International Immunopharmacology 59 (2018) 1–11

Contents lists available at ScienceDirect

International Immunopharmacology

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

The mechanisms of Ag85A DNA vaccine activates RNA sensors through new signal transduction

Jingbo Zhai[a,b], Qiubo Wang[a], Yunfeng Gao[a], Ran Zhang[a], Shengjun Li[a], Bing Wei[a], Yong You[a], Xun Sun[a], Changlong Lu[a,b,*]

[a] Department of Immunology, China Medical University, Shenyang 110122, China
[b] Brucellosis Institute of Inner Mongolia University for the Nationalities, Tongliao 028000, China

ARTICLE INFO

Keywords:
DNA vaccines
Ag85A
RNA sensors
Signal transduction
Antigen presentation
Immunogenicity

ABSTRACT

Low immunogenicity is one of the major problems limiting the clinical use for DNA vaccines, which makes it impossible to obtain a strong protective immune response after vaccination. In order to explore whether Ag85A DNA vaccine could mount more efficiently protective immune response through new RNA sensor and its signal transduction pathway of antigen presentation we designed and synthesized Ag85A gene fragment containing multiple points mutations and transfected the gene fragment into the dendritic cell line (DC2.4) by CRISPR/Cas9. Subsequently, we focused on the changes of RNA sensors RIG-I, Mda-5, and the downstream adaptors MAVS, IRF3, IRF7 and IFN-β. The results indicated the significant increases in the mRNA and protein expression of RNA sensors RIG-I, Mda-5 and related adaptors MAVS, IRF3, IRF7, and IFN-β in the mutant DC 2.4 cells. The flow cytometry results demonstrated that the expression of MHC II on the surface of DC 2.4 significantly increased when compared with that in control. Therefore, it is suggested that Ag85A mutant DNA could release immunogenic message through RNA sensors and related adaptors via non protein pathway. There is at least one RNA signal transduction pathway of Ag85A DNA in DC2.4 cell. The work provides a new mode of action for nucleic acid vaccine to improve immunogenicity and meaningful data for the better understanding of the mechanisms of DNA vaccine.

1. Introduction

DNA vaccine has been widely studied with impressive development [1,2]. Previous studies on DNA vaccine development, immune response, immunological tolerance, and mechanisms of the related products have led to the application of DNA vaccines as one of the choices for treatment for animal diseases. However, the application of vaccines in humans remains limited due the complicity of mechanisms [3,4]. The limited application of DNA vaccines in the clinical trials or use is mainly attributed to low immunogenicity, which hinders strong and effective protective immune response after immunization.

Eukaryotes recognize bacteria, viruses, and other microorganisms by their innate immune system. The cell receptors involved in innate immune response recognize bacterial cell wall components, microbial nucleic acids, and conserved structures in highly conserved protein sequences. These conserved structures belong to pathogen-associated molecular patterns (PAMPs) [5] and are recognized by pattern recognition receptors (PRRs) [5]. Mutual recognition and interaction between PRRs and PAMPs are the key to the initiation of immune response. The discovered PRRs include the Toll-like receptor (TLR) [6] family, retinoic acid-induced gene I-like receptor (RLR) family [7], NOD-like receptor family [8], and C-type lectin receptor family [9]. The RLR family is the most important molecular family recognizing the broad-spectrum microorganism models in cells, including the retinoic acid-induced gene I (RIG-I) [10,11], melanoma differentiation-associated gene 5 (Mda-5), and laboratory of genetics and physiology 2 (LGP2). It was reported that RIG-I and Mda-5 signaling could induce activation of mitochondrial antiviral-signaling protein (MAVS) and oligomerization into prion-like aggregattion, which activates the TANK-binding kinase 1 (TBK1) and I kappa B kinase (IKK). These capabilities maximize the activation of NF-κB, IRF3, and IRF7, which can be transferred into the nucleus and are involved in inducing and coding the antiviral gene of Type I interferons (IFN-α and IFN-β). However so far there is no published work on whether DNA vaccination can activate

Abbreviations: DC, dendrite cells; BMDCs, bone-marrow dendrite cells; APC, antigen presenting cell; PRR, pattern recognition receptor; PAMPs, pathogen-associated molecular patterns; RIG-I, retinoic acid-inducible gene I; Mda-5, melanoma differentiation associated gene-5; MAVS, mitochondrial antiviral signaling; IRF3, interferon regulatory factor 3; IRF7, interferon regulatory factor 7; dsRBD, dsRNA binding domains; CARD, caspase activation and recruitment domain

* Corresponding author at: Department of Immunology, School of Basic Medical Science, China Medical University, Shenyang 110122, China.
E-mail address: cllu@cmu.edu.cn (C. Lu).

https://doi.org/10.1016/j.intimp.2017.11.041
Received 1 September 2017; Received in revised form 26 November 2017; Accepted 28 November 2017

the RNA sensors inside antigen-presenting cells and its related transduction pathway that presents antigenic information to T cells [12].

The (CRISPR)/Cas9 technique is derived from the immune system in bacteria. CRISPR is an immune mechanism caused by degrading invasive virus DNA or other exogenous DNA in bacteria. In bacteria and archaebacteria, the CRISPR system is divided into 3 types. Types I and III require numerous CRISPR-related Cas proteins to exert the effect and Type II requires only one protein [13–15]. The CRISPR/Cas9 system from Streptococcus pyogenes is a system used most widely [16]. Cas9 protein contains 2 nuclease domains that cut the 2 single strands of DNA. Cas9 can combine with crRNA and tracrRNA to form a complex and then invades the DNA through combining with a PAM sequence to form an RNA–DNA complex structure. Cas9 further breaks the target double-stranded DNA. Loading the components expressing sgRNA and Cas9 into a vector can obtain a plasmid expressing both genes at the same time. After being transfected into cells, the target genes can be manipulated. Since CRISPR/Cas system was first reported to modify the genome in mammalian cells, CRISPR/Cas9 has been successfully applied in bacteria, yeast, plants, fish, and mammalian cells and has been the most efficient genome-editing technique [17].

In our previous study, we found that after oral administration of the Ag85A DNA vaccine, various immune responses were induced in the local intestinal tract. Th1 type immune response was mainly located in the local intestinal tract, whereas Th2 type immune response was mainly found in the whole body, which was suggesting that various antigen presentation mechanisms existed in different systems. Reports similar to our results indicated that DCs in different tissues could induce different type of immune response when presenting the same antigen [18,19]. In a followed study we also found that when using the Ag85A DNA vaccine to transfect the DC2.4 cell strain in vitro, the mRNA of A g85A could be detected in instantaneous and stable transfection while Ag85 antigen expression could not be detected. However, the following occurrence suggested that there would be mechanisms of other antigen presentation: the higher levels CD80 and CD86 expression in DC cells bearing Ag85A, the upregulation of MHC II molecule, the increased number of inductive effector T-cells, the enhancement of cytotoxic activity, the increase in IFN-γ secretion and the therapeutic effect of DCs derived from the bone marrow transfected with the Ag85A vaccine on the mouse model for bladder cancer [20]. Similar to most reported DNA vaccines, our Ag85A DNA vaccine could not induce the complete specific immune protection. Therefore, the antigen presentation mechanism of the oral DNA vaccine in the intestinal tract is needed to be explored further.

In this study we designed and synthesized the Ag85A gene segment with multipoint mutation and knocked the mutational Ag85A gene segment into mROSA26 loci of DC 2.4 cell line by CRISPR/Cas9 technique [21–23] and knocked non-mutational Ag85A gene segment into DC 2.4 cell line as the control. We investigated RNA sensors RIG-I and Mda-5 (in the RLR family) and expression levels of downstream adaptors MAVS, IRF3, IRF7, and IFN-β in the related signal transduction pathway in the transfected cell line. We further explored the possible mechanisms of antigen presentation.

2. Materials and methods

2.1. Chemicals

Recombinant murine GM-CSF (Peprotech, USA) and recombinant murine IL-4 (Peprotech, USA); Antibodies used in this work included: Anti-MTB Ag85A Antibody (LS-C285789) was from Lifespan. anti-RIG-I (L-15)(sc-48931); anti-Mda-5 (H-61)(sc-134513); anti-MAVS (C-1)(sc-365333); anti-IRF3 (FL-425)(sc-9082); anti-IRF7 (H-246)(sc-9083); anti-IFN-β (M-17)(sc-17569). McAbs for flow cytometry analysis: anti-MHC class II (Y-Ae) PE (sc-32247 PE) and Goat anti-mouse IgG2a-HRP (sc-2061), which were all products of Santa cruz. IFN-β Elisa kit (ab24324, Abcam); CD80 Elisa kit (ab119578, Abcam); CD86 Elisa kit (ab171338, Abcam). Other chemicals frequently used in our laboratory were all from Sigma or Aldrich.

2.2. Mice and cells

Female C57BL/6 mice (4–6 weeks) were obtained from China Medical University. Mice were maintained in specific pathogen-free conditions and housed in a regulated environment (22 ± 1 °C, relative humidity 55 ± 5%) under a 12 h light/dark cycle. All mice were treated nicely according to the guidelines of the Animal Welfare Committee of China medical university and acclimatized for at least one week prior to the experiments, and all surgical procedures were approved by the committee of experimental animals of China medical university. At the time of experiment, animals were anesthetized with sodium thiamylal, 10 mg/kg, and then euthanized by dislocation of cervical vertebra.

DC2.4 was from China Medical University. *E. coli* competent cells DH5α (9057) and BL21 (9126) were from Takara.

2.3. Primary DCs induction

Bone marrow-derived dendritic cells (BMDCs) were induced from 4 to 6 week-old C57BL/6 mice. The cell suspension was prepared from femurs and tibias. After red blood cells were removed, the bone marrow

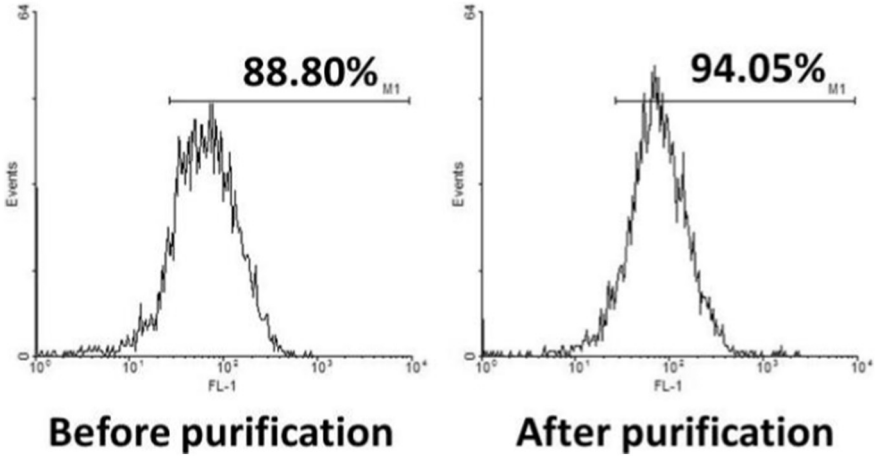

Fig. 1. Primary BMDCs after purification. $CD11c^+$ BMDCs were purified from bone-marrow cells with the method of sequential purification of $CD11c^+$ cells from the $CD11c^-$ fraction and the purity was > 94%.