

Antibody responses against HTLV-1 using nasal and subcutaneous administration of recombinant vaccine loaded in PLGA nanoparticles

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Abstract

The mucosal and systemic immunity of HTLV-1 fusion epitope-loaded PLGA nanoparticles (NPs) was evaluated in a mice model. For this purpose, the multi-epitope chimera including Tax, env, and gag immunodominant HTLV-1 epitopes was encapsulated in biodegradable PLGA NPs. PLGA nanospheres produced by a double emulsion method had a size of < 200 nm and the encapsulation efficiency of chimera antigen was 85%. The release profile of radiolabeled chimera indicated that only 17.4% of chimera were released from PLGA NPs during one month. The PLGA formulations significantly elevated titers of IgG1, IgG2a, and sIgA antibodies relative to the other vaccines. These results revealed that the sustained release of chimera from PLGA as an efficient polymeric system elicited potent cell-mediated and mucosal immunity without inflammatory responses against HTLV-1. Therefore, the appropriate design of vaccine formulation and immunization strategy are crucial factors to construct an efficient vaccine.

Keywords: Recombinant vaccine, PLGA, Mucosal immunity, Cellular response, Antibody assay, HTLV-1.

Introduction

Human T-cell leukemia/Lymphoma virus type 1 (HTLV-1) as an oncogenic retrovirus, is estimated to infect between 15 and 25 million people worldwide. The HTLV-1 infection is endemic in many areas of the world, including Japan, Africa, the Caribbean islands, Central and South America, and some parts of the Middle East such as Iran. The major HTLV-1 diseases are adult T-cell leukemia (ATL) and tropical spastic paraparesis (TSP/HAM), as well as other chronic inflammatory diseases [1–2]. Synthetic peptide-based vaccines have been developed to evoke immune responses with various advantages in comparison to conventional vaccine formulations. Peptide vaccines are produced safely and efficiently to induce cellular and humoral immunity and are chemically stable without oncogenic potential [3]. Nanoparticle-based delivery systems have been developed to elevate the stability, immunogenicity, and intracellular uptake of protein antigens. Among the vaccine delivery systems, polymeric nanoparticles (NPs) such as PLGA, are commonly used to protect peptide vaccines from degradation and facilitate antigen uptake by APCs, to induce robust T-cell immune responses. The biodegradable, biocompatible, and non-toxic PLGA NPs have been approved by the FDA as the controlled release delivery system [4,5]. In the present study, the HTLV-1 fusion epitope was encapsulated in PLGA NPs to assess the immune efficacy of various formulations in an animal model followed by subcutaneous (SC) or nasal administration.

Results Discussion

The fusion peptide comprising immunodominant HTLV-1 epitopes was encapsulated into PLGA NPs to promote immunity against HTLV-1. The spherical structure and smooth surface of PLGA NPs were displayed in Fig. 1, using the scanning electron microscopy technique. The mean intensity diameter of the mentioned vaccine was 186.5 ± 27.1 nm with a polydispersity index below 0.3, based on dynamic light scattering. Surface charge of the formulation was -36.3 ± 5.7 mV and the loading efficiency of (chimera) PLGA was calculated 85.2%, in the present study. The release of chimera from PLGA NPs was slow and only 17.4% of chimera were released during one month, respectively.

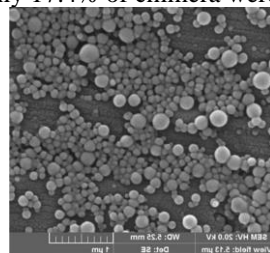


Fig. 1. The surface morphology of PLGA NPs visualized by scanning electron microscope to determine the spherical structure of nanospheres.

The titers of IgG1, IgG2a, and sIgA antibodies were measured by ELISA to identify the type of immunity induced in vaccinated mice by various formulations (Fig. 2). The amounts of IgG1 and IgG2a antibodies were significantly ($p < 0.001$) lower in all control groups such as PBS; blank PLGA NPs; and Trx-tag protein, in comparison to vaccination with test groups including chimera solution; and (chimera) PLGA (Fig. 2a and b). As demonstrated in Fig. 2a, the highest titer of IgG1 ($p < 0.05$) was produced in sera of mice vaccinated by chimera-loaded NPs via SC injection. Fig. 5b indicated that the level of IgG2a subclass was higher ($p < 0.05$) in PLGA formulations compared to vaccines without PLGA NPs by nasal or SC injection. The lower amounts of IgG1 and IgG2a were observed in inoculated mice with chimera solution. Moreover, there was no significant difference ($p > 0.05$) between vaccinated mice by the subcutaneous and nasal routes in all test groups (Fig. 2a and b). The titers of sIgA were higher ($p < 0.05$) in the nasal wash samples of animals inoculated with (chimera) PLGA than the formulations without PLGA NPs via the intranasal route (Fig. 2c). The lowest sIgA titers ($p < 0.05$) were found in SC groups. Additionally, the titers of the sIgA antibody were higher ($p < 0.05$) in all test groups vaccinated through the nasal route compared with control groups. Based on our results, chimera-loaded PLGA NPs skewed Th1/Th2 responses toward cellular and mucosal immunity, with no difference ($p > 0.05$) between the mentioned vaccines.

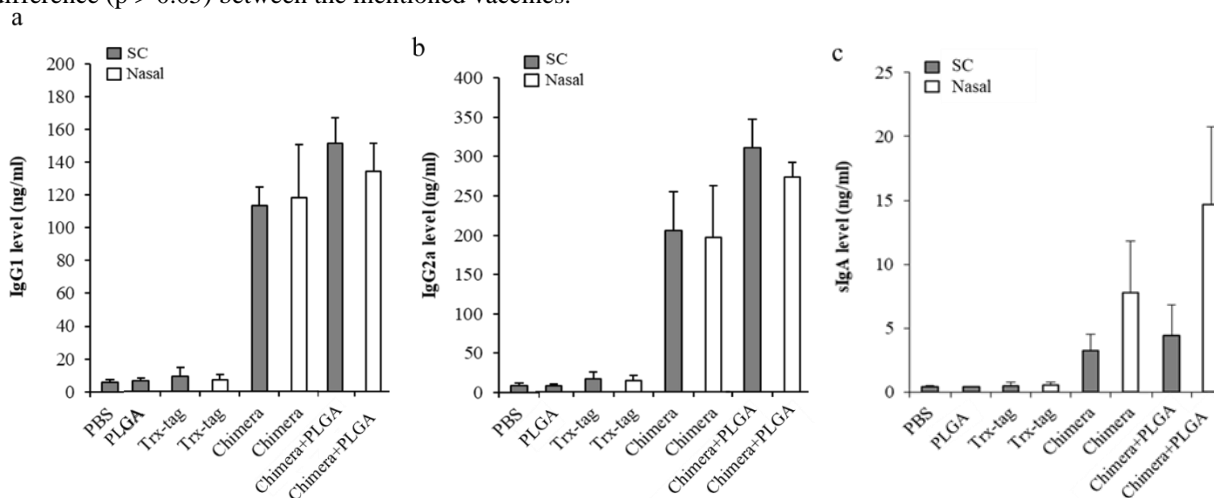


Fig. 2. The level of antibody production in vaccinated BALB/c mice followed by nasal or SC injection. Sera or nasal wash samples of animals were assessed for identification of the titers of IgG1 (a), IgG2a (b), and sIgA (c) by ELISA and data presented as the mean ($n=6$) \pm SD.

The development of effective vaccines is needed to evoke proper immune responses without inflammatory reactions. The vaccines based on peptide or protein subunits have many benefits such as chemical stability and safety without oncogenic properties compared to other vaccines [3]. The present study describes the encapsulation of chimera in PLGA NPs with or without CpG ODN to improve immunity by inducing antigen-specific antibody responses and cytokine productions against HTLV-1. The various vaccine formulations were applied using the nasal or subcutaneous injection in an animal model. According to the antibody assays, the chimera-loaded NPs in the absence or presence of CpG elicited the highest titer of IgG1 and IgG2a in vaccinated mice by intranasal or SC routes. The predominantly higher IgG2a levels determined the induction of strong cell-mediated immune responses by mentioned formulations. Additionally, the high IgG2a/IgG1 ratio illustrated the Th1/Th2 responses toward Th1 immunity in test groups inoculated by nasal or SC injection. The high secretion of sIgA antibody in the nasally immunized groups demonstrated robust mucosal immune responses relative to subcutaneous injection. These results 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 augment mucosal immune response. It has been shown that particulate antigen is better uptaken by microfold (M) cells located in the NALT. 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]. Moreover, the low release of chimera from NPs led to retaining more antigen molecules in particulate form and elicited strong immunity against HTLV-1. Among the antigen delivery systems, PLGA NPs have been considered an efficient polymeric system with potent immunological adjuvant effect to evoke strong immune responses. Similar to the viruses, NPs with a size range between 20 and 200 nm can be quickly trapped by antigen-presenting cells and are efficiently uptaken by dendritic cells to stimulate strong effector T-cell responses during antigen processing and presentation. In contrast, macrophages strongly uptake the particles with a size of approximately 0.5–5 μ m, and the optimal size for DC uptake was determined below 300 nm [8]. Furthermore, the nanoparticles with spherical shapes were more efficient in the interaction with APCs to elicit antibody responses than other particle shapes [8,9]. Based on our results, the sphericity of the PLGA NPs formulated may play a useful role in their immunoadjuvant potential.

Conclusions

Taken together, although the subcutaneous injection of PLGA formulations elicited higher systemic immunity compared to the nasal route, no significant differences were found between intranasal and subcutaneous vaccination of formulations. Based on our study, the SC or nasal vaccination of PLGA formulations particularly nasal administration was efficient to induce potent immune responses against HTLV-1.



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