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Targeting antigens to an invariant epitope of the MHC Class II DR molecule potentiates the immune response to subunit vaccines

Félix Gil[a,1], Mariano Pérez-Filgueira[a,b,2], María G. Barderas[a,1], Carlos Pastor-Vargas[c], Covadonga Alonso[a], Fernando Vivanco[c,d], José M. Escribano[a,*]

[a] Departamento de Biotecnología, INIA, Autovía A6 Km7, 28040 Madrid, Spain
[b] Consejo Nacional de Investigaciones Científicas y Técnicas, Av. Rivadavia 1917, Buenos Aires (1033), Argentina
[c] Department of Biochemistry and Molecular Biology, Universidad Complutense, Madrid, Spain
[d] Departmento de Inmunología, Fundación Jiménez Díaz, 28040 Madrid, Spain

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ABSTRACT

Recombinant subunit and peptidic vaccines in general present a reduced immunogenicity in vaccinated individuals with respect to the whole pathogen from which they derived. The generation of strong immune responses to these vaccines requires the use of potent adjuvants, high antigen doses and repetitive vaccinations. In this report, we document the enhanced antibody response obtained against two recombinant subunit vaccines by means of targeting to antigen-presenting cells by a recombinant single chain antibody. This antibody, named APCH1, recognizes an epitope of MHC Class II DR molecule preserved in different animal species, including humans. We showed that vaccinal antigens translationally fused to APCH1 antibody and produced by recombinant baculoviruses in insect larvae (*Trichoplusia ni*), elicited an increased antibody response in comparison with the same antigens alone or fused to a carrier molecule. These results suggest that targeting of antigens to this invariant MHC Class II epitope has immunopotentiating effects that could circumvent the reduced potency of peptidic or subunit vaccines, opening the possibility of widespread application of APCH1 as a new adjuvant antibody of general use.

1. Introduction

The generation of strong serological responses to protein or peptide antigens in humans or animals usually requires the use of potent adjuvants, most of which cannot be used in commercial vaccines because of deleterious side effects they may cause. The antibody response to most protein antigens requires specific cooperation between B and T cells. In order to deliver the helper signal, $CD4^+$ T cells must recognize processed antigens in the context of the major histocompatibility complex Class II molecules (MHC II) found on the membrane of antigen-presenting cells (APCs).

Antigens may be targeted to APCs through their conjugation to monoclonal antibodies (mAbs) directed against surface molecules on these cells (Barber, 1997; Fossum et al., 1992). Following this idea, different membrane proteins were studied as targets with different effects observed among them (Skea and Barber, 1993). These results were basically related to the chance of each of these surface molecules to be endocyted and the particular pathways they may take upon cellular endocytosis (Snider and Segal, 1989).

In particular, MHC II-targeted antigens were described to enhance antigen presentation and induction of antibody responses against peptide and protein antigens (Carayanniotis and Barber, 1987, 1990; Carayanniotis et al., 1988). This approach was reported to generate potent recombinant subunit vaccines that could be produced in adjuvant-free formulations. Targeting to MHC II molecules also produced specific effects on the Ig isotype profiles induced and even in the generation of long-term memory responses (Skea and Barber, 1993; Snider et al., 1997).

In the present work, we present the development of the recombinant single chain antibody (scFv) APCH1, directed to an invariant epitope of the porcine MHC II DR molecule also preserved in other species. The APCH1 molecule had the ability to drive vaccine antigens to APCs, increasing the specific antibody responses against peptide and subunit vaccine antigens in two different animal models. Our results show that humoral responses were dramatically augmented for peptide antigens fused to APCH1 and that the induced humoral responses can provide protection to the natural host after challenge with virulent rabbit hemorrhagic disease virus (RHDV) when using sub-protective doses of a subunit vaccinal antigen.

* Corresponding author. Tel.: +34 913473917; fax: +34 913478711.
E-mail address: escriban@inia.es (J.M. Escribano).
[1] Present address: Laboratorio de Fisiopatología Vascular, Hospital Nacional de Parapléjicos, SESCAM, Toledo, Spain.
[2] Present address: Instituto de Virología, ClCVyA, INTA, N. Repetto y De Los Reseros s/n, Hurlingham (1686), Buenos Aires, Argentina.

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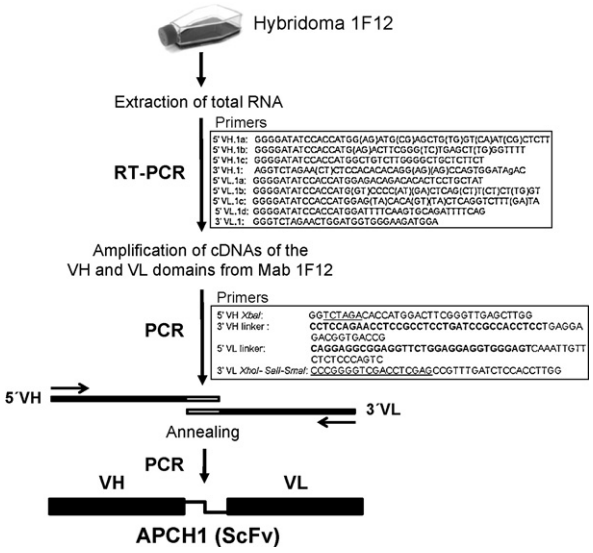

Fig. 1. Cloning strategy of APCH1 single chain antibody from hybridoma 1F12.

2. Materials and methods

2.1. Cloning of VH and VL domain sequences of IF12 and construction of scFv APCH1

The hybridoma producing the monoclonal antibody (mAb) 1F12 (Brodersen et al., 1998; Bullido et al., 1997) with specificity for a porcine MHC II DR molecule epitope was kindly provided by Dr. Javier Domínguez (Biotechnology Department, INIA, Spain). Hybridoma cells were cultured in RPMI 1640 medium supplemented with 0.01 mM piruvic acid (SIGMA), 2 mM L-glutamine and antibiotics. The recombinant single chain (scFv) version of mAb 1F12 was obtained as previously described (Froyen et al., 1993; Chaudhary et al., 1990) (Fig. 1). Briefly, total RNA was extracted from disrupted cells according to the Qiagen RNeasy extraction kit (Qiagen) protocol. All samples were subjected to on-column DNase digestion using the Qiagen RNase free DNase treatment (Qiagen) during RNA isolation. Isolated RNA was quantified and 1 μg was used as a template in a reverse transcriptase reaction using AMV-RT polymerase (Promega) to produce the DNA copies (cDNA) from total mRNA obtained from the hybridoma cells.

Copy DNA fragments corresponding to the mAb 1F12 VH and VL domains were obtained by PCR using as template the cDNA described above. For this purpose, a set of primers comprising constant murine VH and VL sequences was used in different PCR DNA amplifications (Fig. 1).

After sequence analysis of some colonies transformed with pGEM VH and pGEM VL, a new set of primers was designed (Fig. 1). These primers allowed us to fuse the VH and VL domain of MAb IF12 into scFv antibody fragments via a flexible and synthetic hinge of Ser-Gly repetition in different PCR steps. The resulting APCH1 construct (scFv from mAb 1F12) was assembled with the VH-(Gly_4-Ser_3)-VL arrangement and cloned into pGEM-T easy vector to obtain pGEM APCH1, which was used to transform DH5α cells. This vector was used to fuse different vaccine polypeptides in frame with the scFv APCH1 antibody.

2.2. Recombinant baculovirus

Production of recombinant baculovirus expressing the RHDV AST/89 VP60 (Barcena et al., 2000) was previously described (Perez-Filgueira et al., 2007). The DNA sequence encoding for the canine parvovirus (CPV) VP2-derived peptide 2L21 (Langeveld et al., 1995; Langeveld et al., 1994) fused to the β-glucuronidase (βGUS) gene was obtained from plasmid pBI2L21-GUS (Gil et al., 2001). The 2L21-GUS chimeric gene was cloned into the baculovirus transfer plasmid *pBacPak* 8 (Clontech) under control of the polyhedrin promoter, using the restriction enzyme sites *Xba*I and *EcoR*I. DNA sequences for the full length VP60 protein from RHDV AST/89 strain or for the 2L21 epitope flanked by *Xba*I and *Sma*I restriction sites were produced by PCR and translationally fused to 3′ end of the APCH1 gene through a pGem intermediate plasmid (Clontech). Both APCH1-fusion genes were finally transferred to the *pBacPak 8* transfer vector and corresponding recombinant baculoviruses were obtained by homologous recombination as previously described (Oviedo et al., 1997). A *pBacPak 8* vector without insert was used to produce a control baculovirus (BacWT) following the same protocol. Recombinant baculoviruses were propagated and amplified in sf21 insect cells to reach infective titers of $\sim 10^8$ pfu/ml (O'Reilly et al., 1992) and stocks were kept at 4 °C for daily use and at −80 °C for long-term storage.

2.3. Insect growth conditions and inoculation

Trichoplusia ni (*T. ni*; Cabbage looper) larvae were reared following previously described methodology (Gomez-Sebastian et al., 2008; Perez-Filgueira et al., 2006). For all experiments, fifth-instar larvae (last instar larvae before pupation) of about 300 mg weight, were injected near the proleg (forward the body cavity) with 10 μl of recombinant baculovirus diluted to reach the number of pfu per dose selected. Infected larvae were kept in growth chambers at 28 °C and collected at the indicated times. Larvae were immediately frozen and kept at −20 °C until processed.

For time–dose assays, groups of 20 larvae were infected with 10^5, 10^4 or 10^3 pfu of the different baculoviruses, separated into 4 groups of 5 larvae each and finally processed at 24, 48, 72 and 96 hpi. Total protein extracts were analyzed for production of APCH1-fusion proteins by Western blot assay.

2.4. Analysis of protein extracts

Total soluble protein (TSP) fractions were obtained from *T. ni* larvae as described previously (Perez-Filgueira et al., 2006). Total concentration of extracted protein was quantified by Bradford assay (Bradford, 1976).

Quantification of recombinant proteins production in larva was performed by capillary electrophoresis using the Experion system (Bio-Rad, USA) following the manufacturer's instructions. Briefly, crude protein samples (1 μl) were mixed with sample buffer provided, heated and diluted to 1/90 in deionized water, and 6 μl of each diluted sample was loaded in Pro260 chips (Bio-Rad, USA) for analysis.

For Western blot assays, 20 μg of TSP per lane were resolved by SDS-PAGE and transferred into nitrocellulose membranes (Bio-Rad, USA). Membranes were blocked overnight at 4 °C with PBS–0.05% Tween 20 (PBST) and 4% skim milk (blocking buffer, BF) and then incubated at room temperature (RT) for 1 h using monoclonal antibodies (mAbs) 2E11 for VP60 and 3C9 for peptide 2L21 at 1:200 (Ingenasa, Spain). Blots were then washed 3 times with PBST and anti-mouse IgG-horseradish peroxidase (HRP)-labeled conjugate diluted 1:4000 in BF (KPL, UK) was finally added for 1 h as secondary antibody. After extensive washing with PBST, protein bands were detected using ECL Western-blotting detection system on Hyperfilm ECL films (Amersham, USA).

2.5. Analysis of recombinant APCH1-2L21 molecule binding to antigen-presenting cells

To determine whether recombinant APCH1 scFv retained the MHC II-binding capacity; we carried out fluorescence and immuno-