Optimising DNA vaccine technology to prevent HIV-1 infection

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Abstract

The failure of traditional protein-based vaccines to prevent HIV infection highlights the need for novel vaccine strategies. An effective HIV-1 vaccine will need to induce cell-mediated and humoral immune responses at both systemic and mucosal sites.

DNA based vaccines are appealing candidates for novel vaccines because they are inexpensive, stable and simple to manufacture. They also mimic a viral infection, by using cellular machinery to produce vaccine antigens in the same way that viruses hijack the host cell to produce viral proteins and nucleic acids, but are not infectious themselves. DNA vaccines effectively induce both humoral and cell mediated immune responses to viral antigens, however despite being licensed for veterinary use they have so far been insufficiently immunogenic in human trials.

To realise the potential of DNA vaccines, the technology must first be optimised to improve both delivery and immunogenicity. In this thesis I have proposed two novel approaches to optimise DNA vaccines. Firstly, to improve delivery, I have proposed the use of influenza virosomes as a delivery vehicle for the intranasal administration of DNA vaccines. Intranasal vaccination is known to induce pan-mucosal immune responses at distant sites such as the genital mucosa. This concept is particularly important for HIV vaccines, as it is believed that an early immune response at the genital mucosa offers the best chance to control HIV infection before it becomes systemic. In this thesis I have used live imaging of the reporter molecule, luciferase, to confirm efficient intranasal delivery of DNA vaccines by influenza virosomes, and this is the first time that visualisation of this process has been reported. I have also demonstrated that intranasal delivery of virosome-encapsulated DNA can enhance the mucosal immune response.

Secondly, to improve immunogenicity, I have proposed the use of suicide genes to induce cell necrosis and provide an adjuvant effect for DNA vaccines. Necrotic, antigen-positive cells release a range of intracellular factors that signal via receptors on dendritic cells, including Toll-like receptors and Clec9A, and lead to dendritic cell activation and enhanced cross-presentation of antigen. In this thesis I have tested the novel application of the suicide genes NSP4, perforin (PRF) and DTa, which induce cell death by different mechanisms. I have provided evidence that suicide gene-induced necrosis in DNA vaccine-targeted cells provides an adjuvant effect. In this thesis I report that a DNA vaccine that induces cytolytic, rather than apoptotic, cell death enhances CD11c+ CD8α+ dendritic cell activation, broad and multifunctional CD8 T cell responses

and increases protection in mice from challenge with a chimeric virus, EcoHIV. This enhancement was also dependent on the timing of cell death after antigen expression. Thus, cytolytic gene-induced necrosis resulting from co-expression of perforin and an immunogenic protein can significantly improve the immunogenicity of DNA vaccines.

The work in this thesis therefore describes the development of a novel intranasal delivery system and a novel adjuvant system that advance DNA vaccine technology, and improve the potential for DNA to be used as an effective vaccine for HIV-1.

Declaration

I certify that 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. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Abbreviations

(r)Ad5	(recombinant) Human adenovirus type 5
ADCC	antibody-dependent cell-mediated cytoxicity
ADCVI	antibody-dependent cell-mediated viral inhibition
AIDS	Acquired Immunodeficiency Syndrome
bp	base pairs
CFSE	Carboxyfluorescein diacetate succinimidyl ester
CD	Cluster of Differentiation
DAMPs	Damage-associated molecular patterns
DC	Dendritic Cell
DMEM	Dulbecco's Modified Eagle Media
DTa	Diphtheria toxin subunit A
EcoHIV	Ecotropic Human Immunodeficiency Virus (HIV envelope replaced with envelope from murine leukemia virus)
ELISA	Enzyme linked Immuno-sorbent Assay
ELISPOT	Enzyme linked Immuno-sorbent Spot Assay
env	HIV-1 *env* gene encoding DNA sequence for expression of the envelope glycoprotein
env/ENV	HIV-1 envelope gp160 protein that is cleaved into: gp120 surface unit and gp41 transmembrane domain
FCS	Fetal Calf Serum
gag	HIV-1 *gag* gene encoding DNA sequence for expression of gag protein
gag/GAG	HIV-1 p55 gag polyprotein that is cleaved into: p24 capsid, p17 matrix and P7 nucleocapsid
GCV	gancyclovir
GFP	green fluorescent protein
HBS	Hanks buffered Saline
HIV	Human Immunodeficiency Virus
HSV-TK	Herpes simplex virus thymidine kinase
ID	Intradermal injection or vaccination
i.d.	immuno-dominant
IFN-	Interferon
IL-	Interleukin
IM	Intramuscular injection or vaccination

IN	Intranasal vaccination
IP	Intraperitoneal injection or vaccination
IR	Intrarectal vaccination
Iva	Intravaginal vaccination
kb	kilo base
kDa	kilo Dalton
LAV	live attenuated virus
LUC	firefly luciferase
MVA	Modified vaccinia Ankara
NSP4	non-structural protein 4
PAMPs	Pathogen-associated molecular patterns
PBS	Phosphate buffered Saline
PEC	Peritoneal exudate cells
PI	Propidium Iodide
pol	HIV-1 *pol* gene encoding DNA sequence for expression of pol protein
pol/POL	HIV-1 p110 pol polyprotein that is cleaved into: p11 protease, p66/51 reverse transcriptase and p35 integrase
PRF	Perforin
RBC	Red Blood Cells
RES	Restriction enzyme site
RPMI	Roswell Park Memorial Institute Media
SC	Subcutaneous injection or vaccination
SIV	Simian Immunodeficiency Virus
SFU	Spot forming units
TLR	Toll-like receptor
TNF-	Tumour Necrosis Factor

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