

Co-delivery of chimeric peptide vaccine and CpG ODN adjuvant using PLGA nanospheres: Antibody responses against HTLV-1

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Abstract

In the present investigation, the immunogenicity of HTLV-1 fusion epitope-loaded PLGA nanoparticles (NPs) was assessed in the presence of co-encapsulated CpG ODN adjuvant, in a mice model. Accordingly, the recombinant vaccine containing Tax, env, and gag epitopes was encapsulated in PLGA nanoparticles (NPs) with CpG ODN adjuvant to assess the immune efficacy of various formulations in an animal model followed by subcutaneous or nasal administration. PLGA NPs were prepared using a double emulsion method with a size of approximately 190 nm and the encapsulation efficiency was calculated 83.9%. The release profile of radiolabeled chimera indicated that 20% of chimera were released from PLGA NPs during one month. Co-encapsulation of chimera and CpG in PLGA NPs could considerably elicit cell-mediated responses compared to the incorporation of CpG and chimera antigen solution. These findings revealed that the co-delivery of recombinant vaccine and CpG adjuvant using PLGA NPs could induce potent cell-mediated and mucosal immunity without inflammatory responses against HTLV-1. Hence, an appropriate vaccine design and immunization strategy are essential parameters to construct an effective vaccine.

Keywords: Chimeric peptide vaccine, PLGA, CpG adjuvant, Cellular response, Mucosal immunity, Antibody assay, HTLV-1.

Introduction

Human T-cell leukemia/Lymphoma virus type 1 (HTLV-1) is an oncogenic retrovirus that was found to be endemic in some areas of the world such as Japan, Iran, Africa, Caribbean islands, and Central or South America with an incidence of 25 million people worldwide [1,2]. Due to the inability of most peptide vaccines to stimulate potent immune responses, utilization of adjuvants, and proper delivery systems should be considered to boost the efficiency and immunogenicity of candidate vaccines. The oligodeoxynucleotides (ODN) containing unmethylated CpG dinucleotides have been widely used as an immunostimulatory adjuvant in various vaccine formulations for clinical development. CpG ODN triggers TLR9, to improve antigen presentation by activation and maturation of antigen-presenting cells (APCs) such as dendritic cells (DCs) and induce innate and adaptive immunity [3,4]. The binding of CpG ODN and TLR-9 activates cell signaling pathways and stimulates the differentiation and proliferation of B- and T-cells, natural killer (NK) cells, macrophages, and monocytes to secrete immunoglobulin (Ig), and various cytokines. Indeed, ODN containing CpG motifs can elicit a polarized T helper 1 (Th1) immune response, when mixed with antigen or co-encapsulated in vaccine delivery systems. Based on some previous studies, co-delivery and co-localization of CpG ODN and antigen to the same APC could induce high Th1 immune responses [3,5]. In the current study, the immune responses of HTLV-1 fusion epitope-loaded PLGA nanoparticles (NPs) in the presence of CpG ODN adjuvant were evaluated in a mice model followed by subcutaneous (SC) or nasal inoculation.

Results Discussion

The co-delivery of a recombinant vaccine comprising Tax, env, and gag immunodominant HTLV-1 epitopes and CpG adjuvant using PLGA NPs was performed to evoke immune responses against HTLV-1. The spherical structure of PLGA NPs with smooth surfaces was confirmed by the scanning electron microscopy technique. Based on the results of dynamic light scattering, the mean diameter of the mentioned formulation was 189.9 ± 31.7 nm with a polydispersity index (PDI) of 0.289. The surface charge of the formulation was -37.8 ± 6.4 mV and the encapsulation efficiency was $83.1 \pm 4.7\%$. According to the release profile, 20.1% of chimera were released from PLGA nanospheres in the presence of CpG adjuvant within one month. The levels of IgG1, IgG2a, and sIgA antibodies were detected by the ELISA method to identify the type of immunity induced in vaccinated mice (Fig. 1). The titers of IgG1 and IgG2a antibodies were significantly ($p < 0.001$) lower in all control groups such as PBS; CpG alone; blank PLGA NPs; and Trx-tag protein, in comparison with vaccination with test groups including chimera solution; chimera+CpG; and

(chimera+CpG) PLGA NPs (Fig. 1a and b). As shown in Fig. 1a, the highest titer of IgG1 ($p < 0.05$) was generated in sera of mice vaccinated by chimera-loaded NPs with CpG via SC administration. Fig. 1b revealed that the level of IgG2a was higher ($p < 0.05$) in PLGA formulations compared to other vaccines by nasal or SC injection. The lower amounts of IgG1 and IgG2a was found in inoculated mice with chimera solution or chimera+CpG, after SC or intranasal vaccination. Moreover, no significant difference ($p > 0.05$) was observed between the mentioned groups. The titers of IgG1 and IgG2a antibodies were higher ($p < 0.05$) in the (chimera+CpG) PLGA group than the chimera+CpG.

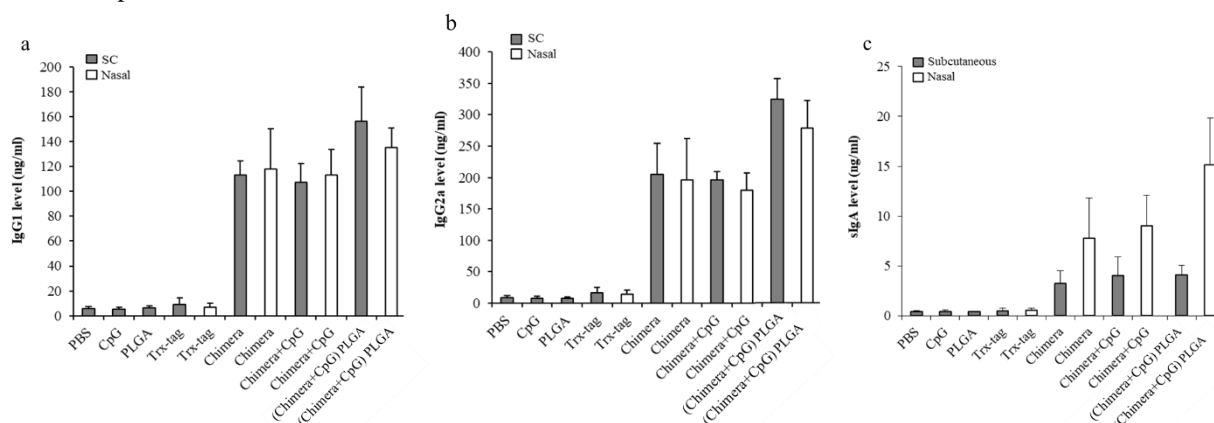


Fig. 1. The titers of antibody production in vaccinated BALB/c mice followed by nasal or SC injection. Sera or nasal wash samples of animals were assessed for identification of the titers of IgG1 (a), IgG2a (b), and sIgA (c) by ELISA and data presented as the mean ($n=6$) \pm SD.

Based on our results, chimera-loaded PLGA with CpG adjuvant skewed Th1/Th2 responses toward cellular and mucosal immunity. Furthermore, the co-administration of chimera and CpG ODN elicited significantly ($p < 0.05$) higher immune responses against HTLV-1, relative to the chimera solution, or incorporation of CpG and chimera vaccine. The predominantly higher IgG2a levels determined the induction of strong cell-mediated immune responses by chimera-loaded PLGA with CpG adjuvant. The high secretion of sIgA antibody in the nasally immunized groups revealed strong mucosal immune responses compared to SC injection. Our findings revealed that the nasal delivery of nanoparticle formulations, could pass through nasal-associated lymphoid tissue (NALT) in the respiratory tract and elevated transmucosal transport to evoke mucosal immune response. The co-delivery of antigen and adjuvant by the NPs gives them particulate entity and results in more uptake and better immune reaction [6]. In this study, the vaccines without PLGA NPs failed to induce potent cellular and mucosal immune responses. In contrast, co-encapsulation of chimera and CpG adjuvant into PLGA NPs stimulated the high titer of sIgA antibody in mice immunized by nasal administration. The mucoadhesive potential of PLGA NPs enhanced their maintenance in the nasal cavity to interact efficiently with NALT M cells and other immune cells [6,7].

Conclusions

Co-delivery of the multi-epitope vaccine and CpG ODN adjuvant by PLGA NPs could significantly induce Th1-biased responses and mucosal immunity in a mice model. The current study proved the significance of chimera and CpG co-delivery by PLGA nanospheres to evoke robust immune responses, which can be performed as a proper candidate to construct a prophylactic or therapeutic vaccine against HTLV-1.

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