ABSTRACT SUMMARY

A novel replication-defective recombinant adenovirus 5 (rAd5) vector-based system for HIV was formulated with chitooligosaccharides and mannosylated polyethyleneimine-triethyleneglycol (man-PEI-TEG), and subsequently evaluated through intranasal delivery. Intranasal delivery of the Chitooligosaccharides-PEI-TEG-rAd5-HIVgag (Oligo-PEI-rAd5-HIVgag) vaccine with a prime-boost regimen strongly elicited both systemic and mucosal immunity.

INTRODUCTION

The Human Immunodeficiency Virus (HIV) epidemic has become one of the most serious health and development challenge in the world [1]. Recent studies have shown that replication-defective recombinant adenovirus (rAd5) was considered as a candidate vector for HIV vaccines. The intranasal vaccination can induce both systemic and mucosal immunity. Indeed, the intranasal immunization with a prime-boost regimen can also be adopted to bypass preexisting anti-Ad5 immunity [2]. However, intranasal immunization has serious problems such as mucociliary clearance and low antigen uptake. Therefore, in the current study some specific functional materials were applied to overcome these barriers. Chitooligosaccharides, as an excellent mucosal adhering adjuvant, were employed to prolong the residence of rAd5 in nasal mucosa. The mannosylated polyethyleneimine can enable vaccine to target dendritic cells (DCs), which are professional antigen-presenting cells with an abundant expression of mannose receptors.

In the present study, the Ad5 expressing HIV gag formulated with chitooligosaccharides and man-PEI-TEG (Oligo-mPEI-rAd5-HIVgag) were evaluated for their transduction abilities to epithelia cells and their potency to generate immune responses after intranasal immunization.

EXPERIMENTAL METHODS

The Oligo-mPEI-rAd5-HIVgag complex was prepared by replication-defective recombinant adenovirus 5 (rAd5) vectors expressing HIV gag coated with chitooligosaccharides and mannosylated polyethyleneimine-triethyleneglycol (man-PEI-TEG) through electrostatic interactions. The physical characteristics of Oligo-mPEI-rAd5-HIVgag complex were determined at 25°C using photon correlation spectroscopy (PCS).

For transduction efficiency assay, Calu-3 and MDCK cells as an in-vitro nasal model were seeded in 24-well plates. Afterwards, naked rAd5-lacz or Oligo/mPEI/Oligo-mPEI-rAd5-lacz with infection (MOI) of 20 were incubated with the cells for 4 h at 37 °C, and then replaced by fresh medium. At 48 h after transduction, the infectivity was analyzed by quantitative detection of β-galactosidase activity.

B6/w mice were inoculated intranasally with 1×1010 viral particles (20μl) of rAd5-HIV gag each group. Two weeks later, mice were boosted intranasally with 1×1011 viral particles of rAd5. Mice were maintained in an upright position hook for 30 min after treatment. At day 28, mice were sacrificed.

The cytotoxicity of antigen-specific CD8⁺ T cells was examined by in vivo CTL assay. Splenocytes isolated from naïve mice were divided equally into two parts and incubated in the presence of synthesized HIV gag peptide or medium only at 37°C for 2h, respectively. The peptide-pulsed cells were stained with 4μM (high) CFSE while non-pulsed part was stained with 0.4μM (low) CFSE. CFSE labeling was then quenched by addition of fetal bovine serum. The equal numbers of two part cells were mixed together and given to recipient mice via tail vein injection. 18h later, spleen cells were prepared and analyzed by flow cytometry.

The HIV gag-specific CD8⁺ cell and CD4⁺ cell responses were determined by measuring the CD8⁺ cell and CD4⁺ cell secreting IL-4 level using intracellular cytokine staining assay. Splenocytes from immunized mice were incubated in the presence of HIV gag peptide for 5 h at 37°C. The splenocytes resuspended in the staining buffer were co-incubated with FITC-labeled anti-mouse CD8a or CD4 or a PE-labeled anti-mouse IFN-γ or IL-4 in the dark for 30min. Finally, the cells were examined by two-color flow cytometry.

The secretion profile of SIgA antibody in mucosa has been shown to indicate mucosal immune responses [3]. After intranasal immunization, the nasal washes were collected from the nostrils by flushing through the nasal cavity with three successive PBS (1%BSA). The concentration of SIgA in nasal washes was measured by ELISA.

RESULTS AND DISCUSSION

The mean particle size, zeta potential and polydispersity index were determined in table 1.

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Zeta potential (mV)</th>
<th>Size (nm)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naked rAd5</td>
<td>-7.2±0.5</td>
<td>103.8±0.5</td>
<td>0.095±0.051</td>
</tr>
<tr>
<td>Oligo-mPEI-rAd5</td>
<td>24.1±0.9</td>
<td>180.5±2.3</td>
<td>0.213±0.033</td>
</tr>
<tr>
<td>Oligo-rAd5</td>
<td>18.4±1.1</td>
<td>178.1±2.2</td>
<td>0.173±0.044</td>
</tr>
<tr>
<td>mPEI-rAd5</td>
<td>23.2±0.8</td>
<td>175.8±1.7</td>
<td>0.145±0.031</td>
</tr>
</tbody>
</table>

Table 1 Zeta potential and size distribution of rAdV and complexes.
As shown in Fig.1, the Oligo-mPEI-rAd5 complex yielded significantly higher transduction efficiency than Oligo-rAd5, mPEI-rAd5 and naked rAd5-lacz in Calu-3 and MDCK cells (*p<0.05, **p<0.01). The results showed that chitooligosaccharides and m-mPEI-TEG jointly increased the efficiency of adenovirus transduction and this effect was stronger than chitooligosaccharides or m-PEI-TEG with adenovirus alone.

![In vitro transduction efficiency](image1)

Figure1. In vitro transduction efficiency of naked rAd5 and complexes in in-vitro nasal models were detected quantitatively by the β-galactosidase enzyme assay system and BCA assay (n=4, *p<0.05, **p<0.01).

To study the efficacy of Oligo-mPEI-rAd5-HIVgag vaccine, we went on in vivo experiments. The antigen-specific CD8+ CTL response was determined after intranasal vaccination. The fluorescent dye carboxyfluorescein succinimidyl ester (CFSE) was used to assess CTL-mediated lysis by monitoring injected HIVgag peptide-pulsed CFSE<sup>high</sup>-labeled splenocyte populations relative to non-pulsed CFSE<sup>low</sup>-labeled populations. As shown in Fig.2, after boosted with an increased dose (10<sup>11</sup>vp), the Oligo-mPEI-rAd5-HIVgag complex enhanced significantly antigen-specific CD8<sup>+</sup> T cell responses while naked rAd5-HIVgag group was still relatively at a low level. Although less potent than intramuscular immunization, the HIVgag-specific cytotoxic lysis induced by intranasal immunization with Oligo-mPEI-rAd5-HIVgag was significantly stronger than naked rAd5-HIVgag group.

![HIVgag-specific cytolytic T cell response](image2)

Figure2. The HIVgag-specific cytolytic T cell response was enhanced by intranasal immunization with Oligo-mPEI-rAd5 complex. The HIVgag-specific cytotoxic CD8<sup>+</sup> T cell response was quantitatively analyzed. (n=5; *p<0.05, **p<0.01)

The HIVgag-specific T cells responses were also monitored by intracellular cytokine staining assay. The IFN-γ producing CD8<sup>+</sup>T cells and IL-4 producing CD4<sup>+</sup>T cells induced by Oligo-mPEI-rAd5-HIVgag immunization were both much more than naked rAd5-HIVgag only (Fig.3).

![HIVgag-specific IFN-γ and IL-4 responses](image3)

Figure3 The Oligo-mPEI-rAd5-HIVgag vaccine promoted IFN-γ secretion of CD8<sup>+</sup>T and IL-4 secretion of CD4<sup>+</sup>T cells. The rates of double positive cells were calculated by the percentages of cytokinespositive specific T cells to total Tcells (n=4; *p<0.05).

The secretory immunoglobulin A (SIgA) has been considered as an important indicator for mucosal immunity. The Oligo-mPEI-rAd5-HIVgag complexes enhanced significantly the HIVgag-specific mucosal SIgA responses in comparison with naked rAd5 groups (Fig. 4).

![Mucosal SIgA responses](image4)

Figure4. The antigen-specific SIgA secretion in the nasal mucosa

CONCLUSIONS

In conclusion, the prime-boost intranasal immunization with the novel Oligo-mPEI-rAd5-HIVgag complex is a potential vaccine candidate for HIV infection eliciting strong cellular and local mucosal immune responses.

REFERENCES


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