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Engineering intravaginal vaccines to overcome mucosal and epithelial barriers

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ABSTRACT

The mucosal surface of the vagina is a primary human immunodeficiency virus (HIV) entry portal, making it an attractive site for HIV vaccination. However, HIV vaccines based on recombinant adenovirus (rAd) do not efficiently cross the mucus layers or underlying epithelium of the vagina. Here we designed nanocomplexes of rAd particles coated with (1) the polyethylene glycol derivative APS to provide a hydrophilic surface that would prevent entrapment in the hydrophobic mucus, and (2) the cell-penetrating peptide TAT to improve transduction efficiency. The optimized rAd-TAT-APS nanocomplexes could achieve the balance of effective mucus-penetrating and cellular transduction. Intravaginal delivery of rAd-TAT-APS encoding HIVgag p24 into mice strongly enhanced HIVgag-specific systemic and mucosal immune responses. This rAd-TAT-APS system may allow effective vaginal delivery of vaccines against HIV and other infectious agents.

1. Introduction

The human immunodeficiency virus (HIV) epidemic remains a significant cause of mortality and morbidity globally [1]. Infection with HIV is associated with progressive depletion of $CD4^+$ T cells and, to a lesser extent, macrophages and dendritic cells [2,3]. This leads to acquired immunodeficiency syndrome (AIDS) [4], in which the individual is severely immunosuppressed and susceptible to opportunistic infections [5,6]. The most frequent mode of transmission is from men to women via the vagina [7], which provides an extensive, exposed mucosal surface for HIV entry [8]. The lack of chemical or physical barriers with which women can block vaginal entry of HIV translates to a high rate of infection of women during sexual intercourse. Mucosal vaccines are far superior to parenteral vaccines for interrupting HIV transmission [9]. Therefore significant efforts have been made to develop HIV vaccines to boost local immune responses in the vaginal mucosa and thereby protect against HIV infection.

Adenovirus, already widely used as a vaccine carrier because of its potent ability to transduce desired genes into the recipient and immune adjuvant properties [10], is attractive as an HIV vaccine vector. Specifically, replication-defective adenovirus of human serotype 5 (rAd5) shows good potential as an HIV-1 vaccination platform [11]. Such a platform must overcome two major barriers to vaccine entry in the vagina. One is the barrier of thick mucus lining the vaginal epithelium, which immobilizes foreign particles in mucus by adhesive electrostatic, hydrophobic, or hydrogen-bonding interactions [12]; subsequent elimination of the particles results in poor therapeutic drug or gene delivery [13]. If particles penetrate slowly through mucus, they are more susceptible to clearance during mucus turnover [14]. The second major barrier is the vaginal epithelium itself, since rAd vaccines efficiently vector the desired gene(s) into the recipient's epithelial cells only when the cells express high levels of the coxsackievirus and rAd receptor (CAR) [15]. However, most epithelial cells and antigen-presenting cells in humans do not express CAR [16].

Overcoming the mucosal and epithelial barriers to rAd-based vaccine entry and transduction of vaginal cells and antigen-presenting cells requires modifying the virus particles appropriately. Since the primary component of mucus is negatively charged mucins, one approach to overcoming the mucus barrier is to modify the nanoparticles with short-chain polyethylene glycol (PEG) of 2–5 kDa [17,18]. Work from our laboratory has shown that coating rAd with the cationic PEG derivative amino-$(EO)_n$-$(AGE)_m$-Cys (APC) containing a 2.8-kDa PEG liner with 5.8 amines helped the

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viral particles traverse vaginal mucus quickly but did not facilitate subsequent uptake by epithelial cells or antigen-presenting cells [19]. Conversely, coating rAd with the cationic PEG derivative APC containing a 4.5-kDa PEG liner with 10.5 amines led to efficient transduction of cells but relatively poor mucus-penetrating ability. These results suggest that PEG density and surface charge must be optimized to ensure that rAd nanocomplexes pass through both mucosal and epithelial barriers.

One approach to improve the transduction of CAR-negative cells is to attach polycationic or amphipathic cell-penetrating peptides to the rAd capsid protein. These peptides include R8, proline-rich peptide and the HIV trans-activating transcriptional activator TAT (CAYGRKKRRQRRR) [20]. These peptides, 7–30 residues long, facilitate delivery of vaccine cargo into target cells, and they are subsequently degraded into individual amino acids, making them harmless to cells [21,22]. Studies *in vitro* with CAR-negative cells indicate that conjugating such peptides to rAd leads to transduced gene expression that is 10- to 100-fold higher than with uncoated rAd [23]. It is unclear whether the same effects are observed *in vivo,* and published procedures for coating rAd with cell-penetrating peptides do not allow control over the amount of peptide on the particle surface, which limits the usefulness of the vaccine platform.

Here we describe an intravaginal vaccination system based on rAd particles non-covalently coated with the anionic PEG derivative amino-$(EO)_n$/$(AGE\text{-}Suc)_m$ (APS) to prevent entrapment in mucus [24], as well as with the TAT peptide to stimulate particle uptake by target cells (Fig. 1). We validated this system by adding the HIVgag p24 gene to rAd nanocomplexes, and then assaying the ability of this vaccine to elicit HIVgag-specific immune responses *in vitro* and *in vivo*.

2. Materials and methods

2.1. Chemicals and biomaterials

Ethylene oxide (EO) was obtained from Xinhua Institute of Active Material (Jiangsu, China). Allyl glycidyl ether (AGE), mercaptosuccinic acid and medroxyprogesterone acetate were purchased from Sigma-Aldrich (Saint Louis, USA). Carboxyfluorescein diacetate succinimidyl ester (CFSE) was purchased from Invitrogen (Carlsbad, CA, USA). Brefeldin A, Alexa Fluor®488 dye (isomeric mixture of carboxylic acid ester) and the following anti-mouse antibodies were obtained from eBioscience (San Diego, CA, USA): PE-labeled antibodies against IFN-γ, IL-4; FITC-labeled antibodies against CD8a and CD4. Goat anti-mouse HRP-conjugated antibody against IgG was purchased from ZSGB-BIO (Beijing, China). Goat anti-mouse horseradish peroxidase (HRP)-conjugated antibody against IgA was obtained from Santa Cruz (CA, USA). HIV gag peptide corresponding to the H-2 Kd-restricted immunodominant CTL epitope (AMQMLKETI) was purchased from GenScript (Piscataway, NJ, USA). Recombinant human HIV-1 gag p24 was obtained from USBiological (Swampscott, Salem, MA, USA).

Serotype 5 adenovirus vector expressing HIV-1 gag p24 was E1/E3-deleted. This vector was kindly provided by Prof. Hildegund C.J. Ertl of Wistar Institute of Anatomy and Biology, Philadelphia, PA, USA). Adenovirus was amplified in the human embryonic kidney cell line 293 T and by CsCl gradient centrifugation. Virus particle number (vp) was determined optical density at 260 nm. Virus stocks were stored at −80 °C before use.

2.2. Cell culture and animals

Immortalized VK2/E6E7 human vaginal epithelial cells obtained from School of Pharmaceutical Science, Southern Medical University were maintained in Defined Keratinocyte-SFM (GIBCO/BRL) supplemented with 1 ml Defined Keratinocyte-SFM Growth Supplement, 0.4 mM $CaCl_2$, 100 units/ml penicillin, and 100 mg/ml streptomycin. DC 2.4 dendritic cells were purchased from the Chinese Academy of Sciences (Shanghai, China) and maintained in RPMI-1640 medium (Hyclone, Life Technologies, Grand Island, NY, USA) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 mg/ml streptomycin. All the cells were incubated at 37 °C with 5% CO_2.

6 to 8 weeks free female BALB/c mice (specific pathogen) were purchased from the Laboratory of Center of Sichuan University. All procedures with animals were approved by Institutional Animal Care and use guidelines.

2.3. Synthesis of APS copolymer

2.3.1. Protection of the amine group of β-aminoethanol with $(Boc)_2O$

β-aminoethanol (7.64 g, 0.125 mol) in 25 ml methanol was mixed with 50 ml water in which sodium hydroxide (10.00 g, 0.25 mol) had been dissolved. This solution was stirred in an ice bath for 10 min, then 25 ml methanol solution of $(Boc)_2O$ (32.70 g, 0.15 mol) was added dropwise. The reaction mixture was stirred at room temperature for 24 h and filtered. The filtrate was extracted several times with ethyl acetate, and the pooled extracts were washed with water until neutral, then washed with saturated salt solution. The solution was dried over anhydrous sodium sulfate, and ethyl acetate was removed by evaporation, leaving the desired Boc-aminoethanol.

2.3.2. Synthesis of Boc-$(EO)_n$/$(AGE)_m$

Boc-aminoethanol (2.64 g, 0.015 mol) was dried for 1 h at 95 °C under reduced pressure with an oil pump and dissolved in 50 ml nonaqueous 1,4-dioxane. Sodium hydride (60%, w/w; 2.76 g, 0.115 mol) was added, and the mixture was stirred at 36 °C for 1 h. Nonaqueous EO (100 ml) was added dropwise via a constant pressure infundibulum connected to a condensing tube (containing recirculating water at 4–8 °C) and a drying tube. The mixture was stirred at 60 °C for 3 days, and the condensing tube was removed. AGE (45 ml) was added to the solution and stirred at 60 °C for another 3 days. The reaction was halted with water, and the solution was adjusted to neutral using hydrochloric acid. Solvents were removed by evaporation. The residue was dissolved in dichloromethane and dried over anhydrous sodium sulfate. The remaining solution was filtered and centrifuged, and the supernatant was condensed by evaporation and precipitated three times using diethyl ether. Dialysis and lyophilization yielded Boc-$(EO)_n$/$(AGE)_m$.

2.3.3. Deprotection of Boc-$(EO)_n$/$(AGE)_m$

Boc-$(EO)_n$/$(AGE)_m$ (2.57 g) was dissolved in 12.5 ml dichloromethane solution containing 40% trifluoroacetic acid. The solution was stirred at room temperature for 2 h, condensed by evaporation and purified further by dialysis for 2 days against distilled water in cellulose with a molecular weight cut-off of 1000 Da. The solution was lyophilized, yielding amino-$(EO)_n$/$(AGE)_m$.

2.3.4. Synthesis of amino-$(EO)_n$/$(AGE\text{-}Suc)_m$

Amino-$(EO)_n$/$(AGE)_m$ (2.10 g) was dissolved in 30 ml methanol and mixed with 5 ml water containing mercaptosuccinic acid (6.04 g, 0.04 mol) and sodium hydroxide (3.24 g, 0.08 mol). The reaction mixture was stirred for 1 day at room temperature under ultraviolet light, and stirred for another 2 days at 37 °C without ultraviolet light. The mixture was dialyzed, ion-exchanged on H^+-type sulfonic acid resin and lyophilized to obtain amino-$(EO)_n$/$(AGE\text{-}Suc)_m$ (APS) as a yellow powder.