

Vaccination By *In Vivo* Expression Of Secretion Adapted Antigens

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DEDICATION

TO

**MY PARENT
MY HUSBAND
AND MY DAUGHTER ASMAA**

I declare that this thesis has been composed by myself and the work there in has not been submitted for a degree at this or any other university.

Asmaa Mohamed Ahmed Elfiky

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Abstract

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Vaccination by *in vivo* expression of secretion adapted antigens

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Four candidate cDNA molecules encoding GST, filamin, GAPDH and *Sm* 21.7 antigens were subcloned in the eukaryotic pSec Tag2 expression vectors for expression and secretion *in vivo*, thus offering a unique approach to immune presentation. Immunization studies were conducted in mice using two DNA delivery routes: first intramuscular using GST and filamin constructs, with or without a booster dose of the corresponding recombinant protein antigens. Second intradermal using GST, filamin, GAPDH and *Sm* 21.7 constructs. Protection levels varied according to the type of DNA construct used, addition of a booster dose of r-protein and to the different routes of administration. While no significant protection was observed for GST and filamin DNA constructs in cases of mice immunized intramuscularly, the addition of a protein booster dose induced protection levels of 59% and 42% for GST and filamin DNA constructs, respectively. On the other hand, intradermal administration of the DNA constructs resulted in statistically significant protection levels of 18%, 37%, 38% and 50% for GST, filamin, GAPDH and *Sm* 21.7, respectively. All 4 vaccine constructs generated a humoral immune response detectable by Western blot analysis against native soluble worm antigen preparation (SWAP) or their corresponding recombinant antigens. The significant levels of protection observed in this study with DNA vaccination demonstrate the promise of an effective intradermal vaccination approach and an intramuscular approach involving priming with a DNA and boosting with recombinant protein.

LIST OF ABBREVIATIONS

ABP	Actin-binding protein
ADCC	Antibody-dependent cell mediated cytotoxicity.
APCs	Antigen presenting cells
BCA	Bicinchoninic acid
BCIP	5-bromo-4-chloro-3-indolyl phosphate.
BSA	Bovine serum albumin
cDNA	Complementary DNA.
CIAP	Calf intestinal alkaline phosphatase
CMV	Cytomegalovirus
CTL	Cytotoxic T cells.
dATP	2'- deoxyadenosine 5'- triphosphate.
dCTP	2'- deoxycytosine 5'- triphosphate.
dGTP	2'- deoxyguanosine 5'- triphosphate.
DNase I	Deoxyribonuclease I
dNTPs	2'- deoxyribonucleoside 5'- triphosphate.
dTTP	2'- deoxythymidine 5'- triphosphate.
<i>E. coli</i>	<i>Escherichia coli</i>
ER	Endoplasmic reticulum
EDTA	Ethylene diamine tetra acetic acid
ELISPOT	Enzyme-Linked ImmunoSorbent spot
FABP	Fatty acid-binding protein

GAPDH	Glyceraldehydge-3-phosphate dehydrogenase
GPI	Glycosylphosphatidylinositol
GST	Glutathione S-transferase
IFN- γ	Interferon-gamma
IL	Interleukin
IPTG	Isopropylthio- β -D-galactoside
IRS	Immunized rabbit serum
IrV5	Irradiated vaccine antigen five
kDa	Kilo dalton
MAP	Multiple antigenic peptide
MBP	Maltose-binding protein
MHC	Major histocompatibility complex
NBT	Nitro Blue Tetrazolium
NRS	Normal Rabbit Serum.
PBMCs	Peripheral blood mononuclear cells
PBS	Phosphate-Buffered Saline
PCR	Polymerase Chain Reaction
PMSF	Phenylmethanesulfonylfluoride
r	Recombinant.
RA	Radiation-attenuated.
RNase A	Ribonuclease A
rSjGST	Recombinant <i>Schistosoma japonicum</i> glutathione S-transferase.

SBSP	Schistosome Biological Supply Program
SDS	Sodium dodecyl (lauryl) sulfate
SDS-PAGE	SDS- polyacrylamide gel electrophoresis.
Sj	<i>Schistosoma japonicum</i>
Sm	<i>Schistosoma mansoni</i> .
SmGPX	<i>Schistosoma mansoni</i> glutathioneperoxidase.
SWAP	Soluble worm antigen preparation
TNF-alpha	Tumor necrosis factor alpha
TPI	Triose phosphate isomerase
TSP-1	Tetraspanin

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