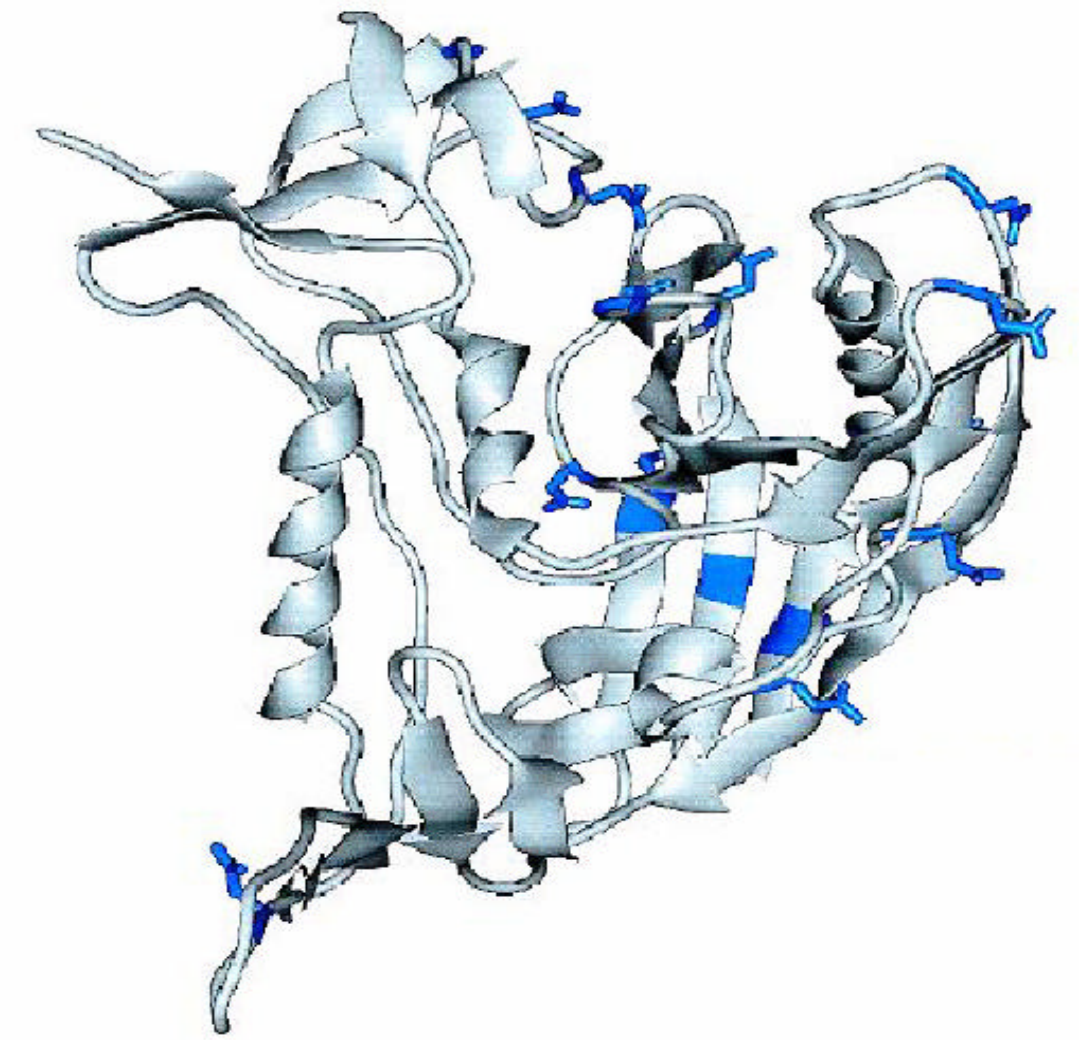

Multiple epitope immunogens (MEI) mimic the variability of the V3 loop of HIV-1 subtype C

Raymond Hewer

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by

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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

MAGISTER SCIENTIAE

in

BIOCHEMISTRY

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 13th 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

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 Freunds adjuvant
CHO	Chinese hamster ovary
CMV	cytomegalovirus
ConA	concanavalin A
DCCD	dicyclohexylcarbodiimide
DNA	deoxyribonucleic acid
ELISA	enzyme linked immunosorbent assay
env	*envelope*
FBS	fetal bovine serum
FA	Freunds adjuvant
FCS	fetal calf serum
FIV	feline immunodeficiency virus

FMDV	foot and mouth disease
Fmoc	9-flournylmethloxycarbonyl
gag	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 Freunds adjuvant
IFN	interferon
Ig	immunoglobulin
IM	intramuscular
IP	intraperitoneal
KLH	keyhole limpet hemacyanin
KS	karposi's sarcoma
LAV	lympadenopathy-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
pol	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 defiency
SISA	Simple Interactive Statistical Analysis
SDS	sodium dodecyl sulfate
SHIV	simian/human immunodefiency virus
SIV	simian immunodefiency virus
TB	tuberculosis
*t*Boc	*tert*-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

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Chapter 1

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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 immunodefiency 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 ≤ 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 ≤ 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 epitoop immunogene (MEI) boots die varieërbaarheid van die V3 gebied van HIV-1 subtipe 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, hoofsaalklik 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 subtipe C (Hewer en Meyer, 2002), na te boots. Anti-MEI humoral immuniteit geinduseer in muise en konyne, produseer antigen herkenbare teenliggaam titers van ≤ 5000 in ensieme-bind immunosorbent assays (ELISA) en stimulasie indices (SI) van 7 in sel struikelblok in die ontwikkeling van `n effektiewe menslike immunoeffektiewe virus tipe 1 (HIV-1) entstof. Die hoe aantal proliferasie toetsing. Plasma polyclonal teenliggaam versamel van HIV / AIDS pasiente in Suider Afrika en Peurto Rico herken die MEI antigen by teenliggaam titers van ≤5000. In vergelykende studies, resulte verkry met die MEI, oortref die vekry 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.