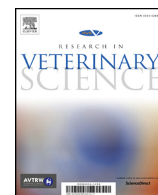
Research in Veterinary Science 111 (2017) 55–62

Contents lists available at ScienceDirect

Research in Veterinary Science

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



Original Research Paper

Targeting antigens to Dec-205 on dendritic cells induces a higher immune response in chickens: Hemagglutinin of avian influenza virus example

David Jáuregui-Zúñiga [a,1], Martha Pedraza-Escalona [a,1], Gerardo Pavel Espino-Solís [a,2], Verónica Quintero-Hernández [a], Alejandro Olvera-Rodríguez [a], Marco Aurelio Díaz-Salinas [b,3], Susana López [b], Lourival Domingos Possani [a,*]

[a] Departamento de Medicina Molecular y Bioprocesos, Instituto de Biotecnología, Universidad Nacional Autónoma de México, UNAM, Apartado Postal 510-3, Cuernavaca, Morelos 62210, México
[b] Departamento de Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México, UNAM, Apartado Postal 510-3, Cuernavaca, Morelos 62210, México

ARTICLE INFO

Article history:
Received 20 September 2016
Received in revised form 18 November 2016
Accepted 2 December 2016
Available online xxxx

Keywords:
Antigen targeting
Avian influenza
DEC-205
Hemagglutinin

ABSTRACT

It is widely known that targeting a variety of antigens to the DEC-205 receptor on dendritic cells (DCs) significantly potentiate immunity. This communication reports the development of a new murine monoclonal antibody (mAb) against the chicken DEC-205, using as immunogen the carbohydrate recognition domain-2 (CRD-2) heterologously expressed. This mAb recognizes a protein band of 250 kDa by immunoprecipitation analysis and shows strong cross-reactivity with human and pig DEC-205. Furthermore, the hemagglutinin (HA) of avian influenza H5N2 virus was cloned and expressed using insect cell-baculovirus expression system. We chemically conjugated the anti-chicken DEC-205 antibody with the highly purified HA to direct the antigen to the dendritic cells and evaluate the immune response elicited in vivo by this conjugate. A single dose of chemical conjugate was sufficient to elicit a strong immune response in chickens as early as fourteen days after priming. In addition, the conjugate induced an earlier and higher response compared to unconjugated HA. These results suggest that the strategy described here has potential to be used in the future design and development of successful vaccines against different chicken infectious diseases with direct impact in biotechnology and veterinary fields.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Dendritic cells (DCs) are highly specialized antigen presenting cells (APCs) that play a key role in maintaining innate and adaptive immune responses. Several DCs subsets have been defined and their functions include antigen capture, processing and presentation to T cells in the context of major histocompatibility (MHC) molecules (Steinman et al., 1997). Previous studies have demonstrated that antibody-mediated targeting proteins to surface receptors on DCs lead to a marked increase in immunogenicity of proteins in vivo (Guo et al., 2000, Jiang et al., 1995 and Steinman, 2008). For this reason, DEC-205 (CD205) was the first DCs receptor used for antigen targeting. The DEC-205/CD205 is a 205 kDa endocytic C-type multilectin transmembrane receptor with a large extracellular domain, which consists of a number of subdomains, including a cysteine rich (CR) domain, a fibronectin type II (FN) domain, and ten contiguous carbohydrate recognition domains (CRDs) (Jiang et al., 1995 and Kato et al., 1998). This receptor is expressed most abundantly by DCs, although it is detected in many different cells and tissues like cortical thymic epithelial cells (Idoyaga et al., 2009 and Kato et al., 2006) and can mediate antigen uptake, processing and presentation (Bozzacco et al., 2007). The cytoplasmic domain of DEC-205 contains a tyrosine based motif (FSSVRY) which is responsible for the initial clathrin-coated pit-mediated endocytosis, while an acidic triad motif (EDE) targets DEC-205 to late endosomes/early lysosomes, allowing endocytosed antigen to reach the vesicles rich in MHC class II molecules (Mahnke et al., 2005). The use of anti-DEC-205 mAbs to improve priming of immune responses has generated great interest since the DEC-205 antigen targeting, in combination with adjuvant that induces DC maturation, results in efficient DCs antigen processing and presentation by MHC class I and II molecules (Bonifaz et al., 2002, Dudziak et al., 2007, Hawiger et al., 2001 and Mahnke et al., 2000). Consequently, the antibody-mediated antigen targeting to DEC-205 is a potential useful strategy for vaccine design and induction of enhanced immune responses against infectious diseases.

“Flu” is a highly contagious, acute viral respiratory disease, produced by the influenza A virus which causes significant morbidity and mortality (Cox and Subbarao, 1999, Glezen, 1982 and Hilleman, 2002).

* Corresponding author.
E-mail address: possani@ibt.unam.mx (L.D. Possani).
[1] These author contributed equally to this work.
[2] Present address: Baylor Institute for Immunology Research, 3434 Live Oak St, Dallas, Texas 75204.
[3] Present address: Laboratory of Human Retrovirology, Institut de Recherches Cliniques de Montréal (IRCM), 110 Pine Avenue West, Montreal, QC H2W 1R7, Canada.

http://dx.doi.org/10.1016/j.rvsc.2016.12.002
0034-5288/© 2016 Elsevier Ltd. All rights reserved.

Influenza virus has been isolated from a variety of animals, including humans, pigs, horses, sea mammals, and birds (Webster et al., 1992). This class of virus has a segmented single-stranded RNA genome contained in a central core of viral proteins which in turn are surrounded by a lipid membrane that contains two glycoproteins: the hemagglutinin and the neuraminidase. Hemagglutinin is the receptor-binding and membrane fusion glycoprotein of influenza virus and the main target for neutralizing antibodies (Chizmadzhev, 2004 and Skehel and Wiley, 2000). The complete hemagglutinin from the H5N2 is composed of 568 amino acids, with a molecular weight of 56 kDa, and is post-translationally cleaved by trypsin into HA1 and HA2 subunits, with the HA1 subunit mediating the initial contact with the cell membrane and HA2 being responsible for membrane fusion (Chizmadzhev, 2004). Infection of poultry with highly pathogenic avian influenza virus can be devastating in terms of economic losses and social disruption (Lau et al., 2004). For this reason, it is important designing new methodologies to enhance the immune response against this and other sicknesses in chicken.

Here we describe the development of a mAb against the carbohydrate recognition domain -2 (CRD-2) of the chicken DEC-205 receptor, produced by heterologous expression in *E coli*. The mAb anti-DEC-205 was purified and used in conjunction with the purified HA recombinant hemagglutinin of the H5N2 virus produced in baculovirus, for production of an effective immunogen capable of generating antibodies against the viral protein. It is suggested that this strategy could be used in the future for the development a new, faster, efficient and safe influenza virus vaccine against H5N2 virus or other sicknesses of poultry.

2. Material and methods

2.1. Expression and purification of CRD-2 of chicken DEC-205

The CRD-2 domain of chicken DEC-205 modified to carry a 6× His tag at the C-terminal region was amplified by polymerase chain reaction (PCR) from a chicken peripheral blood cDNA library using the primers: Forward 5′-CAT GCC ATG GAG TTT TGG AGA CAC GTG AAT ACT C-3′ with a *Nco I* restriction enzyme site and Reverse: 5′-CCA AGC TTG GGT TAA TGA TGA TGA TGA TGA TGC TCA TCC AAT GAG CAA TTT TT-3'with *Hind III* restriction enzyme site. The PCR fragment was cloned into the pET22b (+) plasmid (Novagen, San Diego CA, USA) and the expression of His-tagged CRD-2 domain was done in *E. coli* Rosseta II strain induced by 0.2 mM of IPTG (Sigma-Aldrich Inc., Germany). Although the expression system allows exporting the expressed protein to the periplasm, the CRD-2 domain was purified from inclusion bodies to recover a greater amount of recombinant protein. Briefly, the recombinant protein was dissolved in Tris HCl pH 8.0 and 8 M urea, and the protein solution was loaded onto chromatography columns containing Ni-NTA agarose resin (QIAGEN Inc., Germany.). The resin was washed with successive Tris-HCl buffers pH 8.0 with decreasing concentrations of urea (4, 2, 1 0.5 and 0.2 M) to promote refolding, followed by washes with Tris-HCl pH 8.0 and 250 mM Imidazole, protein was eluted using acetate buffer at pH 4.5. The resulting protein was reduced during 1 h at 52 °C with 100 mM DTT and then subjected to reverse-phase chromatography in a C18 column using the Waters 600E HPLC system. The column was equilibrated with solvent A (0.1% trifluoroacetic acid in water) and eluted applying a concentration gradient toward solvent B (0.1% trifluoroacetic acid containing 100% acetonitrile) from 20 to 80% at a flow rate of 1.6 mL/min at room temperature. Purity was verified by 15% SDS-PAGE and the sequence was performed by sequencing with mass spectrometry analysis (data not shown).

2.2. Cloning, expression, purification and characterization of hemagglutinin from the H5N2 virus

Expression of recombinant hemagglutinin from H5N2 virus (rHA) was accomplished in Sf9 insect cells, using the Bac-to-Bac baculovirus expression system (Invitrogen, San Diego CA, USA). The full length hemagglutinin (HA) encoding gene of H5N2 strain isolated from a RNA's collection (kindly provided by Dr. R. Webster from St. Jude Hospital, Tennessee, USA) was amplified by RT-PCR and cloned into a baculovirus transfer vector (pFAST-BacHT [Invitrogen, San Diego CA, USA]). This vector contains the baculovirus polyhedrin promoter upstream the cloned gene. The cloned gene was verified by DNA sequence analysis. Next, the recombinant pFast vector was used to transform DH10Bac™ *E. coli* strain (Invitrogen, San Diego CA, USA) to generate the recombinant bacmid. The recombinant bacmid was transfected into Sf9 insect cells to generate recombinant baculovirus particles using Cellfectin® II reagent (Invitrogen, San Diego CA, USA). The recombinant baculovirus was grown to prepare virus stock by infecting increasingly larger cultures of Sf9 cells in Sf-900™ II SFM culture medium (Gibco Life technologies, NY, USA), and the viral titer was obtained using a commercial kit (BacPAK™ Baculovirus Rapid Titer Kit; Clontech Laboratories, USA). The virus stock was then used to infect insect cells (1.5×10^6 cells/mL) at a multiplicity of infection (MOI) of 1 to produce rHA for purification. Seventy-two hours post-infection (hpi), infected cultures were centrifuged at 3000 rpm and the supernatant discarded. The cells were homogenized in lysis buffer (25 mM Tris-HCl pH 8.0, 500 mM NaCl, 20 mM imidazole, 1% de Nonident P40, EDTA-free protease inhibitor cocktail). The homogenates were centrifuged at 13,000 rpm for 30 min at 4 °C and the resulting supernatants were loaded onto a column prepared with Ni-NTA resin and washed with 20 column volumes of washing buffer (25 mM Tris-HCl pH 8.0, 500 mM NaCl, 35 mM imidazole). Nickel bound proteins were eluted with elution buffer (25 mM Tris-HCl pH 8.0, 500 mM NaCl, 250 mM imidazole). For rHA characterization, purified protein was analyzed by 9% SDS-PAGE and western blotting using anti-H5 (Abcam Inc., Cambridge, MA, USA) and anti-His (Roche Applied Science, Penzberg, Germany) antibody. Additionally, the rHA hemagglutination titer was determined by hemagglutination assay. Briefly, rHA was diluted to a concentration of 2 μg/100 μL of PBS and 50 μL were placed in the first column of a round bottom 96-well plate. Recombinant HA samples were diluted two-fold along the length of the plate and 50 μL of 1% chicken red blood cells (CRBC) in PBS were added to each well. The plates were incubated at room temperature for 1 h and the agglutination level was recorded. The highest dilution in which agglutination was observed was used to calculate the amount of hemagglutination units per microgram of rHA.

2.3. Immunization of mice with CRD2-DEC-205

Mice were immunized subcutaneously with 25, 50, 100, 150 or 200 μg of purified recombinant CRD-2 protein emulsified with an equal volume of complete Freund's adjuvant (Sigma-Aldrich Inc., Germany) and boosted 5 times at 10 days intervals with incomplete Freund's or alumina adjuvants (Sigma-Aldrich Inc., Germany) emulsion via a similar route. Serum samples were collected from mice a week after each immunization. The titer of anti-CRD-2 antibodies in serum was assessed by indirect ELISA, mentioned below. Five days after the fifth immunization, the mouse with the highest anti-CRD-2 titer was sacrificed and splenocytes were fused to murine myeloma cell line SP2 as described below.

2.4. Production of hybridomas, ascitic fluid, mAb purification and determination of mAb isotype

Mouse splenocytes were fused to mouse SP2 myeloma cells by dropwise addition of 50% polyethylene glycol 1500 (Sigma-Aldrich Inc., Germany) to the cells. The polyethylene glycol 1500 was washed away with serum-free DMEM medium (Gibco Life technologies, NY, USA) and the fused cells were directly resuspended in selective medium HAT (Sigma-Aldrich Inc., Germany) and distributed into 96-well plates. The supernatants from hybridoma-containing wells were collected and screened by indirect ELISA as mention below. Monoclonal cell lines

Download English Version:

https://daneshyari.com/en/article/5544016

Download Persian Version:

https://daneshyari.com/article/5544016

Daneshyari.com