

Table of Contents

Abstract	i
Acknowledgement	iii
Table of contents	iv
List of abbreviations	ix
Chapter 1	
1. Introduction	
1.1. Basic concepts in immunology	2
1.2. Generation of lymphocytes in bone marrow and thymus	2
1.3. T cell development	3
1.4. Innate immunity	3
1.5. Adaptive immunity	4
1.6. Protective immunity	5
1.7. Identification of lymphocytes	5
1.8. T cell mediated immunity	6
1.9. Peptide sequence motifs	6
1.10. Peptide-MHC molecule complex and antigen processing	7
1.11. MHC-peptide complex recognition by T cells	8
1.12. General principles of intracellular signaling pathway	9
1.13. T cell priming	9
1.14. Co-stimulatory molecules in professional antigen presenting cells	9
1.15. General properties of armed T cells	10
1.16. T cell mediated cytotoxicity	11

1.17. Functions of cytokines in cell mediated immunity	12
1.18. CD antigens and cytokines	13
1.19. Immunological memory	14
1.20. Immunity to malaria	14
1.21. Amino acid sequence of SSP2	15
Chapter 2	
2. Hypothesis and Objectives	18
Specific objectives	21
Chapter 3	
3. Materials	
3.1. Animals	23
3.2. Cell lines	23
3.3. Culture media and cell culture	23
3.4. Malarial SSP2 peptides and SSP2 DNA	24
Chapter 4	
4. Methods	31
4.1. PCR – polymerase chain reaction	31
4.2. Method of preparation of agarose gel electrophoresis	32
4.3. Generation of recombinant plasmids	33
4.4. Purification of DNA	34
4.5. Transformation of plasmid into <i>E. coli</i>	34
4.6. Purification of plasmid DNA by miniprep	34
4.7. Retroviral gene transfer system	35
4.8. Affinity chromatography	37
4.9. SDS-polyacrylamide gel electrophoresis	37

4.10. <i>py</i> SSP2 protein expression	38
4.11. SSP2 gene transfection to retrovirus vector	39
4.12. Induction of SSP2 specific polyclonal antibodies using DNA vaccine	40
4.13. Isolation of bone marrow derived dendritic cells of BALB/c mice	41
4.14. Transfection of DCs and RL♂ cells with retrovirus expressing SSP2	42
4.15. Preparation of cytolytic T cells	42
4.16. T cell induction and maintenance	42
4.17. Radio-isotope assay	43
4.18. Enzyme-linked immunosorbent assay	43
4.19. ELISPOT	44
4.20. Fluorescence activated cell scanning (FACS)	45
4.21. Western blot analysis	46
4.22. Intradermal implantation of RL♂-SSP2 cells in BALB/c mice	46
4.23. Statistical analysis	47

Chapter 5

5. Results

5.1. Retrovirus transfected bone marrow cells cultured with GMCSF and IL-4 generated matured dendritic cells	49
5.2. DCs transfected with retrovirus encoding SSP2 had prolonged expression of transfected genes	55
5.3. Immunization with RL♂ tumor cells expressing SSP2 induced A and C peptide-specific IFN- γ CTLs	59
5.4. Immunization with transfected DCs encoding SSP2 antigens induced A and C antigen specific IFN- γ producing CTL	61

5.5. Immunization of retrovirus transfected <i>py</i> SSP2 expressing DCs generated A and C peptide specific T lymphocytes	61
5.6. Complete protection from Intradermal implantation of tumor cells (RL♂-SSP2) in mice immunized with DC encoding SSP2 gene	66
Chapter 6	
6. Discussion	69
7. References	74
8. List of figures	
Figure 1. Cell surface phenotype of bone marrow derived DCs as determined by FACS analysis	54
Figure 2. Enhanced transfection efficacy of retrovirus transfected dendritic cells by prolonged centrifugation	58
Figure 3. Immunization with RL♂ tumor cells expressing SSP2 induced A and C peptide-specific CTLs producing IFN- γ	60
Figure 4. Cytolytic activity of A and C peptide specific CTLs generated by immunization with DCs	65
Figure 5. Tumor growth pattern after intradermal implantation of tumor cells (RL♂-SSP2) in BALB/c mice after immunization with DC encoding SSP2 gene	67
9. List of tables	
Table 1. Types of cytokines released from different types of cells	13
Table 2. Description of five MHC class I-K ^d restricted peptide binding motifs in <i>py</i> SSP2 ₁₋₃₀₃ Protein	25
10. Annexures	
10.1. Annexures;	
Plate 1	
<i>Py</i> SSP2 protein expression in expression vector and agarose gel-electrophoresis apparatus	

10.2. Plate 2.

SSP2 expression in SDS-PAGE, SSP2 DNA insertion in retrovirus Vector and the power supply used in electrophoresis and blotting

10.3. Plate 3.

Construction of DNA vaccine, Confirmation of SSP2 insertion and DNA/RNA analyzer.

10.4. Plate 4.

96 well ELISA plate, immunized and control group of BALB/c mice and their tumor implantation.

10.5. Plate 5.

Tumor growth in control group of BALB/c mice with intradermal tumor cell implantation.

10.6. Presentations and publications

Oral presentation to the 37th joint conference on parasitic diseases August 2002, Nagasaki Japan on;

Induction of antigen specific CD8⁺ T cells by immunization with genetically engineered dendritic cells expressing malarial-surface sporozoite protein 2(SSP2)

Research paper on

Hettihewa L.M. 2003 -Immunization of retrovirus-transfected dendritic cells induces specific cytotoxic T lymphocytes to two distinct malarial peptides presented by K^d molecule *Intern. Immunopharm* 3 (2003) 1401-1411

List of abbreviations

γ	-	gamma
♂	-	male
a	-	adenosine
c	-	cytosine
g	-	guanine
t	-	tyrosine
Ab	-	antibody
Ag	-	antigen
A-E	-	names of the selected SSP2 MHC class I peptides
APC	-	antigen presenting cell
BALB/c	-	Genomic name of the mice
B 7.1/B 7.2	-	cell surface expressing molecules expressed in dendritic Cells when activated
cDNA	-	complementary DNA library
Cr	-	sodium chromate
CS	-	circumsporozoite protein
CTLs	-	cytotoxic T lymphocytes
CD	-	cluster of differentiation
CD 4 ⁺	-	CD4 ⁺ T cells
CD 8 ⁺	-	CD8 ⁺ T Cells
CD11c	-	expression molecule on the surface of DC
DC	-	dendritic cells
DMEM	-	dulbecco's modified eagle medium

DMRIE-C	-	1, 2 dimyristyloxypropyl-3 dimethyl-hydroxy ethyl ammonium bromide
DNA	-	deoxyribo nucleic acids
EGFP	-	green fluorescence p vector
ELISA	-	Enzymed linked immuno-sorbent assay
ELISPOT	-	Enzyme linked immuno-spot assay
FACS	-	fluorescence activated cell sorting
FCS	-	fetal calf serum
IFN- γ	-	interferon gamma
IgG	-	immunoglobulin G
GMCSF	-	growth of marrow cell stimulating factor
IL-2	-	interleukin -2
IL-4	-	interleukin -4
i.p.	-	intra peritonealy
IPTG	-	isopropyl - β -D-thiogalactoside
Kb	-	kilobase
K ^d	-	gene haplotype
KDa	-	Kilo Dalton
Lac Z	-	beta galactosidase gene encoded in E coli expression vector
LB	-	liquid broth
LPS	-	lipopolysaccharides
M 15	-	strain of <i>E coli</i>
mAb	-	monoclonal antibody
M.F.I.	-	mean fluorescence intensity
MHC	-	Major histocompatibility assay

mRNA	-	messenger RNA
NaOH	-	Sodium hydroxide
ORI	-	replication origin
P815	-	name of the mastocytoma cell line
PBS	-	phosphate buffered saline
PGEM3Zf	-	name of the cloning vector
PJW404 reseq	-	Name of the DNA vector
PQE 31	-	name of the protein expression vector
PT67	-	PT 67 packaging cell line for retrovirus transfection
<i>py</i>	-	<i>Plasmodium yoelli</i>
PCR	-	polymerase chain reaction
pMSCV puro	-	puromycin gene transfected murine stem cell retrovirus
RL	-	Radiation induced leukaemia cells
RNA	-	ribonucleic acids
S.E.M.	-	Standard error of mean
SSP2	-	Sporozoite surface protein-2
SDS	-	Sodium dodecyl sulfate
T _H 1	-	type 1 helper T cells
TRAP	-	Thrombospondin related anonymous protein
X-gal	-	5-bromo-4-chloro-3-indolyl- β -D-galactoside