

SUICIDE GENES IN A NOVEL VACCINATION STRATEGY FOR HEPATITIS C

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By

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Abstract

Hepatitis C virus (HCV) is a blood transmitted virus which causes persistent infection of the liver greatly resulting in cirrhosis and hepatocellular carcinoma. Current treatment regimens for HCV, such as pegylated interferon- α and ribavirin in combination, fail to clear the virus in 45-80% of individuals. New therapies under development, including immunotherapies, face many challenges. One of which is the need to induce a broad, multifunctional T-cell response directed against NS3/4A (cell-mediated immunity (CMI)), which is associated with clearance of infection, to enhance the immune response in chronic non-responders to conventional treatment. These individuals tolerate high levels of circulating HCV antigens and fail to clear the virus normally.

Dendritic cells are the only antigen presenting cells able to prime naive T-cells, the central aim of any vaccination strategy. DC detect viral infection by phagocytosis of exogenous viral antigens released from infected, often apoptotic/necrotic cells, process and present these in association with MHC II to CD4+ T-cells and to naive CD8+ T-cells in association with MHC I (cross-presentation). Cross-presentation is proposed to be required for immunity to viruses, as immunity would otherwise only occur if the virus infected the DC (if direct presentation were the only possible mechanism). Therefore, one potential way to enhance the CMI is to enhance cross-presentation through induction of apoptosis or necrosis in vaccine-targeted cells.

The overall aim of this study was to immunise C57BL/6 mice with DNA vaccines encoding HCV immunogens and genes encoding toxic proteins termed "suicide

genes". Induction of apoptosis/necrosis in the vaccine targeted cells was predicted to release the vaccine-derived antigen and enhance cross-presentation.

This thesis explored suicide gene function *in vitro* and testing *in vivo* to examine the effect on the immunogenicity of a DNA vaccine strategy. Initially, suicide genes from a range of bacterial and viral sources were screened *in vitro* by luciferase co-transfection experiments to determine cell-killing ability. A highly toxic gene, diphtheria toxin subunit A (DTA), moderately toxic gene, mouse mutant perforin (MMP) and poorly toxic gene, rotavirus non-structural protein 4 (NSP4) were selected and each was cloned into a dual-promoter vector, then used to transfect HEK293T and Cos-7 cells. Alamar blue and additional luciferase experiments confirmed the cytotoxic effects. Finally, the mode of cell death was determined as apoptosis for DTA, necrosis for MMP and apoptosis and necrosis for NSP4 by lactate dehydrogenase (LDH) assay and Annexin V/Propidium iodide flow cytometry. DTA was later confirmed to inhibit protein synthesis and thus was not pursued *in vivo*.

The HCV NS3 and NS3/4A serine protease genes were cloned under a second promoter in each dual-promoter suicide gene construct and protein expression was detected by western blot analysis. These DNA vaccines were used to immunise C57BL/6 mice to compare the immunogenicity by IFN- γ ELISPOT. NS3 was selected as the more immunogenic candidate. Addition of NSP4 to the NS3 encoding vector enhanced immunogenicity slightly, although MMP did not.

Declaration of Originality

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Abbreviations

a.a.	amino acids
AEC	Animal Ethics Committee
ALT	alanine aminotransferase
Anti-HCV	hepatitis C antibody
Anti-C	antibody to HCV core protein
Anti-E1	antibody to HCV envelope protein 1
Anti-E2	antibody to HCV envelope protein 2
AP	Antarctic phosphatase
APC	antigen presenting cell
Bcl-xL	protein produced by B cell lymphoma 2 gene
bp	base pairs
BSA	bovine serum albumin
C	HCV core protein
CD81	cluster of differentiation 81
cDNA	complementary deoxyribonucleic acid
CMI	cell-mediated immune response
Cos-7	African green monkey kidney cell line
CTL	cytotoxic T lymphocytes
DAA	direct acting antiviral (drug)

DAMP	damage associated molecular pattern
DC	dendritic cell
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dNTP	deoxynucleotide triphosphate
DT	diphtheria toxin
DTA	diphtheria toxin subunit A
E1	HCV structural protein E1
E2	HCV structural protein E2
ELISA	enzyme-linked immunosorbent assay
EMCV	encephalomyocarditis virus
ER	endoplasmic reticulum
EtOH	ethanol
FHL	familial haemophagocytic lymphocytosis
GCV	ganciclovir
GCV-DP	ganciclovir di-phosphate
GCV-MP	ganciclovir mono-phosphate
GCV-TP	ganciclovir tri-phosphate
GTP	guanosine triphosphate
h	hour

HA	haemagglutinin
HAART	highly active anti-retroviral therapy
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HEK293T	human embryonic kidney cells producing SV40 Large T-antigen
HeLa cells	Henrietta Lacks immortal cervical carcinoma cell line
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HIV	Human Immunodeficiency Virus
HLA	human leukocyte antigen
HMGB1	high mobility group box 1 protein
HMP	human mutant perforin (12 amino acids truncated at C-terminus of protein)
HSP	heat shock protein
HSV	herpes simplex virus
Huh-7	human hepatocellular carcinoma cell line 7
HVR	hypervariable region
ID	Intradermal
IFN- α	interferon alpha
IL-2	interleukin 2
IL	interleukin

IM	Intramuscular
IRES	internal ribosome entry site
IRF	interferon regulatory factor
Kb	kilo base (pair)
LB	Luria Broth
LDL	low density lipoproteins
LPS	lipopolysaccharide
MCP-I	monocytes chemoattractant protein-1
MgCl ₂	magnesium chloride
MHC I	major histocompatibility complex class I
MHC II	major histocompatibility complex class II
Min	minute
MIP-I	macrophage inflammatory protein-1
mL	millilitre
mM	milli-molar
MMP	mouse mutant perforin (12 amino acids truncated at C-terminus of protein)
MRI	magnetic resonance imager
mRNA	messenger ribonucleic acid
MVA	Modified Vaccinia Ankara

NAD	nicotinamide adenine dinucleotide
ng	nanogram
NK cells	natural killer cells
nt	nucleotide
NS	non-structural (protein)
NS3	HCV non-structural protein 3
NS3/4A	HCV non-structural protein 3 and 4A
NSP4	rotavirus non-structural protein 4
NTPase	nucleotide triphosphatase
O/N	overnight
ORF	open reading frame
PAMP	pathogen associated molecular pattern
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PHA	phytohaemagglutinin
PKR	protein kinase RNA-activated
PRR	pattern recognition receptor
p/s	photons per second
RC	replication complex
RIG-I	retinoic acid inducible gene I

RIP-I	receptor interacting protein I
RNA	ribonucleic acid
RNase	ribonuclease
rpm	revolutions per minute
RT-PCR	reverse-transcription polymerase chain reaction
SCID	severe combined immunodeficiency
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
Sec	second
SIV	Simian Immunodeficiency Virus
SLO	streptolysin O
SOC	super optimal culture
SR-B1	human scavenger receptor class B type 1
ssRNA	single-stranded ribonucleic acid
STAT-6	signal transducer and activator of transcription 6
SVR	sustained virological response
TBE	Tris borate EDTA
TEMED	Tetramethylethylenediamine
TGF- β	transforming growth factor beta
T _H 1	T helper cell class 1 response

T _H 2	T helper cell class 2 response
TIR	Toll/Interleukin-1 receptor
TK	thymidine kinase
TLR	toll-like receptor
TMD	transmembrane domain
TNF- α	tumour necrosis factor alpha
UTR	untranslated region
μ g	microgram
μ L	microlitre
μ M	micromolar
VLDL	very low density lipoprotein
VP	viral protein
VPC	vector producing cell
WCH	Women's and Children's Hospital, North Adelaide