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Comparison of DNA vaccines producing HIV-1 Gag and LAMP/Gag chimera in rhesus macaques reveals antigen-specific T-cell responses with distinct phenotypes

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ABSTRACT

Optimized DNA expression vectors encoding the native HIV-1 Gag or a fusion of Gag with the lysosomal membrane associated protein 1 (LAMP) were compared for immunogenicity upon intramuscular DNA delivery in rhesus macaques. Both vaccines elicited $CD4^+$ T-cell responses, but with significant differences in the phenotype of the Gag-specific cells: the native Gag induced $CD4^+$ responses with a phenotype of central memory-like T cells ($CD28^+$ $CD45RA^-$), whereas the LAMP/Gag chimera induced $CD4^+$ responses with effector memory phenotype ($CD28^-$ $CD45RA^-$). Antigen-specific T cells producing both IFN-γ and TNFα were found in the animals receiving the native Gag, whereas the LAMP/Gag chimera induced humoral responses faster. These results demonstrate that modification of intracellular Gag trafficking results in the induction of distinct immune responses. Combinations of DNA vectors encoding both forms of antigen may be more potent in eliciting anti-HIV-1 immunity.

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1. Introduction

The major histocompatibility complex type II (MHC II) processing compartment (MIIC) is a lysosomal-like organelle that plays an important role regulating T-cell antigen processing and presentation. Targeting antigens directly to the MIIC compartment of professional antigen-presenting cells (APCs) have been shown to successfully enhance and modulate the immunogenicity of several antigens [1–15]. Lysosomal associated membrane protein-1 (LAMP-1) normally is present in MIIC of immature APCs. The effects of LAMP-1 targeting on the elicited immune responses have been analyzed in immunization studies in several mouse strains, macaques, and in humans. Several laboratories have reported that targeting antigens with the tyrosine based motif (YQTI) of LAMP-1 [1–3] can enhance the immune responses against a variety of antigens from many pathogens, including HPV-16 E7 and E6; HIV-1 Gag, Env gp120, Env gp160, and Nef; West Nile virus preM-E; dengue 2 preM-E; SARS coronavirus N; listeriolysin "O"; and tumor antigens such as the thyroid hormone receptor (TSHR), human telomerase reverse transcriptase (hTert) and human melanosomal antigen (MAGE-3) [4–15]. In general, the LAMP-1/antigen chimeras were shown to elicit broader repertoire of antigen-specific $CD4^+$ T-cell responses, greater functional avidity, augmented proliferative response and ability to secrete a variety of interleukins. The increased $CD4^+$ T-cell mediated responses produced by the LAMP-1/antigen formulation are thought to medi-

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ate enhancement in B cell and $CD8^+$ T-cell responses, as well as the development of immunological memory. When compared to the non-targeted molecules, LAMP-1/antigen chimera elicited higher antibody titers, increased antibody affinity and neutralizing activity, as well as expansion of the number of recognized B-cell epitopes. Similarly, the $CD8^+$ responses were found elevated in several LAMP-1/antigen chimeric systems, as assessed by tetramer staining, IFN-γ enzyme-linked immunospot (ELISPOT), cytotoxicity assays, and the functional avidities and T-cell response repertoires of $CD8^+$ cells. The longevity of the immunological memory of B cells and $CD8^+$ cells was also increased in animals immunized with LAMP-1/chimeras [4–15].

In previous studies, vaccination of mice with DNA plasmid expressing the LAMP/Gag chimera showed strong, broad cellular and humoral immune responses [9,11–14]. This vaccine construct was also tested in a pilot study using five rhesus macaques, and induced humoral and cellular immune responses, which were associated with a sustained activation of B lymphocytes as well as $CD4^+$ and $CD8^+$ T cells [12].

Here, we performed a direct comparison of the immune responses elicited upon intramuscular DNA injection of plasmids producing the native Gag or the human LAMP/gag chimera in a cohort of 22 Indian rhesus macaques. Both of the plasmids contain the RNA/codon optimized HIV-1 *gag* sequence [16–18], and were cloned into the same plasmid backbone, suitable for clinical studies. The results show that each of the HIV-1 antigen formulation delivered as naked DNA vaccines have unique immunogenic properties and induce qualitatively distinct cellular and humoral immune responses.

2. Materials and methods

2.1. Plasmids

pCMV-gag.kan plasmid contains the RNA optimized HIV-1 *gag* p55 gene from HXB2 inserted into the mammalian expression plasmid pCMV.kan [16,19], which consists of a plasmid backbone optimized for growth in bacteria, the human cytomegalovirus (CMV) promoter, the bovine growth hormone (BGH) polyadenylation site and the kanamycin resistance gene. The RNA optimized *gag* gene contains multiple nucleotide changes that destroy the previously identified RNA inhibitory/instability sequences but do not affect the coding potential [16–18]. The pCMV-LAMP/gag.kan plasmid contains the optimized HIV-1 *gag* between the human LAMP luminal domain and the LAMP transmembrane and cytoplasmic (TM/cyt) tail domain [12]. This modified LAMP/gag DNA vector has a backbone that does not contain the AAV-ITR sequences or the penicillin resistance gene used in previous studies [9,11–14]. Plasmids used for vaccination of rhesus macaques had DNA purity of 96% and endotoxin levels less than 0.33 EU/mg and were obtained with support from the NIH/NIAID *Reagent Resource Support Program for AIDS Vaccine Development*, Quality Biological, Inc. (Principal Investigator, Ronald Brown).

2.2. Analysis of protein expression

DCEK cells, a line of murine fibroblasts doubly transfected with class II MHC E^k and type 1 intercellular adhesion molecule (ICAM-1) [20,21], were plated in 6-well plates (2×10^6 cells/well) and transfected with plasmid DNA (4 μg) using the FuGENE™ 6 (Roche Applied Science, Indianapolis, IN) transfection reagents according to the manufacturer's instructions. The protein concentration for each cell extract was quantified prior to Western blot analysis and equal amounts of proteins were loaded per well. Western immunoblot analysis and antigen capture enzyme-linked immunosorbent assay (ELISA) were performed as described previously [11].

2.3. Immunization of macaques

Twenty-two healthy 4- to 8-kg male Indian rhesus macaques were maintained in the non-human primate facility of Southern Research Institute, Frederick, MD, USA. Animal care and treatment were in accordance with standards approved by the Institutional Animal Care and Use Committee, according to the principles set forth in the Guide for the Care and Use of Laboratory Animals, National Research Council, National Academy Press, 1996. Each animal was immunized via the intramuscular (IM) route five times at weeks 0, 4, 14, 24 and 36 with 2 mg of optimized *gag* or LAMP/gag plasmid using a biojector. HIV-1 Gag-specific humoral and cellular immune responses were determined as described in Fig. 2. Isolation of peripheral blood mononuclear cells (PBMCs) and HIV-1 Gag-specific IFN-γ ELISPOT assay were performed as described [12]. The ELISPOT assays were considered valid only when the negative controls had less than 5 spot forming cells (SFC) and the positive controls more than 500 SFC. Responses were considered positive when the sample produced more than 10 spot and the signal in the presence of peptides minus two standard deviations was higher than the signal in the medium control sample plus two standard deviations. Mucosal samples including mouth swabs, nasal and rectal washes were collected periodically. Antibody titers were determined as described [14], using either a 1:100 dilution of the individual sera samples collected over time or end-point dilutions using a pool of the sera collected at 2 weeks post vaccination 3 and 5, respectively. The reported optical density (OD) corresponds to the value minus three times the OD value of a non-immune serum. The reported titers correspond to the reciprocal of the highest serum dilution that gave a three times higher OD value than the corresponding dilution of a non-immune serum. The determinations were performed in duplicate.

2.4. Flow cytometric analysis

PBMC were resuspended at a density of 10^6 cells/ml in RPMI-1640 supplemented with 10% FBS, 100 U/ml penicillin/streptomycin and 2 mM L-glutamine, in the presence or absence of a pool of HIV-1 gag 15-amino acid (aa) peptides overlapping by 11-aa (114 peptides spanning the entire Gag sequence, at a final concentration of 1 μg/ml for each peptide). The peptide stimulation was performed without the addition of costimulary anti-CD49 or antiCD28 antibodies. As positive control, cells were treated with the enterotoxin B of *Staphylococcus aureus* (SEB) at a final concentration of 20 ng/ml. Cells were treated for 6 h with monensin to prevent protein secretion, and cell surface staining was performed using the following antibody cocktail: CD3, CD4, CD8, CD45RA and CD28. Cells were washed twice, and fixed and permeabilized with Cytofix/Cytoperm (BD, Pharmingen) according to the manufacturer's instructions. In some experiments, an alternative surface staining adding to the cocktail a monoclonal antibody against CCR7 was performed. Intracellular cytokine detection was performed using a cocktail of antibodies against IFN-γ, IL-2 and TNFα labeled either with the same fluorochrome (APC) or with FITC, APC and PE Cy7, respectively. For each sample at least 10^5 T cells were acquired in the FACSAria flow cytometer (BD, Pharmingen) and the data were analyzed using the FlowJo platform (Tree Star, Inc., Ashland, OR). Typical background in the absence of peptide stimulation was below 0.02%. Peptide stimulated samples were considered positive if they were at least two-fold higher than the medium control.