NOVEL RECOMBINANT DNA AND LIVE VIRUS VACCINES TO PREVENT OR CONTROL HIV-1 INFECTION

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Chapter 1. Introduction

Chapter 4. Human rhinovirus-A1 as an expression vector

Abstract

Background

Vaccination is the most cost effective and long-term solution to the global human immunodeficiency virus (HIV) pandemic. The HIV Gag and Tat proteins are attractive components of a HIV vaccine as immune responses targeting these proteins confer protective benefits against HIV infections in humans. This thesis has developed two innovative candidate HIV vaccines viz. a DNA vaccine encoding oligomerised and secreted Tat (pVAX-sTat-IMX313), and a recombinant live human rhinovirus serotype A1 (HRV-A1)-based vaccine encoding Gag and Tat (rHRV-Gag/Tat).

Methods

To construct pVAX-sTat-IMX313, Tat was fused with the oligomerisation domain of IMX313 to form Tat heptamers and linked to the leader sequence of tissue plasminogen activator to ensure that the bulk of oligomerised protein is secreted. To develop the rHRV-Gag/Tat vaccine, initially, the full length *tat* gene and 5 discrete overlapping fragments corresponding to the full length *gag* gene were individually inserted into the junction between the HRV-A1 genes encoding structural and non-structural proteins (P1/P2 junction) to ensure that the exogenous HIV Gag or Tat proteins were separated from the recombinant polyprotein using the HRV encoded 2Aprotease enzyme. Thus, one recombinant HRV encoding Tat (rHRV-Tat) and 5 rHRVs each encoding a unique Gag fragment (rHRV-Gag1-5) were generated. The individual rHRVs were then mixed into a single cocktail vaccine (rHRV-Gag/Tat), purified and titrated for inoculation in mice.

The immunogenicity of these vaccines was evaluated in female BALB/c mice that received up to five intradermal injections of pVAX-sTat-IMX313 (50 μg per dose) at 2 weekly intervals in one study. In another study, mice were vaccinated intranasally with 2 doses ($5x10^6$

TCID50/dose) of the rHRV-Gag/Tat followed by a single 50 μg booster dose of a cocktail DNA vaccine containing pVAX-sTat-IMX313 and pVAX-Gag-Perforin. Vaccine-induced immune responses were examined 2 weeks after the last dose by antibody ELISA, *in-vitro* Tat transactivation neutralization, IFN-γ ELISpot, $KdGag_{197\text{-}205}$ tetramer staining and intracellular cytokine staining assays.

Results

Data showed that fusing Tat with IMX313 results in complete heptamerisation of Tat. Furthermore, the data suggested that pVAX-sTat-IMX313 vaccination elicited higher titers of serum neutralizing Tat-specific IgG, secretory IgA (sIgA) in the vagina and CMI responses, and showed superior control of ecotropic HIV (EcoHIV) infection, a surrogate murine HIV challenge model, compared with animals vaccinated with other DNA vaccines tested in this study. Human rhinovirus serotype A1 (HRV-A1) was successfully engineered into a replication-competent genetically stable recombinant vector to deliver a mucosally-targeted vaccine, rHRV-Gag/Tat, by inserting exogenous HIV *gag* and *tat* sequences into the HRV-A1 genome. Finally, intranasal administration of 2 doses of rHRV-Gag/Tat followed by a single DNA booster dose induced superior poly-functional Gag-specific CD8 T cell responses in the spleen (systemic) and mesenteric lymph nodes (mucosal), higher Tat-specific serum IgG and sIgA in the vagina, and effective control of EcoHIV infection compared to other vaccination regimens tested in this study.

Conclusion

First, the data support the inclusion of IMX313 as a molecular adjuvant for Tat-based HIV DNA vaccines. Second, the data demonstrated that intranasal vaccination with rHRV-Gag/Tat followed by a single DNA booster dose is effective in eliciting HIV-specific immunity pan-mucosally and systemically. Collectively, the data support further testing of the pVAX-sTat-

IMX313 and rHRV-Gag/Tat vaccines in macaques, preferably in a heterologous prime-boost vaccination strategy, and results from these studies might influence future HIV clinical trials.

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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List of abbreviations and acronyms

AIDS: acquired immune deficiency syndrome

Ad5: Adenovirus serotype 5

APCs: Antigen presenting cells

ADCVI: Antibody-dependent cell-mediated virus inhibition

ADCC: Antibody-dependent cellular cytotoxicity

ADCP: Antibody-dependent cellular phagocytosis

ADCD: Ab-dependent complement deposition

Ad5: Adenovirus serotype 5

APOBEC-3G: Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like-type 3G

~: Approximately

cDNA: Complementary DNA

CCR5: Chemokine receptor 5

CCR5 Δ32: CCR delta 32

$CD4^+$: Cluster of differentiation 4 positive

$CD8^+$ Cluster of differentiation 8 positive

Δ *Nef:* delta Nef

DNA: Deoxyribonucleic acid

DCs: Dendritic Cells

DC-SIGN: Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin

ELISA: Enzyme-linked immunosorbent assay

ELISpot: Enzyme-linked immunosorbent spot assay

eIF-4GI: Eukaryotic initiation factor 4GII

ESCRTs: Endosomal sorting complexes required for transport

gp120: Glycoprotein 120

gp41: Glycoprotein 41

g: Gram

≥: Equal to or greater than

HIV-1 or HIV-2: Human immunodeficiency virus type 1 or 2

HIV LTR: HIV long terminal repeats

HLA: Human leukocyte antigen

HAART: Highly active anti-retroviral therapy

HeLa cells: Henrietta Lacks cells

HEK cells: Human embryo kidney cells

HCV: Hepatitis C Virus

IRES: Internal ribosome entry site

IN: Intra nasal

IFN-γ: Interferon gamma

IL-2: Interleukin-2

IL4: Interleukin-4

IL-7: Interleukin-7

ISCOMs: Immune stimulating complexes

IAVI: International AIDS Vaccine Initiative

sIgA: Secreted immunoglobulin A

IgG: Immunoglobulin G

IgE: Immunoglobulin E

I.e: That is to say

Kb: kilo base

kDa: kilo dalton

<: Less than

LRAs: Latency reversing agents

LEDGF/p75: Lens epithelium-derived growth factor/p75

MHC-I/II: Major histocompatibility complex class I or II

MHC-E: Major histocompatibility complex class E

MPER: Membrane proximal external region

ml: millilitre

mg: milligram

µl: micro litre

mRNA: messenger RNA

MSM: Men-who have-sex with men

Nabs: Neutralizing antibodies

NF-kB: Nuclear factor NF-κB

NFAT: Nuclear factor of activated T-cells

NK cell: Natural killer cells

%: Percentage

/: Per

RNA: Ribonucleic acid

STDs: Sexually transmitted diseases

SIV: Simian immunodeficiency

SIVmac251: Simian immunodeficiency for macaques strain 251

SIVmac239: Simian immunodeficiency for macaques strain 239

SIVsm E660: Simian immunodeficiency for macaques strain E660

SIVsmm: Simian immunodeficiency virus for sooty mangabeys

Th1 and 2: Type 1 and Type 2 immune responses

TNF-α: Tumor necrosis factor-alpha

UTR: untranslated region

UNAIDS: United Nations Programme on HIV and AIDS

Viz: namely

WHO: World Health Organisation