Comparative Immunology, Microbiology and Infectious Diseases 34 (2011) 259–265

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

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

Avian CD154 enhances humoral and cellular immune responses induced by an adenovirus vector-based vaccine in chickens

Oliberto Sánchez Ramos[a,*,1], Alain González Pose[b,1], Silvia Gómez-Puerta[a], Julia Noda Gomez[c], Armando Vega Redondo[c], Julio César Águila Benites[b], Lester Suárez Amarán[e], Natalie C. Parra[d], Jorge R. Toledo Alonso[d]

[a] Department of Pharmacology, Faculty of Biological Sciences, University of Concepción, Chile
[b] Animal Biotechnology Department, Center for Genetic Engineering and Biotechnology (CIGB), P.O. Box 6162, Havana 10600, Cuba
[c] Department of Virology, National Center for Animal and Plant Health (CENSA), Apdo 10, San José de Las Lajas, Havana, Cuba
[d] Department of Physiopathology, Faculty of Biological Sciences, University of Concepción, Chile
[e] Laboratory of Gene Therapy of Viral Hepatitis, Division of Gene Therapy and Hepatology, Center for Applied Medical Research (CIMA), Pamplona, Navarra, Spain

ARTICLE INFO

Article history:
Received 13 March 2010
Received in revised form
18 November 2010
Accepted 29 November 2010

Keywords:
CD154
Influenza vaccine
Adenoviral vector
Immune response
Molecular adjuvant
H5N1

ABSTRACT

Recombinant adenoviral vectors have emerged as an attractive system for veterinary vaccines development. However, for poultry vaccination a very important criterion for an ideal vaccine is its low cost. The objective of this study was to test the ability of chicken CD154 to enhance the immunogenicity of an adenoviral vector-based vaccine against avian influenza virus in order to reduce the amount of antigen required to induce an effective immune response in avian. Chickens were vaccinated with three different doses of adenoviral vectors encoding either HA (AdHA), or HA fused to extracellular domain chicken's CD154 (AdHACD). Hemagglutination inhibition (HI) assay and relative quantification of IFN-γ showed that the adenoviral vector encoding for the chimeric antigen is able to elicit an improved humoral and cellular immune response, which demonstrated that CD154 can be used as a molecular adjuvant allowing to reduce in about 50-fold the amount of adenoviral vector vaccine required to induce an effective immune response.

1. Introduction

Recombinant adenoviruses (rAds) have emerged as a promising alternative to conventional veterinary vaccines. rAds have many advantages, such as: the ability to replicate at high titers in complementing cell lines, they can be administered orally and can infect dividing and nondividing cells. They are relatively cheap, easy to produce and can be used as a tool to discriminate vaccinated from infected animals [1]. The strong immunogenicity is a very important desired characteristic of adenovirus-based vaccines. rAds elicit an innate immune response which promotes the production of proinflammatory cytokines and the differentiation of immature dendritic cells (DCs) into professional antigen-presenting cells (APCs) [1]. They also trigger the activation of antigen-specific CD4 and CD8 T cells [2,3].

The ability of adenoviral vectors to induce an effective immune response against high pathogenic avian influenza virus (HPAIV) has been assessed in several previous studies [4–6]. Most of theses studies employed a human adenovirus serotype 5 vector containing the HA gene of a HPAIV and administered in doses between 1×10^8 and 5×10^8 plaque-forming units (PFU). These high doses allow overcome the inherent poor immunogenicity of HA molecule.

* Corresponding author at: Department of Pharmacology, Faculty of Biological Sciences, University of Concepción, P.O. Box 160C, Concepcion, Chile. Tel.: +56 41 2661191; fax: +56 41 2216558.
E-mail address: osanchez@udec.cl (O.S. Ramos).
[1] These authors contributed equally to this work.

0147-9571/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cimid.2010.12.001

However, from the productive point of view, these doses may constitute a drawback for the successful application of adenoviral vector-based vaccines in the agricultural sector for the control of HPAIV. An approach to avoid this limitation could be the development of more immunogenic molecules of HA able to induce an enhanced humoral and cellular immune response, allowing to reduce the adenoviral dose required to provide a protective immune response.

CD154 is a glycoprotein expressed as a type II integral membrane protein on the surface of activated T cells, basophils, and mast cells. This molecule belongs to the necrosis factor (TNF) superfamily and has been defined as the most important costimulator for activating APCs [7]. Its receptor CD40, which also belongs to the TNF superfamily [8], is a surface protein that is expressed on B cells, DCs, macrophages, Langerhans cells and nonhemopoietic cells, including endothelial cells, fibroblasts, and epithelial cells. CD40–CD154 interactions between DCs and T cells provide signals for activation and maturation of DCs [9]. CD154 ligation of CD40 on B cells influences various stages in B cell development [6,9,10], including secretion of cytokines and Ig isotype switching [11]. Several recent studies have shown that murine CD154 administered individually, or as a fusion protein can function as a molecular adjuvant enhancing both immune responses and disease protection [12–15]. In avian species, the immunostimulatory capacity of CD154 has been also demonstrated by immunization of ducks as a model for infection of humans with hepatitis B virus [16]. This previous report suggests that the role of CD154 in the regulation of adaptive immune responses had already evolved before the divergence of birds and mammals [16]. Specifically in chickens, the CD154 coding sequence has been cloned and sequenced, and its role in B cell development and differentiation have been also investigated [17].

In this study we combine the adjuvant capacity of CD154 with a delivery system based on adenoviral vectors to create a veterinary vaccine that could potentially be used against HPAIV. We postulated that the extracellular domain of chicken CD154 fused to a H5 gene from HPAIV could bind to CD40 on the APC cell surface and enhance antigen processing and presentation. To test this hypothesis, we constructed two adenoviral vectors encoding the HA (AdHA) or the chimeric molecule HA–CD154 (AdHACD). Our results indicate that the *in vitro* expressed HA–CD154 chimeric molecule is able to multimerize, which should allow it to interact more efficiently with the CD40 receptor on the APC cells surface. Additionally, the humoral and cellular immune response induced by both vectors was compared after an immunization trial in chickens. We found that immunization with the adenoviral vector encoding HA–CD154 induces a cellular immune response and HA-specific antibody titers significantly higher than that induced by the adenoviral vector encoding HA alone.

2. Materials and methods

2.1. Gene synthesis

The HA and CD154 molecules were chemically synthesized by the company GeneArt Inc. (Germany). The primary protein sequences were taken from the database of the National Center for Biotechnology Information (NCBI) using the access numbers AY818135 (for HA) and AJ243435 (for CD154).

Synthetic HA gene encode for the first 537 amino acids of hemagglutinin from the highly pathogenic influenza virus A/Viet Nam 1203/2004. Restriction sites *Kpn*I and *Xho*I were incorporated in the 5′ end of the coding sequence. With the aim of increasing the translation efficiency, a Kozak consensus sequence was incorporated just before the start codon. An *Eco*RI restriction site was added just before the stop codon, and an *Eco*RV restriction site was also incorporated after the stop codon.

The coding sequence for extracellular domain of chicken CD154 molecule comprised the amino acids from 52 to 272. A segment of four repeated Gly-Gly-Gly-Gly-Ser units was included on the amino terminal end to ensure the molecule flexibility. Restriction sites *Eco*RI and *Sal*I were included at the 5′ and 3′ end, respectively. A stop codon was located just before the *Sal*I restriction site.

The HA artificial gene was digested with *Kpn*I and *Eco*RV and inserted into the similarly digested expression vector pAEC-SPT [18]. The resulting vector was denominated pHA. Vector pHA and synthetic CD154 gene were digested with endonucleases *Eco*RI and *Sal*I and ligated to generate the vector pHA-CD, encoding the chimeric protein HA–CD154.

2.2. Adenoviral vector construction

Replication defective adenoviral vectors were built based on the AdEasy system [19]. The plasmid pAdTrack-CMV was employed as transfer vector, which contain an additional transcriptional unit for green fluorescent protein (GFP). Coding sequences for HA and for HA–CD154 were removed by digestion with *Xho*I and *Sal*I endonucleases, and cloned on the XhoI site in the pAdTrack-CMV vector. Resulting vectors (ptrack-HA and ptrack-HACD) were linealized by digestion with *Pme*I and co-electrophoresed with the pAdEasy-1 vector into the bacterial strain BJ5183. The recombinant viral genomes were digested with the *Pac*I endonuclease and transfected into the complementation cell line HEK-293. Appearance of comet-like fluorescent plaques to about seven days after transfection was considered the primary evidence of viral particles generation. The resultant adenoviral vectors (AdHA and AdHACD) were amplified up to about 5×10^{11} plaque forming units (PFU) and stored at −70 °C until use.

2.3. In vitro expression assay and protein detection

Mammary epithelial cells HC11 were seeded in 100 mm plates at a density of about 2.5×10^5 cells/cm^2 in DMEM supplemented with 10% fetal calf serum, epidermal growth factor (10 ng/ml) and insulin (10 μg/ml). After 3 days of confluence, cells were infected with the AdHA or AdHACD adenoviral vectors at a multiplicity of infection (MOI) of 25. Five hour later the medium was replaced with fresh DMEM devoid of fetal calf serum. Seventy-two hours later, the culture medium was harvested and assayed for protein detection. 250 μl of the harvested medium were precipitated with trichloroacetic acid (TCA) and the protein