Veterinary Immunology and Immunopathology 212 (2019) 9–14

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

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

Construction and expression of a single-chain variable fragment antibody against chicken DEC 205 for targeting the bacterial expressed hemagglutinin-neuraminidase of Newcastle disease virus

David Jáuregui-Zúñiga[a,*], Martha Pedraza-Escalona[b], Rubén Merino-Guzman[c], Lourival D. Possani[a]

[a] Departamento de Medicina Molecular y Bioprocesos, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Apartado Postal 510-3, Cuernavaca, Morelos 62210, México
[b] CONACYT-UDIBI-ENCB-Instituto Politecnico Nacional. Unidad Profesional Lázaro Cárdenas, Prolongación de Carpio y Plan de Ayala s/n, Col. Santo Tomás, Ciudad de México 11340, México
[c] Departamento de Medicina y Zootecnia de Aves, Facultad de Medicina Veterinaria y Zootecnia Universidad Nacional Autónoma de México, UNAM, C.U., Ciudad de México 04510, México

ARTICLE INFO

Keywords:
Antigen targeting
DEC 205
Hemagglutinin-neuraminidase
Newcastle virus
ScFv

ABSTRACT

Targeting antigens to endocytic receptors on the surface of dendritic cells is a new strategy for increasing the adaptive immune response. The objective of the current study was the construction and bacterial expression of a recombinant antibody single-chain fragment variable (ScFv) directed against chicken DEC 205, an endocytic receptor, for use in the genetic fusion of antigens. In particular, we use as antigen the hemagglutinin-neuraminidase (HN) of Newcastle disease virus. Our results show that inoculation of chickens with HN genetically fused to the ScFv anti-DEC 205 induced an evidently higher immune response against HN, in contrast to inoculation with unconjugated HN. In addition, neutralizing antibodies against Newcastle disease virus were detected only in the serum from chickens immunized with HN fused to ScFv anti-DEC 205. Inoculated fused antigens to ScFv against endocytic receptor DEC 205 resulted in a greater antibody-specific anti-HN production compared with antigens applied alone. The results of this study show that the strategy described here has the potential to be used in the development of more effective vaccines against infectious diseases in chickens.

1. Introduction

Dendritic cells (DCs) are efficient stimulators of B and T lymphocytes, and they regulate the innate and acquired immunity against pathogens. They are particularly efficient for capturing, processing, and presenting antigens (Steinman and Banchereau, 2007). Previous studies in mice have described that targeting different antigens to surface receptors on DCs using specific antibodies, in combination with adjuvants that induce DC maturation, leads to a noticeable enhancement in the efficiency of uptake and presentation of antigens on MHC I and MHC II complexes. This results in an increase of at least two orders of magnitude in the activation and proliferation of antigen-specific CD4 + and CD8 + T lymphocytes (Mahnke et al., 2000; Steinman et al., 2003; Boscardin et al., 2006).

DEC 205 is a C-type multilectin transmembrane endocytic receptor that is highly expressed on the surface of immature DCs (Mahnke et al., 2000) and helps with the uptake and processing of antigens. The 205-kDa molecular weight includes approximately 7 kDa of carbohydrates found in eight glycosylation branching variants, all of which are N-linked (Swiggard et al., 1995). The endocytic receptor DEC 205 has been employed for targeted antigens to DCs in murine, human, and chicken studies for immune response enhancement (Mahnke et al., 2000; Hawiger et al., 2001; Bonifaz et al., 2002; Dudziak et al., 2007; Jáuregui-Zúñiga et al., 2017). This strategy has been shown to be effective against viral (Cheong et al., 2010; Coconi-Linares et al., 2013), bacterial (Do et al., 2012), and parasitic infections (Matos et al., 2013).

In another way, several studies have shown the use of complete monoclonal antibodies to target antigens to DCs. In contrast, single-chain fragment variable (ScFv) antibodies are less frequently used in target antigens. However, the use of ScFvs, rather than complete antibodies, offers several advantages for antigen targeting. For example, their small size improves tissue penetration (Yokota et al., 1992;

* Corresponding author.
E-mail address: dajazu@yahoo.com (D. Jáuregui-Zúñiga).

https://doi.org/10.1016/j.vetimm.2019.05.001
Received 6 November 2018; Received in revised form 3 May 2019; Accepted 5 May 2019
0165-2427/ © 2019 Elsevier B.V. All rights reserved.

Colcher et al., 1998; Weisser and Hall, 2009) and reduces immunological negative side effects due to the lack of an Fc domain (Demangel et al., 2005). In addition, ScFv expression in heterologous systems is easier and less expensive than that of whole antibodies (Weisser and Hall, 2009).

Newcastle disease (ND) is a highly contagious viral disease that generates a large economic loss in the poultry industry worldwide, which is characterized by acute morbidity and high mortality (Leeuw and Peeters, 1999; Alexander, 2000). The causal agent, Newcastle disease virus (NDV), is a virulent variant of avian paramyxovirus type 1 (Lamb and Parks, 2007; Alexander and Senne, 2008). The NDV genome is a nonsegmented single strand of negative-sense RNA, which is composed of six genes, encoding the nucleocapsid protein, phosphoprotein, matrix protein, fusion protein, hemagglutinin-neuraminidase (HN), and large polymerase protein (Aldous et al., 2003). The fusion and HN proteins are the main targets for neutralizing antibodies (Romer-Oberdorfer et al., 2003; Huang et al., 2004; Panda et al., 2004b). Live and inactivated vaccines have been widely used since the 1950s; despite this, an average of 60 countries reported ND outbreaks each year from 2013 to 2015 (Dimitrov et al., 2017). However, as evident from the multiple outbreaks occurring worldwide, current vaccination strategies are not fully efficacious, and research and development toward formulation of an optimal ND vaccine are still needed.

In the present study, we constructed a fusion protein using an ScFv derived from monoclonal antibody (mAb) that is directed against the DEC-205 receptor and the HN from NDV to improve the protective immunity against NDV in chickens. This construction should provide a targeting platform that enhances immunogenicity and is easier to produce.

2. Materials and methods

2.1. ScFv anti-DEC 205 construction

The previously obtained 2F2 mAb, which recognizes the carbohydrate domain recognition (CDR-2) domain of chicken receptor DEC 205 (Jáuregui-Zúñiga et al., 2017), was used to generate the construction of the ScFv anti-DEC 205. The coding sequence of the variable regions of the mAb anti-DEC 205 was amplified by polymerase chain reaction (PCR). The set of primers used is listed in Table 1. First, F1 and R1, and F2 and R2 pair of primers were used to perform separate PCR reactions to amplify the VL and VH sequences, respectively. The PCR products were purified and used as the template in a gene splicing by overlap extension PCR (SOE-PCR) using F1 and R2 pair of primers. F1 and R2 primers were designed to include NcoI and BamHI sites, respectively. R2 primer was designed to include an E tag epitope (GAPVPYPDPLEPR). A complementary overlapping sequence encoding a flexible linker of 12 amino acids (GGGGSGGGGSGGGGS) was added to R1 and F2 primers to splice the genes VL and VH. The final product from the SOE-PCR reaction (722 pb) was purified and digested using NcoI and BamHI restriction enzymes (Thermo Scientific). The digested DNA was subsequently cloned into pET22b (+) vector (NOVAGEN) excised with the same restriction enzymes. The pET22b (+) vector carries an N-terminal pelB signal sequence for periplasmic localization. The appropriate insertion of the coding sequence for the ScFv into pET22b (+) vector was corroborated by PCR. The ScFv was expressed in *Escherichia coli* BL21 at 22 °C overnight and purified by osmotic shock followed by affinity chromatography with Ni-NTA Sepharose beads (Qiagen). The process of protein production and purification was followed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis, whereas the protein identification was completed with anti-His (1:1000) (Roche) and anti-E (1:1000) (Abcam) antibodies by western blot analysis, and the N-terminal sequence was performed with mass spectrometry analysis for confirm the identification (data not shown).

2.2. Western blot for ScFv anti-DEC 205 characterization

The ability of ScFv anti-DEC 205 to recognize the DEC 205 receptor was evaluated by western blot. The bursa of Fabricius from 12-week-old chickens was homogenized in 10 ml of ice-cold RIPA extraction buffer (10 mM Tris-Cl, pH 8.0, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, 140 mM NaCl) in a prechilled mortar. The macerated sample was centrifuged at 25,000*g* for 10 min. The supernatant was collected and concentrated by precipitation with 10% trichloroacetic acid at 4 °C O/N. The pellet was washed twice with ice-cold acetone and air dried. The precipitated sample was solubilized with 100 µl of 2x Laemmli buffer and quantified with the 2-D Quant Kit (GE Healthcare), and 100 µg was then subjected to 10% SDS-PAGE under reducing conditions and blotted onto a polyvinylidene difluoride (PVDF) membrane. In the same way, 200 ng of the purified CRD-2 domain (previously obtained by Jáuregui-Zúñiga et al., 2017) was subjected to 15% SDS-PAGE under reducing conditions and blotted onto a PVDF membrane. Both the DEC 205 and CRD-2 domain were detected in PVDF membranes using the ScFv anti-DEC 205 as primary Ab at a concentration of 3 µg/ml and overnight incubation at 4 °C. A mouse anti E-tag (1:1000) (Abcam) was used as secondary Ab. The signal was revealed using an anti-mouse HRP mAb (1:1000) (Abcam) and diaminobenzidine solution (Sigma Aldrich).

2.3. Amplification of HN gene of NDV

RNA was isolated from virus collected from allantoic fluid from chicken specific pathogen free (SPF) eggs inoculated with NDV (LaSota strain) using SV Total RNA Isolation kit (Promega). One microgram of RNA was used to generate cDNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). First-strand cDNA was synthesized using a specific primer (HN Rev, listed in Table 1) for HN from NDV. The entire HN gene was amplified for PCR using the HN For and HN Rev primers listed in Table 1. The HN gene was directionally cloned between BamHI and XhoI sites into pET22b (+) plasmid and ScFv-pET22b construction previously digested with the same enzymes. PCR reactions using the HN-specific primers (HN For and HN Rev) and

Table 1
List of Primers used.

Primer	Sequence
F1	5'-CATCCATGGCTGGTGAGAGGGTTACTATGAGC-3'
R1	5'-CCACCGCCAGAGCCACCTCCGCCTGAACCGCCTCCACCTTTTATTTCCAGC TTGG-3'
F2	5'-GGCGGAGGTGGCTCTGGCGGTGGCGGATCGGAGGTTCAGCTGC-3'
R2	5'-CAGGGATCCATACGTGGTTCCAGCGGATCTGGATAAGGCACAGGTGCTCCA GAGACAGTGACCAGAGTCCCTTG-3'
HN For	5'-GGCGGATCCCATGGGGGCTAGCACACCTAG-3'
HN Rev	5'-GGCAAGCTTGCCAGACCTGGCTTCTCTAAC-3'
T7 For	5'-TTAATACGACTCACTATAGG-3'
T7 Rev	5'-CCGCTGAGCAATAACTAGC-3'

pET22b (+) primers (T7 For and T7 Rev, listed in Table 1) were carried out to confirm the appropriate insertion of the amplified coding sequence for the HN into scFv-pET22b vector. The recombinant ScFv anti-DEC 205:HN and HN alone were successfully expressed in *E. coli* BL21 and purified by affinity in an Ni-NTA agarose resin (Qiagen). Moreover, the binding activity and specificity of the ScFv anti-DEC 205:HN were determined by western blot in the same way for the ScFv anti-DEC 205 alone as previously described.

2.4. Immunization of chickens and detection of the anti-HN antibodies

Chicken immunization was done as previously reported by Jáuregui-Zúñiga et al. (2017). Briefly, 2-week-old SFP chickens (ALPES S.A. de C.V.) were maintained in the animal facilities of IBT-UNAM. All animals were housed and handled in accordance with institutional guidelines for animal welfare, and the project was approved by the Bioethical Committee of IBT-UNAM. Groups of 4 chickens were injected subcutaneously with a single dose of 100 µg of ScFv anti-DEC 205, 100 µg of ScFv anti-DEC 205:HN, or 100 µg of recombinant unconjugated HN, using in all groups 50 µg of *E. coli* lipopolysaccharide (LPS) (Sigma Aldrich) as adjuvant in phosphate-buffered saline (PBS). Two control groups were stablished: one group was injected with 50 µg of *E. coli* LPS (Sigma Aldrich), and one group was injected only with PBS.

For all groups, blood samples were collected from the wing vein 1 day before immunization and 7, 14, 21, and 28 days after injection. The HN-specific IgY antibodies in chicken sera were detected by indirect enzyme-linked immunosorbent assay (ELISA). ELISA plate wells were coated with 50 µl of purified HN (2 µg/mL), incubated overnight at 4 °C, washed with PBST, and blocked for 2 h at 37 °C with 100 µl of 3% (w/v) bovine serum albumin (Sigma-Aldrich). Wells of the first column were coated with 100 µl of serum samples (diluted 1:20) and further 2-fold serial dilutions applied until 1:40,962 and incubated for 2 h at 37 °C, then washed and incubated with 100 µl of HRP-conjugated rabbit anti-chicken (Abcam) diluted (1: 5000) for 1 h at 37 °C. Plates were washed, and 100 µl OPD solution (Thermo Scientific) was added. The absorbance was measured (without stop solution addition) at 450 nm using a HALO MPR-96 plate reader (Dynamica) after 30 min of incubation at room temperature. Any background correction was used for the data.

2.5. Hemagglutination inhibition and virus neutralization assays

To detect the presence of neutralizing antibodies against Newcastle virus, the hemagglutination inhibition (HI) and virus neutralization assays were performed. The HI test was carried out as described in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (World Organisation for Animal Health (OIE, 2017) using 10 hemagglutination units; the sera were tested without preadsorption with chicken red blood cells. Titers of 8 or more were regarded as positive. Virus neutralization test was performed using SPF embryonated chicken eggs as previously described by Thayer and Beard (1988). Briefly, 10-fold serial dilutions of LaSota NDV strain, with a starting titer of 10^{-7}, were incubated with sample sera diluted by 1/2, and then the serum-virus mixtures in each dilution were inoculated into 5 SPF embryonated chicken eggs. Eggs were also inoculated with the corresponding titers of virus alone in parallel. Endpoints were calculated by the Reed and Muench method (1938). A neutralization index (NI) was calculated, representing the difference (in logarithmic units) in viral titer between virus alone and the corresponding virus-antiserum mixture. Sera with an NI of 2 or greater were considered positive.

2.6. Statistical analysis

For statistical analysis, two-way analysis of variance (ANOVA) followed by a multiple-comparisons Tukey test were used to determine statistical significance on Prism 7.0 software (GraphPad, La Jolla, CA, USA). p values of ≤ 0.05 were considered significant. The D'Agostino-Pearson test was used to analyze the normal distribution of the data before the ANOVA analysis.

3. Results and discussion

It is known that targeting antigens to DCs significantly potentiates immunity (Mahnke et al., 2000; Steinman et al., 2003; Boscardin et al., 2006). One of the first DC receptors selected for this approach was decalectin DEC 205. In a previous work, we described a specific mAb against chicken DEC 205 and demonstrated that the inoculation of avian influenza hemagglutinin chemically conjugated with the mAb anti-DEC 205 increased the immune response in comparison with the unconjugated antigen (Jáuregui-Zúñiga et al., 2017). The chemical cross-linking of proteins has a low efficiency (40% in average) with this technique, and achieving a consistent and efficient coupling of antigens and antibodies is difficult, which is a major concern for vaccine development. Therefore, it is reasonable to search for better methods of conjugating antigens to mAb. Antigen genetic fusion to ScFvs and its expression in bacterial systems is a good option because it is more consistent, efficient, and cost-effective than chemical cross-linking. For this reason, we constructed the ScFv from the mAb anti-DEC 205 previously obtained. The ScFv anti-DEC 205 gene was constructed in the VL-VH orientation (Fig. 1). VL (297 pb) and VH (377 pb) domains were amplified by PCR (Fig. 2A) and assembled by SOE-PCR to generate the ScFv construct VL-VH (722 bp) (Fig. 2A) with a flexible linker (GGGGSGGGGSGGGGS). The construction was cloned into the plasmid pET22b (+) (which introduces a 6His-tag to the protein) and transformed into *E. coli* BL21 for expression and purification of ScFv anti-DEC 205. The molecular weight of expressed recombinant protein was expected to be approximately 28 kDa. After affinity purification, the final purity of the ScFv anti-DEC 205 protein was up to 95% (Fig. 2B).

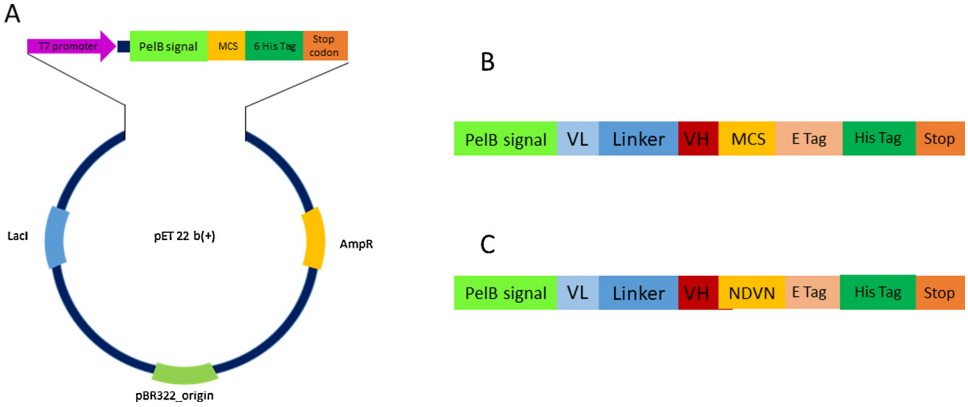

Fig. 1. Constructions of ScFv anti-DEC 205 and ScFv anti-DEC 205:HN. (A) The pET-22b(+) vector carries an N-terminal pelB signal sequence for periplasmic localization, plus C-terminal 6His tag sequence. (B) ScFv anti-DEC 205 construction contains the VL (variable domain of the light chain), the VH (variable domain of the heavy chain), and the MCS (multiple cloning site); the N-terminal pelB signal sequence, the C-terminal E,-tag and the 6His-tag are shown. (C) ScFv anti-DEC 205:HN construction contains the VL, VH, NDVHN (Newcastle disease virus hemagglutinin-neuraminidase); the N-terminal pelB signal sequence, the C-terminal E-tag and the 6His-tag are shown.

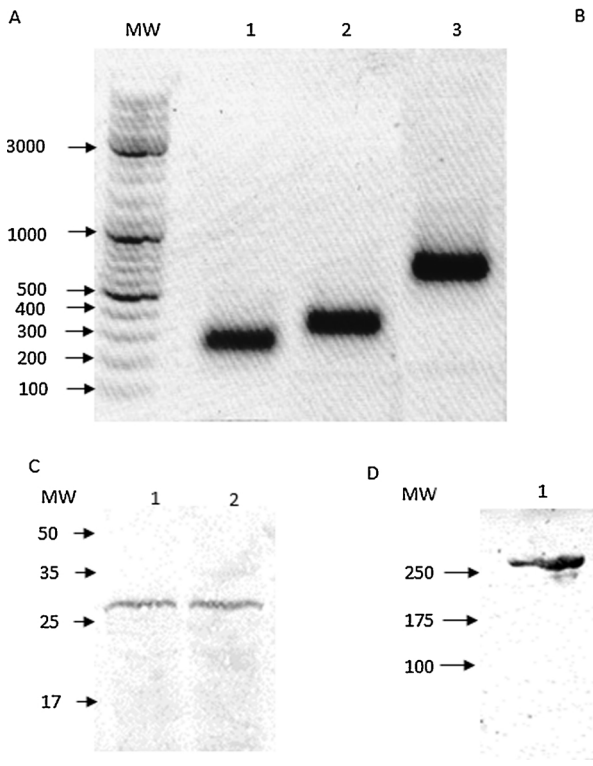

Fig. 2. Construction, purification, identification, and characterization of the ScFv anti-DEC 205. (A) Amplifications of VH and VL gene. Line 1, amplified VL (297 pb); line 2, amplified VH (377 pb); line 3, VL-VH fusion by SOE-PCR (722 bp). (B) Expression and affinity purification of ScFv anti-DEC 205. Line 1, total protein from the *E. coli* cells after IPTG induction; line 2, purification of ScFv anti-DEC 205 by Ni-NTA affinity chromatography. (C) Identification of ScFv anti-DEC 205 protein by western blot. Line 1, identification of ScFv anti-DEC 205 with anti-E tag antibody; line 2, identification of ScFv anti-DEC 205 with anti-His tag antibody. (D) Detection of the DEC 205 present in the chicken bursa of Fabricius lysates with the ScFv anti-DEC 205; line 1, DEC-205 band was recognized by ScFv anti-DEC 205; an anti-E mouse Ab was used for ScFv anti-DEC 205 detection. (E) Detection of the CDR-2 domain of the DEC 20 with the ScFv anti-DEC 205. Line 1, CDR-2 was recognized by ScFv anti-DEC 205; an anti-E mouse Ab was used for ScFv anti-DEC 205 detection.

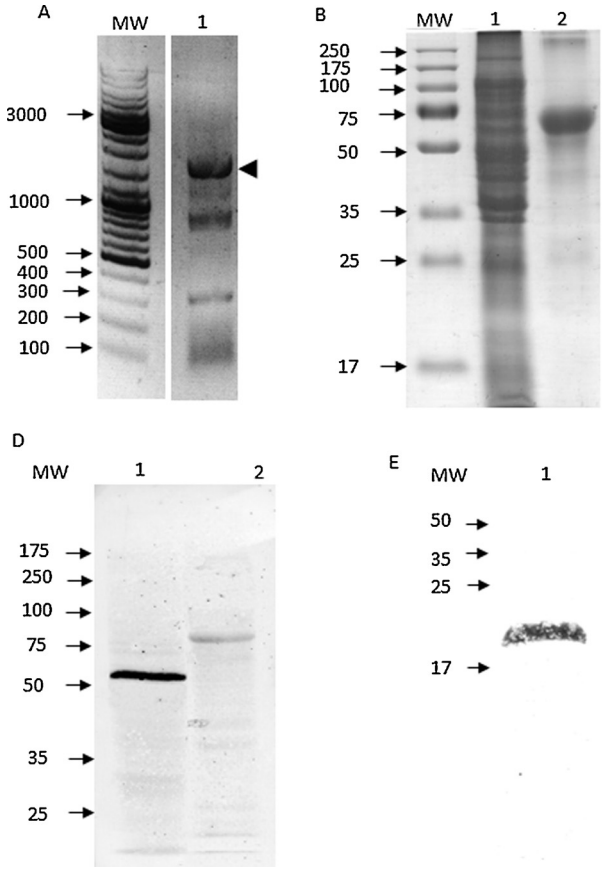

Fig. 3. Construction, purification, identification, and characterization of the ScFv anti-DEC 205:HN. (A) Amplifications of the HN gene. Line 1, amplified HN gene is indicated with the arrowhead (1.6 kb). (B) Expression and affinity purification of HN. Line 1, total protein from the *E. coli* cells after IPTG induction; line 2, purification of HN by Ni-NTA affinity chromatography. (C) Expression and affinity purification of ScFv anti-DEC 205:HN. Line 1, total protein from the *E. coli* cells after IPTG induction; line 2, purification of ScFv anti-DEC 205:HN by Ni-NTA affinity chromatography. (D) HN and ScFv anti-DEC 205:HN identification by western blot analysis. Line 1, HN detection by anti E tag Ab; line 2, ScFv anti-DEC 205:HN detection by anti E tag Ab. (E) Detection of DEC 205 present in the chicken bursa of Fabricius lysates by western blot with the ScFv anti-DEC 205:HN; line 1, detection of DEC 205 by ScFv anti-DEC 205:HN;, an anti-E mouse Ab was used for ScFv anti-DEC 205:HN detection. (F) Detection of CDR-2 domain with the ScFv anti-DEC 205:HN; line 1, identification of the CDR-2 domain by ScFv anti-DEC 205:HN; an anti-E mouse Ab was used for ScFv anti-DEC 205:HN detection.

The identity of the purified protein was corroborated by western blot with anti His and anti E-tag antibodies (Fig. 2C). In addition, the cDNA of HN from NDV (1.6 kb) was obtained by reverse transcription PCR from the negative-stranded viral RNA isolated from purified virus (LaSota strain) (Fig. 3A) and cloned in frame in the C-terminal of the ScFv anti-DEC 205 into the ScFv-pET 22b construction. In the same way, HN was cloned in the pET22b (+) plasmid to be used as control. The HN and the ScFv anti-DEC 205:HN were expressed in *E. coli* BL21

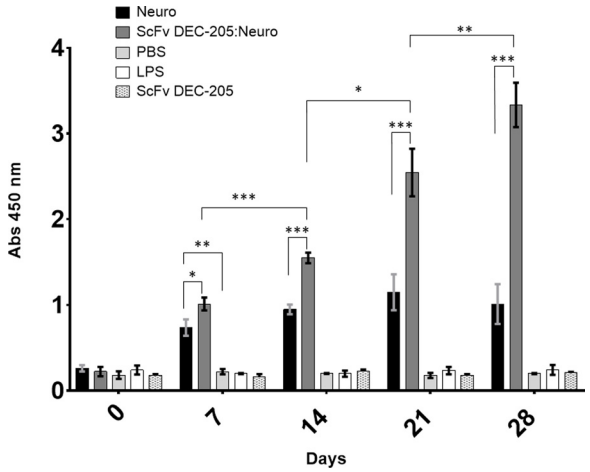

Fig. 4. Detection of anti-HN antibodies. One group of chickens was immunized with the ScFv anti-DEC 205:HN, and one group was inoculated with unconjugated HN. *E. coli* LPS was used as adjuvant in both groups. One group was inoculated with *E. coli* LPS alone, and finally, one group was inoculated with PBS alone as a control. Chicken serum was obtained at day 0, 7, 14, 21, and 28 after priming (DAP). An indirect ELISA assay was used for anti HN Abs analysis. A demonstrative measurement of a serial dilution (1:1215) of sera is shown. Bars represent the mean absorbance with standard deviation, obtained within each experimental group; three technical and four biological replicates were included. Two-way ANOVA followed by a multiple-comparisons Tukey test were used to determine statistical significance ($*p < 0.05$; $**p < 0.01$, $***p < 0.001$).

and purified. The molecular weights of the expressed recombinant proteins were expected to be approximately 60 kDa and 88 kDa for HN and ScFv anti-DEC 205:HN, respectively. After purification, the final purity of the HN and ScFv anti-DEC 205:HN was estimated to be up to 95% in both cases (Fig. 3B and C). The identity of the purified proteins was corroborated with a western blot analysis with anti E-tag antibodies in the case of ScFv-HN and anti His in the case of the HN alone (Fig. 3D).

The ability of ScFv anti-DEC 205 and the fusion ScFv anti-DEC 205:HN to recognize DEC 205 was analyzed by western blot. We used both the ScFv anti-DEC 205 and the ScFv anti-DEC 205:HN as primary Ab and an anti E-tag as secondary Ab to identify the receptor DEC 205 present in the bursa of Fabricius lysates from chickens. A band was detected in the membrane, and the signal was identified at approximately 250 kDa, which coincides with the molecular weight previously reported for DEC 205 (Staines et al., 2013; Jáuregui-Zúñiga et al., 2017) (Figs. 2D and 3 E). In addition, to verify the ability of ScFv anti-DEC 205 and the fusion ScFv anti-DEC 205:HN to recognize DEC 205, both constructions were used as primary Ab for the identification of the recombinant CRD-2 domain from DEC 205 (blotted in a PVDF membrane) and an anti E-tag as secondary Ab. A band of approximately 17 kDa was detected in the membrane, which corresponds to the weight of the recombinant CRD-2 domain from DEC 205 (Jáuregui-Zúñiga et al., 2017) (Figs. 2E and 3 F). The previous results indicate that the ScFv version of the mAb anti-DEC 205 retains its ability to recognize the DEC 205 in lysates of the bursa of Fabricius in chicken. Likewise, the fusion of HN of NDV at the carboxyl terminal of the ScFv does not affect its capability to recognize the DEC 205.

Chickens were used to establish whether inoculation with the ScFv anti-DEC 205:HN could induce a higher immune response in comparison with the unconjugated HN. Different groups of chickens were inoculated once with ScFv anti-Dec205, ScFv anti-DEC 205:HN, with unconjugated HN, with *E. coli* LPS, or with PBS alone. The presence of anti-HN IgY antibodies in the different sera was evaluated by ELISA. There were no detectable antibodies of anti-HN in the sera from preimmunized chickens of any group. The presence of antibodies against HN was detected only in chickens inoculated with ScFv anti-DEC 205:HN fusion and unconjugated HN 7 days after priming (Fig. 4), indicating that the immune response against HN is not due to ScFv only or LPS action and that a single dose of recombinant HN or ScFv anti-DEC 205:HN was enough to produce an immune response. On the other hand, the immune response increased over time in chickens inoculated with ScFv anti-DEC 205:HN fusion, with the highest response at 28 days after immunization. On the other hand, the immune response in chickens inoculated with HN alone remained equal over time (Fig. 4).

In addition, the HI test showed that only the serum from chickens inoculated with the ScFv anti-DEC 205:HN fusion 28 days after priming presented antibodies against NDV capable of preventing attachment of the virus to red blood cells. The highest dilution of serum that inhibited hemagglutination was 1:32. Therefore, only the serum from chickens inoculated with the ScFv anti-DEC 205:HN fusion 28 days after priming and the serum from chickens inoculated with HN alone 28 days after priming (as a control) were used for the virus neutralization test. The NI calculated for the serum from chickens inoculated with HN alone 28 days after priming was 0.8, meanwhile the NI calculated for the serum from chickens inoculated with the ScFv anti-DEC 205:HN 28 days after priming was 3.5. We consider an index greater than 2 as positive; therefore, neutralizing antibodies were present only in sera from chickens 28 days after priming with ScFv anti-DEC 205:HN. The absence of detectable neutralizing antibodies in sera at earlier times (7, 14, and 21 days after priming) is probably due to the nonglycosylated nature of the protein expressed in *E. coli* (Panda et al., 2004a). The same reason can explain the absence of detectable neutralizing antibodies in sera from chickens immunized with HN alone.

In summary, the results of this work demonstrated that inoculation of recombinant antigens fused to the ScFv version of the mAb anti-DEC 205 expressed in *E. coli* induces a higher antibody-specific anti-HN production in chickens and promotes the production of neutralizing antibodies in comparison with the inoculation of *E. coli*–expressed recombinant antigen alone. The use of this construction would facilitate the expression, purification, and fusion of different antigens and could be used to development new effective vaccines against several avian diseases.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgements

This work was partially supported from a grant 215350 of the National Council of Science and Technology of México, given to Centro Lavin para el Desarrollo de Innovación y Transferencia de Tecnología. The authors acknowledge the technical assistance of Martín Patiño Vera and Mario Trejo Loyo.

References

Aldous, E., Mynn, J., Banks, J., Alexander, D., 2003. A molecular epidemiological study of avian paramyxovirus type 1 (Newcastle disease virus) isolates by phylogenetic analysis of a partial nucleotide sequence of the fusion protein gene. Avian pathology 32, 237–255.

Alexander, D., 2000. Newcastle disease and other avian paramyxoviruses. Rev. Sci. Technol.-Off. Int. Epizoot. 19, 443–455.

Alexander, D., Senne, D., 2008. Newcastle disease, other avian paramyxoviruses, and pneumovirus infections. In: Saif, Y.M., Fadly, A.M., Glisson, J.R., McDougald, L.R., Nolan, L.K., Swayne, D.E. (Eds.), Diseases of Poultry, 12th ed. Iowa State University Press, Ames, pp. 75–116.

Bonifaz, L., Bonnyay, D., Mahnke, K., Rivera, M., Nussenzweig, M.C., Steinman, R.M., 2002. Efficient targeting of protein antigen to the dendritic cell receptor DEC-205 in the steady state leads to antigen presentation on major histocompatibility complex class I products and peripheral CD8+ T cell tolerance. J. Exp. Med. 196, 1627.

Boscardin, S.B., Hafalla, J.C.R., Masilamani, R.F., Kamphorst, A.O., Zebroski, H.A., Rai, U., Morrot, A., Zavala, F., Steinman, R., Nussenzweig, R., Nussenzweig, M.C., 2006. Antigen targeting to dendritic cells elicits long-lived T cell help for antibody responses. J. Exp. Med. 203, 599–606.

Cheong, C., Choi, J.H., Vitale, L., He, L.Z., Trumpfheller, C., Bozzacco, L., Do, Y., Nchinda, G., Park, S.H., Dandamudi, D.B., Shrestha, E., Pack, M., Lee, H.W., Keler, T., Steinman, R.M., Park, C.G., 2010. Improved cellular and humoral immune responses in vivo following targeting of HIV Gag to dendritic cells within human anti-human DEC205 monoclonal antibody. Blood 116, 3828–3838.

Coconi-Linares, N., Ortega-Dávila, E., López-González, M., García-Machorro, J., García-Cordero, J., Steinman, R.M., Cedillo-Barrón, L., Gómez-Lim, M.A., 2013. Targeting of envelope domain III protein of DENV type 2 to DEC-205 receptor elicits neutralizing antibodies in mice. Vaccine 31, 2366–2371.

Colcher, D., Pavlinkova, G., Beresford, G., Booth, B.J., Choudhury, A., Batra, S.K., 1998. Pharmacokinetics and biodistribution of genetically-engineered antibodies. Q. J. Nuclear Med. 42, 225–241.

Demangel, C., Zhou, J., Choo, A.B., Shoebridge, G., Halliday, G.M., Britton, W.J., 2005. Single chain antibody fragments for the selective targeting of antigens to dendritic cells. Mol. Immunol. 42, 979–985.

Dimitrov, K.M., Afonso, C.L., Yu, Q., Miller, P.J., 2017. Newcastle disease vaccines-a solved problem or a continuous challenge? Vet. Microbiol. 206, 126–136.

Do, Y., Didierlaurent, A.M., Ryu, S., Koh, H., Park, C.G., Park, S., Perlin, D.S., Powell, B.S., Steinman, R.M., 2012. Induction of pulmonary mucosal immune responses with a protein vaccine targeted to the DEC-205/CD205 receptor. Vaccine 30, 6359–6367.

Dudziak, D., Kamphorst, A.O., Heidkamp, G.F., Buchholz, V.R., Trumpfheller, C., Yamazaki, S., Cheong, C., Liu, K., Lee, H.W., Park, C.G., Steinman, R.M., Nussenzweig, M.C., 2007. Differential antigen processing by dendritic cell subsets in vivo. Science 315, 107–111.

Hawiger, D., Inaba, K., Dorsett, Y., Guo, M., Mahnke, K., Rivera, M., Ravetch, J.V., Steinman, R.M., Nussenzweig, M.C., 2001. Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo. J. Exp. Med. 194 (769).

Huang, Z., Panda, A., Elankumaran, S., Govindarajan, D., Rockemann, D.D., Samal, S.K., 2004. The hemagglutinin-neuraminidase protein of Newcastle disease virus determines tropism and virulence. J Virol. 78, 4176–4184.

Jáuregui-Zúñiga, D., Pedraza-Escalona, M., Espino-Solís, G.P., Quintero-Hernández, V., Olvera-Rodríguez, A., Díaz-Salinas, M.A., López, S., Possani, L.D., 2017. Targeting antigens to Dec-205 on dendritic cells induces a higher immune response in chickens: hemagglutinin of avian influenza virus example. Res Vet Sci. 111, 55–62.

Lamb, R., Parks, G., 2007. Paramyxoviridae: the viruses and their replication. In: Knipe, D.M., Howley, P.M., Griffin, D.E., Lamb, R.A., Martin, M.A., Roizman, B., Straus, S.E. (Eds.), Fields Virology, 5th ed. Lippincott Williams & Wilkins, Philadelphia, pp. 1449–1496.

Leeuw, O.S., Peeters, B.P.H., 1999. Complete nucleotide sequence of Newcastle disease virus: evidence for the existence of a new genus within the subfamily paramyxovirinae. J. Gen. Virol. 80, 131–136.

Mahnke, K., Guo, M., Lee, S., Sepulveda, H., Swain, S.L., Nussenzweig, M., Steinman, R.M., 2000. The dendritic cell receptor for endocytosis, DEC-205, can recycle and enhance antigen presentation via major histocompatibility complex class II-positive lysosomal compartments. J. Cell Biol. 151, 673.

Matos, I., Mizenina, O., Lubkin, A., Steinman, R.M., Idoyaga, J., 2013. Targeting leishmania major antigens to dendritic cells in vivo induces protective immunity. PLoS One 8 (6), e67453.

Panda, A., Elankumaran, S., Krishnamurthy, S., Huang, Z., Samal, S.K., 2004a. Loss of N-linked glycosylation from the hemagglutinin-neuraminidase protein alters virulence of Newcastle disease virus. J. Virol. 78, 4965–4975.

Panda, A., Huang, Z., Elankumaran, S., Rockemann, D.D., Samal, S.K., 2004b. Role of fusion protein cleavage site in the virulence of Newcastle disease virus. Microb Pathog. 36, 1–10.

Reed, L.J., Muench, H., 1938. A simple method of estimating fifty per cent endpoints. Am. J. Hyg. 27, 493–497.

Romer-Oberdorfer, A., Werner, O., Veits, J., Mebatsion, T., Mettenleiter, T.C., 2003. Contribution of the length of the HN protein and the sequence of the F protein cleavage site to Newcastle disease virus pathogenicity. J. Gen. Virol. 84, 3121–3129.

Staines, K., Young, J.R., Butter, C., 2013. Expression of chicken DEC205 reflects the unique structure and function of the avian immune system. PLoS One 8, e51799.

Steinman, R.M., Banchereau, J., 2007. Taking dendritic cells into medicine. Nature 449, 419–426.

Steinman, R.M., Hawiger, D., Nussenzweig, M.C., 2003. Tolerogenic dendritic cells. Annu. Rev. Immunol. 21, 685–711.

Swiggard, W.J., Mirza, A., Nussenzweig, M.C., Steinman, R.M., 1995. DEC-205, a 205 kDa protein abundant on mouse dendritic cells and thymic epithelium that is detected by the monoclonal antibody NLDC-145 Purification, characterization and N-terminal amino acid sequence. Cell. Immunol. 165, 302–311.

Thayer, S.G., Beard, C.W., 1988. Serologic procedures. In: Swayne, D.E., Glisson, J.R., Jackwood, M.W., Pearson, J.E., Reed, W.M. (Eds.), A laboratory manual for the isolation and identification of avian pathogens, 4th ed. American association of avian pathologist, Kennett Square.

Weisser, N.E., Hall, J.C., 2009. Applications of single-chain variable fragment antibodies in therapeutics and diagnostics. Biotechnol. Adv. 27, 502–520.

World Organisation for Animal Health (OIE), 2017. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Available at:. OIE, Paris. http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.03.14_NEWCASTLE_DIS.pdf.

Yokota, T., Milenic, D.E., Whitlow, M., Schlom, J., 1992. Rapid tumor penetration of a single-chain Fv and comparison with other immunoglobulin forms. Cancer Res. 52, 3402–3408.