<b>83</b>
Figure 3.11. Helical wheel of the b-MEI-s construct	<b>84</b>
Figure 3.12. LC-ESMS spectrum of the b-MEI-s construct	<b>86</b>
Figure 3.13. HPLC chromatogram of the b-MEI-s construct	<b>87</b>
Figure 3.14a. The response of anti-MEIV3b4 antibodies against MEIV3b4 as antigen	<b>89</b>
Figure 3.14b. The comparison of subcutaneous and intraperitoneal immunizations	<b>90</b>
Figure 3.14c. The response of anti-MEIV3b4 antibodies against MEIV3b4 and HIV-1 subtype C whole virus.	<b>91</b>
Figure 3.15. Proliferation of negative control and MEIV3b4 mouse splenocytes in response to MEIV3b4 as stimulus	<b>93</b>
Figure 3.16. Proliferation of splenocytes isolated from MEIV3b4-immunized rabbits	



in response to MEIV3b4 and control protein	94
Figure 3.17. SDS electrophoretogram of anti-gp120 mAb produced in hybridoma cells	97
Figure 3.18. The response of anti-peptide antibodies against their inducing peptide constructs	102
Figure 3.19. Response to the four synthetic peptide constructs by plasma antibodies from different sources within Gauteng, South Africa	103
Figure 3.20. Response to the four synthetic peptide constructs by plasma antibodies from Venda and Puerto Rico	104

## List of Tables



UNIVERSITY  
JOHANNESBURG

### **Chapter 1**

Table 1.1. HIV/SIV proteins and their corresponding size, function and localization	13
Table 1.2. AIDS defining conditions	20

### **Chapter 3**

Table 3.1. Summary of selected properties and characteristics of the synthetic peptide and peptide constructs utilized	69
Table 3.2. Kyte-Doolittle hydrophobicity values assigned to each amino acid and the MEIV3b4 sequences of most and least hydrophilicity	70
Table 3.3. Theoretically derived characteristic values of the MEIV3b4 construct	76
Table 3.4. The effect of adjuvant on immunization style	88
Table 3.5. End point titers of mouse and rabbit antibodies in response to various antigens	95

Table 3.6. Summary of the stimulation indices (S.I.) obtained as a measure of cell proliferation	98
Table 3.7. Antibody titers of patient plasma against the four synthetic peptide constructs.	101



---

## Abstract

---

# **Multiple epitope immunogens (MEI) mimic the variability of the V3 loop of HIV-1 subtype C**

by

Raymond Hewer

**Promoter:** Dr. Debra Meyer  
**Department:** Department of Chemistry and Biochemistry  
**Degree:** M.Sc. Biochemistry

Hypermutation of the viral genome has been cited as a leading difficulty in the development of an effective human immunodeficiency virus type 1 (HIV-1) vaccine. The high number of errors made by the reverse transcriptase (RT) enzyme and the absence of RT proofreading mechanisms during HIV-1 replication leads to HIV-1 nucleotide sequence drift most frequently observed in the envelope (env) gene and expressed in env gene products. A multiple epitope immunogen (MEI) was designed and synthesized to mimic the hypervariability observed within the third variable (V3) region of HIV-1 subtype C (Hewer and Meyer, 2002). Anti-MEI humoral immunity induced in mice and rabbits, produced antigen-recognizing antibody titers of  $\leq 5000$  in enzyme linked immunosorbent assays (ELISA) and stimulation indices (SI) of 7 in cell proliferation assays. Plasma polyclonal antibodies collected from HIV / AIDS patients in Southern Africa and Puerto Rico recognized the MEI antigen at antibody titers of  $\leq 5000$ . In comparative studies, results obtained with the MEI surpassed those obtained using other peptides representing variable and conserved regions. Immunogenic constructs representing multiple viral protein sequences, such as the MEI, can be beneficial components of preventative and therapeutic HIV-1 vaccines.

---

### Samevatting

---

**Meervoudige epitoope immunogene (MEI) boots die varieërbaarheid van die V3 gebied van HIV-1 sub tipe C na**

deur

## Raymond Hewer

**Studieleier:** Dr. Debra Meyer  
**Departement:** Department of Chemistry and Biochemistry  
**Graad:** M.Sc. Biochemistry

Hiperverandering van die virus genotipe was gesiteer as 'n vername afwykings is veroorsaak deur die reverse transcriptase (RT) ensiem en die afwesigheid van die RT proeflees meganismes gedurende HIV-1 replikasie lei tot HIV-1 kern sekwensie drif, hoofsaaklik waargeneem in die envelope (env) gene en weergegee in envelope gene produkte. 'n multiple epitope immunogens (MEI) was ontwerp en saamgestel om die hipervariansie, waargeneem in die 3de variant (V3) streek van HIV-1 sub tipe C (Hewer en Meyer, 2002), na te boots. Anti-MEI humoral immuniteit geinduseer in muise en konyne, produseer antigen herkenbare teenliggaam titers van  $\leq 5000$  in ensieme-bind immunosorbent assays (ELISA) en stimulasie indices (SI) van 7 in sel struikelblok in die ontwikkeling van 'n effektiewe menslike immuno-effektiewe virus tipe 1 (HIV-1) entstof. Die hoe aantal proliferasie toetsing. Plasma polyclonal teenliggaam versamel van HIV / AIDS pasiente in Suider Afrika en Puerto Rico herken die MEI antigen by teenliggaam titers van  $\leq 5000$ . In vergelykende studies, resulte verkry met die MEI, oortref die verkry deur die gebruik van peptide weergegee in veranderlike en konserwatiewe streke. Immunogenic samestellings weergegee deur meervoudige virale proteïen volgorde soos die MEI, kan voordelige komponente van voorkomende en terapeutiese HIV-1 entsof wees.