STRATEGIES FOR THE DEVELOPMENT OF RECOMBINANT PORCINE ADENOVIRUS-BASED VACCINES AGAINST HEPATITIS C VIRUS

A thesis submitted to the University of Adelaide in fulfilment of the requirements for the degree of

Master of Philosophy (Medical Sciences)

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February 2016

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Abstract

Hepatitis C virus (HCV) infects over 200 million people worldwide and results in persistent infection of approximately 80% of cases. Consequently, HCV is a leading global contributor to severe liver disease. Although, direct-acting antiviral agents (DAA) are available, these drugs do not prevent re-infection and cost in excess of US $80, 000 per patient. Thus, the development of an inexpensive HCV vaccine represents the most effective measure to reduce the current epidemic. Patients who recover following acute HCV infections provide valuable clues for vaccine design. These patients develop a broad and sustained cell-mediated immunity (CMI) against multiple HCV proteins, in particular the non-structural (NS) proteins. Therefore, the focus of this thesis was to develop novel vaccine strategies to elicit CMI against HCV NS proteins which may mimic the immunity that facilitates spontaneous control of acute HCV infection.

Dendritic cells (DC) are crucial to initiate CMI making these cells attractive targets for any vaccine. Recombinant porcine adenoviruses (rPAV) appear capable of targeting DC as rPAV encoding influenza virus antigens have been shown to elicit both humoral and CMI. Furthermore, rPAV are not infectious nor replicate in humans making them safe.

The potential of rPAV to elicit HCV-specific CMI is unknown. Therefore, I attempted to construct a cytolytic rPAV encoding a lytic protein as the cytolytic nature of viruses significantly augments immunogenicity due to the release of natural adjuvants known as danger associated molecular patters (DAMPs) from infected cells. As a prelude to this aim, the lytic activity of two cytolytic proteins, namely adenovirus death protein (ADP) from AD5 and mouse perforin (PRF) were compared following

transfection of HEK293T cells with pVAX DNA encoding ADP or PRF (chapter 3). In two independent cell death assays (viz. luciferase assay and lactate dehydrogenase release assay), it was determined that PRF was more lytic, making it a suitable protein to be encoded in a lytic rPAV HCV vaccine.

I then attempted to modify the rPAV shuttle DNA to facilitate cloning and expression of HCV NS antigens. Initially, the efficiency of the truncated CMV (tCMV) promoter and the full-length CMV (fCMV) promoter to drive eGFP expression was compared. Using flow cytometry and confocal microscopy, the tCMV promoter was shown to be more efficient. In addition, the rPAV shuttle DNA was modified to contain a multiple cloning site to facilitate gene insertion. Subsequently, recombinant PAV plasmids encoding PRF controlled by the tCMV or SV40 promoters were constructed, but recombinant virus was not recovered. This is most likely because PRF killed the cells prior to virus assembly. Subsequently, several experiments were conducted (Chapter 5) with a reporter virus encoding LUC and eGFP and established that rPAV production requires early passage cells (P11) cultured for 10-14 days post transfection. Based on these results, a non-lytic rPAV devoid of PRF was constructed to elicit CMI to conserved HCV NS proteins (NS4B and NS5B).

Although, the construction of a cytolytic rPAV failed, the use of rPAV vector encoding NS4B and NS5B proteins is the next logical step to best augment the protective CMI. The construction of this rPAV vaccine accomplished during this thesis may lead to future preclinical studies.

Declaration of originality

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Harshwardhan Jagdale May 18th 2016

Acknowledgements

I would like to sincerely thank my principal supervisor, Professor Eric Gowans for providing me the opportunity to come to Australia and complete my Master of Philosophy in his laboratory. I would like to appreciate the effort and time he had invested in me over these two years to make me think like a scientist.

I would like to thank my co- supervisors Dr. Danushka Wijesundara and Dr. Branka Grubor Bauk for their intellectual inputs in designing of my experiments throughout the candidature. I would like to thank our collaborators Prof. Suresh Tikoo and Prof. Ilia Voskoboinik for providing us the resources for carrying out this work. I would also like to thank the Hospital Research Foundation for providing me a scholarship without which I could have never come to Adelaide, Australia. I would also like to thank all the current and past members of the Virology laboratory who helped me in conducting my research work.

I appreciate all the help that I could get from the friends I made at the Basil Hetzel Institute while living here for past two years. I must mention a special thanks to Dr. Pallave Dasare for the crucial editing support that she provided for my thesis.

Lastly, but not the least, I would like to thank my wife, Arunima Saraf, my loving parents (Yogesh and Kalpana Jagdale) and parents in law (Arun and Pinki Saraf) who have been a strong support and provided me the mental resolve to get through my studies. I also thank my Australian family, Mayank, Chloe, Heidi, Chris, Leanne and my little niece Athena who have loved and supported me throughout my stay here in Adelaide.

List of Abbreviations/Acronyms

a.a.	amino acids
ADCC	antibody dependent cell cytotoxicity
ADP	adenovirus death protein
Ad2	human adenovirus serotype 2
Ad5	human adenovirus serotype 5
AdC3	chimpanzee adenovirus serotype 3
AdC6	chimpanzee adenovirus serotype 6
AdC68	chimpanzee adenovirus serotype 68
APC	antigen presenting cell
Anti HCV	hepatitis C antibody
BCG	bacillus calmette-guerin
bp	base pairs
BSA	bovine serum albumin
°C	degree celsius
CAR	coxsackie adenovirus receptor
CD4	cluster of differentiation 4
CD8	cluster of differentiation 8
CD81	cluster of differentiation 81
CMI	cell mediated immunity
CMV	cytomegalo virus
CPE	cytopathic effect
CTL	cytotoxic T lymphocytes
DAA	direct acting antivirals
DAMPs	damage associated molecular patterns
DAPI	4’, 6-diamidino-2-phenylindole
DC	dendritic cell
DMEM	dulbecco’s modified eagle medium
DMSO	di-methyl sulphoxide
dNTP	di-nucleotide triphosphates
DNA	deoxyribonucleic acid
E	early genes (adenovirus)
E1	HCV structural protein E1

E2	HCV structural protein E2
EDTA	ethylenediaminetetraaceticacid
eGFP	enhanced green fluorescent protein
ER	endoplasmic reticulum
EtOH	ethanol
FCS	fetal calf serum
FITC	fluorescein isothiocyanate
FMDV	foot and mouth disease virus
g	gram
gt	genotype
h	hour
hi fi	high fidelity
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HEK293T	human embryonic kidney cells producing SV40 T antigen
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid
HIV-1	human immunodeficiency virus-1
IFN-α	interferon alpha
IFN-β	interferon beta
IFN-γ	interferon gamma
IFN-λ	interferon lambda
IFN-κ	interferon kappa
IgG	immunoglobulin
IRES	internal ribosome entry site
IRF	interferon regulatory factor
ISGs	interferon stimulatory genes
ITR	inverted terminal repeat
Kb	kilo bases (nucleotide base)
kDa	kilo Dalton
L	late genes (adenovirus)
LAV	live attenuated virus
LB	luria broth
LDL	low density lipoprotein

LDH	lactate dehydrogenase
LUC	firefly luciferase
LPS	lipopolysaccharide
M	molar
MCS	multiple cloning site
MEM	minimum essential medium
MHC-I	major histocompatibility complex class I
MHC-II	major histocompatibility complex class II
MLP	major late promoter
ml	milli litre
mM	milli molar
mg	milli gram
µl	micro litre
µg	micro gram
min	minutes
mRNA	messenger ribonucleic acid
MSU	monosodium urate crystals
MVA	modified vaccinia Ankara
Nabs	neutralizing antibodies
NK	natural killer cell
NKT	natural killer T cell
nm	nanometres
NS	HCV non-structural protein
NTR	non translating region
OD	optical density
Opti MEM	reduced serum medium
ORF	open reading frame
PAMPs	pathogen associated molecular patterns
PAV3	porcine adenovirus serotype 3
PBS	phosphate buffer saline
PCR	polymerase chain reaction
12 del PRF	mouse perforin (12 amino acids truncated at C terminus)
PRR	pattern recognition receptor
%	percentage

RNA	ribonucleic acid
RdRp	RNA dependent RNA polymerase
RPM	revolutions per minute
RT	room temperature
sec	second
SEM	standard error of mean
SOC	super optimal culture
SV40	simian virus 40
SVR	sustained virological response
Taq	*thermophilus aquaticus*
Tm	melting temperature
TCR	T cell receptor
Treg	T regulatory cells
TLR	toll like receptor
TNF-α	tumor necrosis factor alpha
U	unit (restriction enzyme unit)
UTR	untranslated region
VR1BL	fetal porcine retina cells