Biologicals 46 (2017) 6–10

Contents lists available at ScienceDirect

Biologicals

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

Improvement influenza HA2 DNA vaccine cellular and humoral immune responses with Mx bio adjuvant

Sina Soleimani [a, b], Shahla Shahsavandi [b, *], Omid Maddadgar [a]

[a] Faculty of Veterinary Medicine, Tehran University, P.O. Box 14155-6453, Tehran, Iran
[b] Razi Vaccine & Serum Research Institute, Agricultural Research Education and Extension Organization, P.O. Box 31975-148, Karaj, Iran

ARTICLE INFO

Article history:
Received 2 August 2016
Received in revised form
29 November 2016
Accepted 29 November 2016
Available online 24 December 2016

Keywords:
Influenza
Immune responses
HA2 subunit
Mx
Bio adjuvant

ABSTRACT

Immunization with DNA vaccines as a novel alternative to conventional vaccination strategy requires adjuvant for improving vaccine efficacy. The conserved immunogenic HA2 subunit, which harbors neutralizing epitopes is a promising vaccine candidate against influenza viruses. In this study, for the first time we explore the idea of using host interferon inducible Mx protein to increase the immunogenicity of HA2 H9N2 influenza DNA vaccine. The potency and safety of the Mx adjuvanted-HA2 vaccine was evaluated in BALB/c mice by different prime-boost strategies. To assess the effect of the vaccination on the virus clearance rate, mice were challenged with homologous influenza virus. Administration of the adjuvanted vaccine and boosting with the same regimen could effectively enhance both humoral and cellular immune responses in treated mice. These data demonstrated that Mx as host defense peptide can be potentiated for improving influenza vaccine efficacy.

1. Introduction

Antibody response plays an important role in protection against influenza virus infection. The newly emerging antigenic variants that contain antigenic variations may evade from existing antibody responses against influenza viruses. The antigenic properties are specially determined by the surface glycoprotein, hemagglutinin (HA), which comprises major neutralizing epitopes. HA-encoded DNA vaccine induces broader antibody and cytotoxic T lymphocytes (CTL) responses in animal models [1–4]. From previous decades development of influenza DNA vaccines has been of interest because their significant potential to induce both humoral and cellular immune responses. Dendritic cells transport viral antigens into the lymph nodes, where naïve T cells are converted into influenza antigen-reactive $CD8^+$ or $CD4^+$ CTLs [5,6]. Increase in the level of $CD8^+$ CTL and the cellular immune response lead to effective immunity to influenza viruses provided by virus-coding DNA vaccines including HA, NP, M especially M2e, and NA antigens [7–11]. Among the viral antigen, HA is considered as most (or highly) immunogenic. The HA0 precursor is synthesized as a single peptide which is cleaved into HA1 and HA2 by specific host protease and which mediate virus-host membrane fusion following the cleavage activation. Compared with the occurrence of antigenic drifts in HA1 globular head, HA2 subunit is highly conserved [12,13]. Neutralizing antibodies recognize stalk domain of HA2 subunit which elicit sufficient protection against infection and cross-react with the HA of other subtypes [2–4,14]. However, in spite of ability of influenza DNA vaccines to raise CTL response and induce protection in animal models the vaccines have not been widely approved indicating that the choices of adjuvant may need more considerations [15]. Generally the main mechanism underlying adjuvant actions is immune cell recruitment. Adjuvant induces responses which mimic the natural infection and provides long-term protection [5,15,16]. The components act by diverse mechanisms which include enhancing the delivery of antigen, improving magnitude of the immune response, directing antigen presentation by the major histocompatibility complex or providing immune stimulatory signals that potentiate the immune response [6]. Because of post vaccination side effects with synthetic adjuvants, novel strategy is under evaluation which includes use of biological proteins such as cytokines, bacterial derivatives, host defense peptides and immune system regulator proteins to improve immunogenicity of vaccines [14,17–19]. The experimental approaches clearly support that formulation of influenza vaccine with biological adjuvant may be an alternative way to obtain improved protection against the viruses. These adjuvants safely

* Corresponding author.
E-mail address: s.shahsavandi@rvsri.ac.ir (S. Shahsavandi).

http://dx.doi.org/10.1016/j.biologicals.2016.11.004
1045-1056/

induce immune response that can effectively deal with its target antigen. In our previous studies we evaluate candidate Mx protein, a member of dynamin-like large guanosine triphosphatases (GTPases), which are involved in induction of interferon and regulation of immune system required for eliciting immune responses against influenza virus [20,21]. In this study the induction of immune responses following administration of Mx adjuvanted-HA2 DNA vaccine with different DNA prime- DNA boost strategies was evaluated in mice.

2. Materials and methods

2.1. HA2 DNA vaccine and Mx adjuvant construction

Datasets of HA2 nucleotide H9N2 influenza subtypes from their emergence to 2014 were derived from the NCBI database and aligned using ClustalW with default parameters. The conserved HA2 sequence with 571 bp in length was determined by BioEdit program. Viral RNA was extracted from a H9N2 virus (JX456181.1) by Ribospin™ (GeneAll, South Korea). The coding region was then amplified with the designed primer, HA2F: 5′-GGATCC-CATGGCTGCAGATAGGGATA-3′ and HA2R: 5′-CCATGGTTATATA-CAAATGTTGCACCT-3′ carrying the appropriate restriction enzyme sites and cloned into the pcDNA3.1 vector (Invitrogen, USA) between the *Bam*HI and *Nco*I sites. Plasmid DNA was propagated in *Escherichia coli* and purified using EndoFree®Plasmid Mega Kit (Qiagen, Germany) according to the manufacturer's instructions. Concentration of the purified plasmid was adjusted to 1 μg/μl for immunization trials. All Mx sequences from *Homo sapiens*, *Mus musculus*, and *Gallus gallus* were downloaded from the NCBI database and aligned by the ClustalW algorithm. Based on our *in silico* study [20], a conserved sequence encode the motif 13SGKSSVLEALSGVALPR30 in interferon induced domain revealed the better results in inducing B-cell and T-cell immune responses against HA2 H9N2 influenza viruses. Thus the primers MxF: 5′-CCATGGGATTGCGGTGATTGGCGA -3′and MxR: 5′-GGGCAGCGGGTCACAATGGAATC-3′ were designed so that the fragment can be amplified. Cloning procedure was performed as described in this section. The correct sequence of the each cassette within pcDNA3.1 was confirmed by sequencing.

2.2. Immunizations with HA2 DNA vaccine and Mx construct

For the immunization studies, the HA2 DNA vaccine to Mx adjuvant ratio was estimated to be 7:1 based on DNA mass following previous dose finding study. The formulated mixture was named HA2/Mx adjuvanted vaccine. For immunization, seventy female BALB/c mice 7 weeks of age divided in seven groups comprising of four treatment and three control groups which were kept in separate cages. The treatment groups received HA2 DNA vaccine and Mx adjuvant in different prime-boost schedule as follows: group D: HA2/Mx, group E: prime with HA2/Mx and boost with Mx, group F: prime with HA2/Mx and boost with HA2 and group G: prime with HA2/Mx and boost with HA2/Mx. The control include group A received normal saline, group B received only Mx, and group C received only HA2 DNA vaccine. All animals were immunized intramuscularly in quadriceps muscle region and the boosts injected performed after 14 and 28 days after the primary dose. Sera were collected 3 days before injections and at 7, 14, 28, 42, 56 and 70 days post injection (pi). Two weeks after the last immunization, three mice of each group were challenged intranasally with 100 MID_{50} (mouse infectious doses) from homologous H9N2 virus (A/chicken/Iran SS7/2011) and residual lung virus titers evaluated. Four days later, the lung tissues of euthanized mice were collected, homogenized in PBS, and titrated for virus infectivity in MDCK cells. Titers were expressed as the mean $\log_{10}$ $TCID_{50}$/ml (tissue culture infectious dose) ± the standard deviation of three mice per group.

2.3. Immunogenicity evaluation

The antibody was determined by hemagglutination inhibition (HI) assay by standard methods using 4 HA unit of virus and 0.5% chicken red blood cells, and virus neutralization (VN) assay using live H9N2 influenza virus at a concentration of 10^2 $TCID_{50}$/ml. The neutralizing antibody titer was defined as the reciprocal of the highest serum dilution at which the infectivity of 100 $TCID_{50}$ of virus for MDCK cells was completely neutralized in 50% of the wells. Induction of cellular immune responses were evaluated using a 3-(4,5-dimethylthiazol-2-thiazolyl)-2,5-diphenyltetrazolium bromide, thiazolyl-blue (MTT) assay. The spleen from each mouse per group at 28 pi was processed as follows: in a 96-well flat-bottom plate, the splenocytes (1×10^6 cells per well) were stimulated with mitogen phytohemagglutinin A (PHA) at final concentration of 5 μg/ml 0.5 mg concanavalin A (ConA), 1.25 μg HA2. After 72 h incubation at 37 °C in a 5% CO_2 humid incubator, 20 μl MTT was added to each well and the plates were incubated for further 4 h. The supernatant from each well was carefully aspirated and formazan crystals were solubilized by addition of 100 μl dimethyl sulfoxide to each well and incubating plates for 30 min at 37 °C. The absorbance at a wavelength of 570 nm was measured and the values expressed as means ± standard error of the mean for three independent experiments. For T-cell induction studies results were indicated as a stimulation index (SI): the ratio of the average optical density (OD) value of wells containing antigen-stimulated cells to the average OD value of wells containing cells only and no antigen.

Protection from challenge with homologous virus after vaccination was examined according to the viral titers in lungs of infected mice. Lung tissues were aseptically extracted from euthanized mice (n = 3/group) and homogenized in minimal essential medium (MEM) supplemented with antibiotics. MDCK cells were seeded in 96-well cell culture plates and inoculated with each of ten-fold serial dilutions of lung homogenates. The culture procedure was continued for 48 h at 37 °C. The virus titer was calculated by the Reed and Muench method and expressed as the 50% $TCID_{50}$.

2.4. Safety evaluation

The HA2 DNA vaccine containing Mx adjuvant was tested for safety in the pre-clinical studies. The weight of individual mice was monitored weekly and mice were checked for any local reaction in the site of injection.

2.5. Statistical analysis

The data from immunized mice with those from control groups was analyzed by ANOVA (SPSS ver. 11).

3. Results

3.1. HA2 DNA vaccine and Mx adjuvant generation

The HA2 DNA vaccine and Mx adjuvant plasmids constructed in pcDNA3.1 and their correct sequences were confirmed by sequencing.

3.2. Immunogenicity evaluation

The ability of HA2 DNA vaccination, with and without Mx to