Vaccine 32 (2014) 1670–1677

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Vaccination with a *piggyBac* plasmid with transgene integration potential leads to sustained antigen expression and CD8$^+$ T cell responses

Pietro Bertino[a,*], Johann Urschitz[b], FuKun W. Hoffmann[a], Bo Ra You[c], Aaron H. Rose[a], Woo Hyun Park[c], Stefan Moisyadi[b,d], Peter R. Hoffmann[a]

[a] Department of Cell and Molecular Biology, John A. Burns School of Medicine, University of Hawaii, Honolulu, HI, USA
[b] Department of Anatomy, Biochemistry and Physiology, John A. Burns School of Medicine, University of Hawaii, Honolulu, HI, USA
[c] Department of Physiology, Medical School, Chonbuk National University, Jeonju, South Korea
[d] Manoa BioSciences, Honolulu, HI 96822, USA

ARTICLE INFO

Article history:
Received 10 October 2013
Received in revised form 20 January 2014
Accepted 22 January 2014
Available online 7 February 2014

Keywords:
DNA vaccine
Cell mediated immunity
Tumor
Cancer
piggyBac
Transposase

ABSTRACT

DNA vaccination with plasmid has conventionally involved vectors designed for transient expression of antigens in injected tissues. Next generation plasmids are being developed for site-directed integration of transgenes into safe sites in host genomes and may provide an innovative approach for stable and sustained expression of antigens for vaccination. The goal of this study was to evaluate in vivo antigen expression and the generation of cell mediated immunity in mice injected with a non-integrating plasmid compared to a plasmid with integrating potential. Hyperactive *piggyBac* transposase-based integrating vectors (p*mhy*GENIE-3) contained a transgene encoding either eGFP (p*mhy*GENIE-3-eGFP) or luciferase (p*mhy*GENIE-3-GL3), and were compared to transposase-deficient plasmids with the same transgene and DNA backbone. Both non-integrating and integrating plasmids were equivalent at day 1 for protein expression at the site of injection. While protein expression from the non-integrating plasmid was lost by day 14, the p*mhy*GENIE-3 was found to exhibit sustained protein expression up to 28 days post-injection. Vaccination with p*mhy*GENIE-3-eGFP resulted in a robust CD8$^+$ T cell response that was three-fold higher than that of non-integrating plasmid vaccinations. Additionally we observed in splenocyte restimulation experiments that only the vaccination with p*mhy*GENIE-3-eGFP was characterized by IFNγ producing CD8$^+$ T cells. Overall, these findings suggest that plasmids designed to direct integration of transgenes into the host genome are a promising approach for designing DNA vaccines. Robust cell mediated CD8$^+$ T cell responses generated using integrating plasmids may provide effective, sustained protection against intracellular pathogens or tumor antigens.

1. Introduction

DNA vaccination has emerged as a potentially effective means of inoculating a host with one or more genes encoding immunogenic proteins from pathogens or tumors [1–3]. Once the desired genes are introduced into the host cells and gain access to the transcriptional and translational machinery, production of the proteins within the cells allows processing into epitopes and presentation by major histocompatibility complex I and II (MHC-I and -II). By utilizing the host cell to generate relatively high levels of target proteins, a robust cell mediated immune response involving CD8$^+$ T cells specific for those particular proteins can be generated. These stimulated effector cells will then recognize and attack cells infected with intracellular pathogens or cancer cells overexpressing certain tumor antigens while ignoring healthy cells [4]. It remains unclear whether the immune system will also eliminate the cells with integrated transgenes. The specificity and nonreplicating properties of DNA vaccines may offer strategic advantages over traditional vaccine approaches such as attenuated live microbes, inactivated pathogens, or purified proteins. While these traditional approaches for developing vaccines have succeeded for conferring protection against many pathogens, new approaches are likely needed for other pathogens such as HIV-1 or for tumor vaccines.

Plasmids used to deliver genes for vaccination have conventionally consisted of non-integrating DNA vectors due to the potential hazards of insertional mutagenesis and unknown positional effects for vectors that integrate randomly [5]. However, the design of safely integrating plasmids involve user-defined directed integration that, if successful, would improve the safety

Abbreviations: eGFP, enhanced green fluorescent protein; Luc, luciferase.

* Corresponding author. Tel.: +1 808 692 1734; fax: +1 808 692 1970.
E-mail address: pbertino@hawaii.edu (P. Bertino).

0264-410X/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.vaccine.2014.01.063

of insertional therapies. While current transposon vector systems are semi-random at best, the goal is to develop plasmids that are designed to insert into loci that are well-suited for gene transfer and defined as genomic safe harbors. Integrations within these sites would thus not be associated with adverse effects such as proto-oncogene activation or tumor suppressor inactivation [6,7]. For example, user-defined directed transposition to the CCR5 genomic safe harbor was demonstrated in ex vivo cells and single-copy clones harboring targeted integrations were isolated [8]. Integrating plasmids utilize transposons as mobile genetic elements that are capable of self-directed excision and subsequent reintegration within the host genome. Transposases play a key role in the use of these plasmids due to their capacity to recognize and bind to the inverted repeat elements flanking transposons, cut this DNA segment from the donor and reinsert it to the recipient genome. Transposase elements such as *piggyBac*, *Sleeping Beauty* and *Tol2* catalyze these reactions and have shown potential as tools for the stable integration of transgenes when used in the binary plasmid mode [9]. Recent modifications to the transposase and/or the terminal repeats of the transposon have increased their integration efficiency, and/or specificity in ex vivo cell systems, but have not yet achieved the ultimate goal of safe harbor integration in vivo [10–12]. Transgene transmittance to daughter cells was shown in ex vivo cell systems [8], but questions remain regarding the sustained expression of antigen would be achieved in vivo or if antigen expressing cells would be targeted for elimination by the immune system.

If safe integration of desired genes into the host genome can be achieved using these engineered plasmids, they may serve as an invaluable tool for gene delivery in applications such as combating genetic disease, cancer therapy, or vaccination. Standard plasmids containing CMV promoter-driven antigen expression have in some cases demonstrated the ability to generate expression in some tissues for extended periods, but the goal is to improve expression to more consistently sustained levels that lead to stronger immune responses. New approaches involving minicircle DNA for more sustained transgene expression have led to more effective $CD8^+$ T cell responses [13]. Also, the magnitude and the contraction phase of the $CD8^+$ T cell response following intradermal DNA immunization was shown to be regulated by the duration rather than the initial exposure to antigen [14]. Cytomegalovirus (CMV) infection, even with a strain limited to a single cycle, drives an inflation of $CD8^+$ T cell memory [15], and the development of CMV plasmids delivered intramuscularly have shown sustained expression and may prove to be an effective vaccine vector. The use of plasmids containing the *piggyBac* transposase for vaccines has not been thoroughly investigated. In fact, it is unclear whether vaccination with a plasmid that promotes the stable integration of a gene encoding an immunogenic protein provides stronger cell mediated immunity compared to comparable non-integrating plasmids. In this study, we set out to compare p*mhy*GENIE-3 plasmid, which has integrating potential, to a non-integrating p*mhy*GENIE-3-ΔPB plasmid for their ability to promote sustained GFP and luciferase protein expression and generate $CD8^+$ T cell responses in mice. The results of this study will provide the framework for developing safe integrating plasmids as effective vaccine modalities.

2. Materials and methods

2.1. Mice

Female BALB/c mice at 4–8 weeks of age were purchased from Charles River (Hollister, CA, USA) and maintained in a specific pathogen-free animal facility for at least 1 week before experiments. Procedures were performed in accordance with institutional guidelines approved by University of Hawaii Institutional Animal Care and Use Committee (IACUC).

2.2. Plasmids and lipids

Liposomes containing DOTAP and DOPE in a 1:1 molar ratio were prepared as follows. The lipid mixture in chloroform was dried under a stream of nitrogen as a thin layer in a 10-mL round-bottomed tube. The lipid film was hydrated in PBS to give a final concentration of 10 mg/mL. The multilamellar vesicles obtained were then sonicated with Avanti sonicator (Avanti Polar Lipids, Alabaster, AL, USA) and incubated for 10 min with plasmid DNA before subcutaneous (s.c.) injection of 20 μg each of

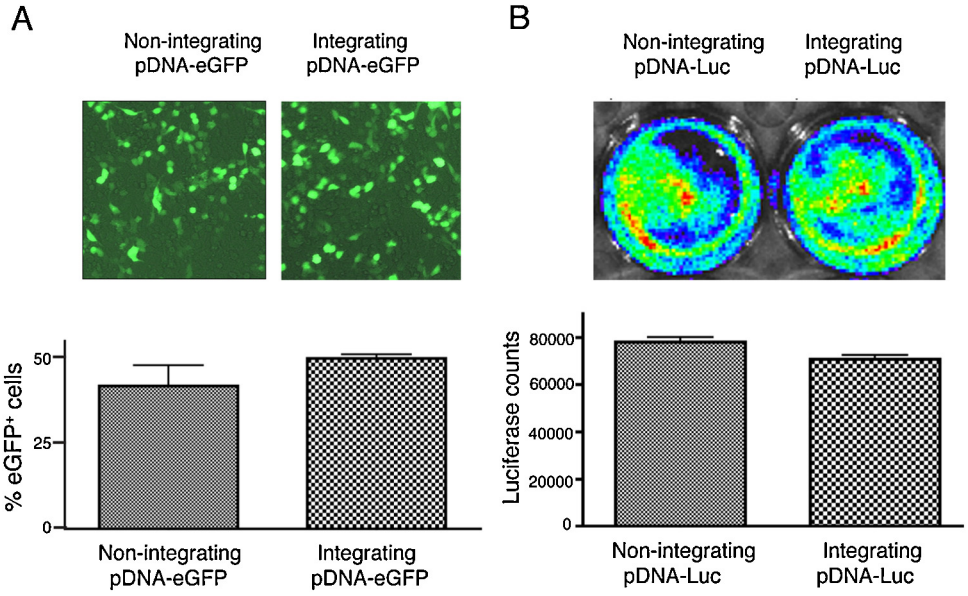

Fig. 1. p*mhy*GENIE-3 and p*mhy*GENIE-3-ΔPB show equivalent in transfection efficiency and protein expression. HEK293 (1×10^6) cells were transfected with 2 μg of each plasmid, (A) p*mhy*GENIE-3-eGFP and p*mhy*GENIE-3-ΔPB-eGFP or (B) p*mhy*GENIE-3-GL3 and p*mhy*GENIE-3-ΔPB-GL3. Cells were monitored over time for percent cells with eGFP signal or luciferase activity as described in Section 2. Data are shown for 48 h post-transfection and represent mean + S.E.M. ($N = 3$), means were compared using a Student's *t*-test with significance at $^* P < 0.05$. In both cases, no significant differences were found. Results are representative of two independent experiments.