

Nasal and subcutaneous administration of recombinant vaccine loaded in PLGA nanoparticles: Cytokine responses against HTLV-1

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Abstract

The HTLV-1 fusion epitope containing Tax, env, and gag epitopes was encapsulated in PLGA nanoparticles (NPs) to assess the immune efficacy of various formulations in an animal model followed by subcutaneous or nasal administration. PLGA NPs fabricated by a double emulsion method had a size less than 200 nm and the encapsulation efficiency of chimera antigen was 85%. The release profile of radiolabeled chimera indicated that 17.4% of chimera were released from PLGA NPs within one month. Encapsulation of chimera in PLGA NPs could considerably elicit cell-mediated responses in comparison to other vaccines. The PLGA formulations significantly increased secretion of IL-10, and IFN- γ cytokines and also decreased the levels of TGF- β 1 production compared to the other vaccines. The present investigation highlights the significance of chimera delivery by PLGA nanospheres to elicit robust Th1 and mucosal responses, which may be utilized as an appropriate candidate to develop the HTLV-1 vaccine.

Keywords: Recombinant vaccine, PLGA, Cellular response, Cytokine assay, HTLV-1.

Introduction

Human T-cell leukemia/Lymphoma virus type 1 (HTLV-1) is estimated to infect approximately 25 million people worldwide. The major HTLV-1 diseases are adult T-cell leukemia (ATL) and tropical spastic paraparesis (TSP/HAM), as well as other chronic inflammatory diseases [1]. The HTLV-1 genome of 9032 nucleotides contains the gag, pol, env genes, and pX region, that pX encodes Tax and Rex proteins, which are crucial for viral replication, and regulation of mRNA splicing, respectively. Indeed, the structural proteins of HTLV-1 are encoded by gag and env genes. Gag proteins are sufficient to assemble and release virus particles, while pol proteins are required for transcription and protease functions. Additionally, envelope glycoproteins comprising gp21 and gp46 subunits are exposed at the HTLV-1 surface and infected host cells to access the immune system of the host during the infectious process and stimulate systemic immune responses in HTLV-1 infected individuals. The stability of the HTLV-1 genome provides a promising approach to design and construct an effective vaccine against HTLV-1 [2,3]. The biodegradable, biocompatible, and non-toxic PLGA NPs have been approved by the FDA as the controlled release delivery system [4]. The potent adjuvant effect of PLGA nanospheres was confirmed by various studies using a model antigen such as tetanus toxoid or diphtheria, which is associated with their ability to be effectively taken up by APCs. The small size and large surface area of polymeric particles are significant factors in determining the rate of antigen release and the type of immunity. According to previous investigations, the PLGA NPs with a size of about 200 nm, are optimal to interact with DCs and induce a strong cellular immune response [5,6]. In the present study, the immunogenicity of HTLV-1 fusion epitope-loaded PLGA nanoparticles (NPs) was assessed in a mice model using subcutaneous (SC) or nasal inoculation.

Results Discussion

The recombinant peptide vaccine comprising Tax, env, and gag immunodominant HTLV-1 epitopes was loaded into PLGA NPs to evoke immune responses against HTLV-1. The spherical structure and smooth surface of PLGA NPs were confirmed by the scanning electron microscopy (SEM) technique. According to the results of dynamic light scattering, the mean diameter of the PLGA formulation was 186.5 ± 27.1 nm with a polydispersity index (PDI) less than 0.3. Surface charge of the formulation was -36.3 ± 5.7 mV and the loading efficiency of the chimera encapsulated in PLGA NPs was 85.2%, in the present study. Based on the release profile, only 17.4% of chimera were released from PLGA nanospheres during one month. The amounts of various cytokines (IFN- γ , IL-10, IL-4, and TGF- β 1) were determined in immunized mice with different formulations after SC or intranasal administration. The titer of IFN- γ , IL-10, and IL-4 ($p < 0.05$) was lower in PBS buffer solution; blank PLGA NPs, and Trx-tag than the test groups containing chimera solution, and (chimera) PLGA. As shown in Fig. 1a, a higher amount of IFN- γ secretion ($p < 0.001$) was observed in animals inoculated by (chimera) in comparison with vaccines without PLGA NPs. Additionally, no significant ($p > 0.05$) difference was found between subcutaneous and intranasal vaccination. The highest level of IL-4 ($p < 0.05$) was determined in the groups that received (chimera) PLGA through SC injection followed by chimera formulations (Fig. 1b). Fig. 1b indicated that the concentration of IL-4 was low (< 11.5 pg/ml) in immunized groups and also was lower than phytohaemagglutinin (PHA)-stimulated lymphocytes ($p < 0.05$). The secretion of IL-10

production was higher ($p < 0.001$) in groups inoculated with PLGA formulations than in the multi-epitope chimera vaccine via intranasal or SC injection (Fig. 1c). The level of TGF- β 1 production was significantly low ($p < 0.001$) in groups received (chimera) PLGA formulations (Fig. 6d). The level of TGF- β 1 was significantly ($p < 0.001$) high in chimera solution with no difference ($p < 0.01$) between intranasal and SC vaccination.

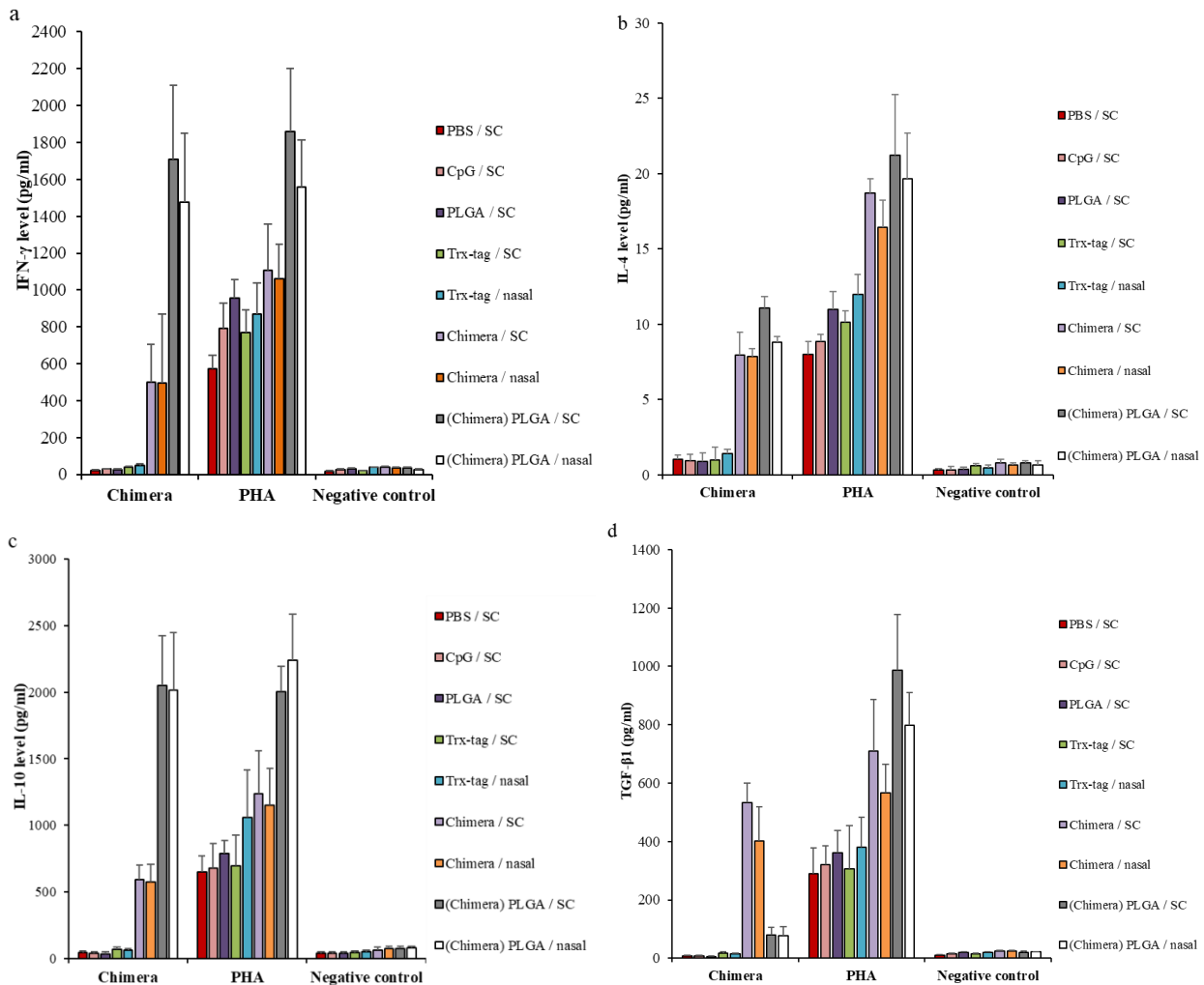


Fig. 1. Cytokine secretions in inoculated animals with vaccine formulations through nasal or SC injection. The levels of IFN- γ (a), IL-4 (b), IFN- γ /IL-4 ratio (c), IL-10 (d), and TGF- β 1 (e), were detected via ELISA and the values presented as the mean ($n=6$) \pm SD.

Based on the cytokine assays, the chimera-loaded PLGA NPs was efficient in secreting IFN- γ and IL-10 as well as low levels of IL-4 and TGF- β 1 cytokines in immunized mice by SC or intranasal administration. According to the potential of PLGA NPs in the localization of adjuvant, the PLGA formulations containing CpG elicited a high production of Th1 cytokines, relative to the co-administration of CpG and chimera. The levels of IFN- γ were 499.83 ± 205.41 and 497.34 ± 273.56 (pg/ml) in mice inoculated by multi-epitope chimera through intranasal or SC injection, in contrast to 1726.62 ± 431.72 and 1475.80 ± 383.08 (pg/ml) for the (chimera) PLGA, respectively. Our results revealed that the delivery of chimera by NPs was capable of elevating the immunogenicity of a chimera to induce robust cell-mediated immunity against HTLV-1. The low IL-4 titers generated by Th2 cells indicated the predominant Th1 responses in all immunized mice with various vaccines. Moreover, the high IFN- γ /IL-4 ratio confirmed that PLGA NPs vaccines effectively primed Th1-skewed immunity and suppressed Th2 responses. Indeed, the efficient vaccine can promote IFN- γ production by the activation of Th1 cells that permit noncytolytic inhibition and clearance of intracellular pathogens. On the other hand, the function of Th1 cells could be regulated by secreting IL-10 as an immunoregulatory cytokine to avoid autoimmune states and inflammatory diseases. The anti-inflammatory cytokine IL-10 is produced by different types of immune cells such as Th cells, DCs, macrophages, B cells, etc. The CD4+Th1 subset induces the IFN- γ and IL-10 secretion to autoregulate immune responses [7,8]. Additionally, the high concentration of IL-10 (100 ng/ml) might downregulate the secretion of proinflammatory cytokine IFN- γ in infected patients with HTLV-1 [9]. Based on our results, the (chimera) PLGA formulation promoted the secretion of IFN- γ and IL-10 responses to elicit a potent Th1 response, anti-inflammatory processes and could help HTLV-1 clearance. Transforming growth factor β 1 cytokine has an immunosuppressive property to prevent the proliferation and

differentiation of Th1, Th2, and B cells to inhibit the generation of various cytokines and innate immune system cells, leading to inflammatory activity and improving disease progress [10].

Conclusions

Encapsulation of chimera in PLGA NPs could considerably elicit cell-mediated responses in an animal model. The present study highlights the significance of chimera delivery by PLGA nanospheres to elicit robust Th1 responses, which may be utilized as an appropriate candidate to develop the HTLV-1 vaccine. Therefore, the proper design of vaccine formulation and immunization strategy are crucial factors to construct an efficient vaccine.

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