Evaluating the Immuno-modulatory Effects of a Natural Adjuvant as a Novel Vaccine Strategy

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Abstract

Introduction: Traditional vaccine strategies are inefficient at protecting against more complex pathogens including HIV, therefore novel vaccine technologies are required. DNA vaccines are attractive as they are relatively cheap and easy to manufacture, but reduced immunogenicity in humans is a limitation. The key to improving DNA vaccine immunogenicity is enhanced DNA uptake and localised inflammatory responses, which may be achieved with the use of an appropriate adjuvant. HSP70 is a damage associated molecular pattern (DAMP) that binds to and activates dendritic cells, making HSP70 a potential natural adjuvant. The immunogenicity of a DNA vaccine encoding HIV gag and HSP70 was examined in which the latter was genetically modified to encode cytoplasmic, fusion, secreted or membrane-bound forms of HSP70, controlled by a separate promoter within the same construct, ensuring delivery of the antigen and adjuvant to the same cell. Furthermore, immune responses induced by DNA vaccines encoding multiple antigens compared with a single antigen were measured.

Methodology: C57Bl/6 mice were vaccinated three times at two weekly intervals with 10 μg doses of DNA via the intradermal route. Gag-specific T cell responses were determined by EliSpot, intracellular cytokine staining (ICS) and proliferation assays. Short term and long term protection was evaluated by challenge with EcoHIV, a chimeric HIV that can infect mouse, but not human, leukocytes.

Results: A bicistronic vector containing the CMV and SV40 promoters was produced. The CMV promoter was shown to be approximately 10-fold stronger than the SV40 promoter using the eGFP reporter gene. Therefore, initially, the HIV gag antigen was cloned downstream of the CMV promoter and the wild type and modified HSP70 genes were inserted downstream of the SV40 promoter, to ensure that the adjuvant properties of HSP70 targeted antigen-positive cells. Membrane-bound or secreted HSP70 significantly enhanced gag-specific T cell responses in vaccinated mice and increased the breadth of T cell responses as determined by the recognition of otherwise subdominant gag epitopes. The adjuvant effect of membrane-bound or secreted HSP70 also significantly improved the ability of HIV-specific T cells to secrete multiple cytokines, a characteristic thought to be important for vaccine efficacy. Finally, inclusion of genes encoding membrane-bound HSP70 resulted in increased protection as

shown by a significant reduction in the titre of EcoHIV in peritoneal exudate cells after virus challenge, compared with mice vaccinated with the gag-only DNA.

To increase the expression of membrane-bound or secreted HSP70, these genes were also inserted downstream of the CMV promoter, while expression of gag was controlled by the SV40 promoter. However, this significantly decreased gag-specific T cell responses as shown by EliSpot and ICS analysis. Furthermore, DNA vaccines which included the HIV pol gene with gag compared with gag alone reduced the gag-specific T cell responses in vaccinated mice and reduced the protection in mice challenged with EcoHIV.

Conclusion: This study demonstrated that expression of membrane-bound or the secreted form of HSP70 from a DNA vaccine encoding HIV gag significantly improved gag-specific T cell function, multifunctionality and proliferation. Most importantly, the adjuvant effect of membrane-bound HSP70 increased protection in mice challenged with EcoHIV.

Declaration

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Abbreviations

ADCC	Antibody-dependent cell-mediated cytotoxicity
AIDS	Acquired immunodeficiency syndrome
APOBEC	Apolipoprotein B mRNA editing enzyme-catalytic polypeptide-like
ART	Antiretroviral therapy
CAT-1	Cationic amino acid transporter-1
CD	Cluster of differentiation
CFSE	Carboxyfluorescein succinimidyl ester
CMV	Cytomegalovirus
Crfs	Circulating recombinant forms
CTL	Cytotoxic T lymphocyte
DAMPs	Damage associated molecular patterns
DCs	Dendritic cells
eGFP	enhanced green fluorescent protein
ELISA	Enzyme-linked immunosorbent assay
EliSpot	Enzyme-linked immunosorbent spot
GALT	Gut-associated lymphoid tissue
HAART	Highly active antiretroviral therapy
HDACi	Histone deacetylase inhibitors
HEK 293T	Human embryonic kidney 293T cells
HESN	Highly-exposed sero-negative
HIV	Human immunodeficiency virus
HSP70	Heat shock protein 70
hTfR	Human transferrin receptor
ICS	Intracellular cytokine staining
IFN-γ	Interferon-γ
IL-2	Interleukin-2
LB	Luria broth
LPS	Lipopolysaccharide
LTNP	Long term non-progressors
LTR	Long terminal repeat
MCS	Multiple cloning site
MHC	Major histocompatibility complex
MLV	Murine leukaemia virus
NHP	Non-human primate
PBMC	Peripheral blood mononuclear cell
PCP	*Pneumocystis carinii* pneumonia
PCR	Polymerase chain reaction
PEC	Peritoneal exudate cell
PHA	Phytohaemagglutinin
PRR	Pattern recognition receptor

qRT-PCR	Real-time quantitative PCR
SFU	Spot forming units
SHIV	Simian/human immunodeficiency virus
SIV	Simian immunodeficiency virus
SV40	Simian virus 40
TAA	Tumour-associated antigen
TLR	Toll-like receptor
TNF-α	Tumour necrosis factor α