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Design, expression, and characterization of a novel dendritic cell-targeted proteins

Yu Wang [1], Xiao Ming Zhu [1], Fang Wang [1], Shuai Wu, Zi Cheng Wang, Zhi Yan Du, Jin Qi Yan*, Ji Yun Yu*

Beijing Institute of Basic Medical Sciences, 27 Tai Ping Road, Beijing 100850, China

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ABSTRACT

In vivo approaches to inducing an effective immune response focus on targeted antigen (Ag) delivery to dendritic cells (DCs). In this study, we developed a new method of targeting plasmid DNA and/or the antigen (Ag)–antibody (Ab) complex to DCs via the DC receptor DEC-205, also known as cluster of differentiation CD205. We cloned and expressed a recombinant protein composed of mouse DEC-205-specific single-chain fragment variable region (mDEC-205-scFv), the streptococcal protein G (SPG) IgG-binding domain and cationic peptide (CP), which named mDEC205-scFv-SPG-CP (msSC). In vitro, the recombinant protein msSC can specifically bind to DCs through the section of mDEC-205-scFv, and bound the Ag–Ab complex via SPG as well as plasmid DNA through electrostatic bonding with CP in vitro. In addition, msSC functioned in a manner similar to anti-DEC-205 monoclonal Ab and bound to mouse bone marrow-derived DCs. It was demonstrated in vivo that msSC can target plasmid DNA to DCs, resulting in efficient uptake and expression. Moreover, msSC can form a complex with pGL3-CMV and transport it to draining lymph nodes when injected in vivo. These results indicate that msSC can be used as a carrier protein for vaccine delivery to DCs via formation of plasmid DNA-Ag–Ab ternary complexes.

1. Introduction

Various methods are used to deliver known antigen (Ag) to a site of infection or tumor formation [1], including electroporation of plasmid DNA injected into muscle, Ag–antibody (Ab) coupling, and loading dendritic cells (DCs) with tumor-associated Ags or whole tumor cells in vitro [2–4]. However, these techniques have several drawbacks such as non-specific Ag targeting and dilution or degradation of the Ag as well as high cost, and are often complex procedures that require special equipment [1]. Recent strategies for inducing an effective immune response in vivo have focused on targeted delivery of Ags to cluster of differentiation CD40 [5] or the DC-specific intercellular adhesion molecule 3-grabbing non-integrin in the surface of DCs [6]. However, these molecules lack specificity as they are expressed by monocytes, eosinophils, and fibroblasts. Nonetheless, CD205 can be used to specifically target the DC receptor DEC-205, which is an endocytic receptor belonging to the mannose receptor family that is highly expressed in DC cells [7,8]. Ag coupled to an anti-DEC205 monoclonal Ab induced an Ag-specific immune response that eliminated metastatic melanoma in a mouse model [4]. While effective, this method depends on consistent and efficient coupling between Ag and Ab [1].

In the present study, the DC-targeted recombinant protein msSC was constructed to improve the efficiency of vaccine uptake by DCs and hence enhance the immune response. msSC consists of mouse DEC-205-specific single-chain fragment variable region (mDEC-205-scFv) [1,9], streptococcal protein G (SPG) immunoglobulin (Ig) G-binding domain [10,11], and cationic peptide (CP) [12,13]. The mDEC-205-scFv domain can specifically bind the DC surface molecule DEC-205, thereby targeting the Ag to DCs. mSC contains an SPG domain that binds IgG to enable efficient binding to Ag–Ab complexes [14–16], as well as a cationic peptide that is capable of binding negatively charged plasmid DNA. This fusion protein has several advantages. First, it can decrease non-specific binding to receptors or cells and improve DNA uptake and Ag-presenting capacity. Secondly, it can bind plasmid DNA and Ags simultaneously to synergistically induce an immune response, thereby promoting cellular and humoral immunity.

* Corresponding authors.
E-mail addresses: yanjinqi@sohu.com (J.Q. Yan), yujyun@126.com (J.Y. Yu).
[1] These authors contributed equally to this work.

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We demonstrate that msSC can bind Ag–Ab complexes through the SPG domain and plasmid DNA through electrostatic coupling. In addition, msSC functioned in a manner similar to anti-DEC-205 monoclonal Ab bound to mDEC-205, effectively binding to mouse bone marrow-derived (mBM)DCs, and enhanced the uptake of plasmid DNA by DCs and the transport of plasmid DNA to draining lymph nodes when injected in vivo. These results indicate that msSC can be used for targeted delivery of vaccines to DCs to eliminate infections or tumors.

2. Materials and methods

2.1. Ethics statement

Pathogen-free female BALB/c mice were obtained from the Experimental Animal Center of Military Medical Sciences. Animals used in this study were 4–6 weeks old and maintained in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication no. 85-23, Revised 1996). Experimental procedures conformed to international guidelines for the care and use of laboratory animals and were approved by the Animal Ethics Committee of Beijing Institute of Basic Medical Sciences.

2.2. Plasmids and reagents

pUC57-Amp was obtained from Genewiz, Inc. (South Plainfield, NJ, USA). pET-28a was from Novagen (Billerica, MA, USA). pGL3-CMV containing the luciferase reporter gene for in vivo DC targeting of msSC was purchased from Promega (Beijing, China). pSVK-HBVA (16-kb DNA with hepatitis B virus [HBV] fusion Ag) for the gel retardation assays was constructed by our laboratory. *Escherichia coli* DH5α was used for cloning and transformation of recombinant plasmids. *E. coli* BL21 (DE3) pLysS from a laboratory stock was used for protein expression. Monoclonal rat anti-mouse DEC205 Ab was purchased from eBioscience (San Diego, CA, USA). Mouse anti-His tag antibody, horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG, and HRP-conjugated human and mouse IgG were purchased from Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd. (Beijing, China). Recombinant HB surface Ag (HBsAg) was obtained from Beijing Wantai Bio-Pharmaceutical Co., Ltd. (Beijing, China). Human HB immunoglobulin (HBIg) was obtained from the Chinese Biotechnology Company.

2.3. Media and growth conditions

DH5α cells transformed with pUC57-Amp were incubated in Luria broth (LB) with 100 μg/ml ampicillin at 37 °C. BL21 (DE3) pLysS cells transformed with pET-28a (+) were incubated in LB with 50 μg/ml kanamycin at 37 °C. Protein expression was induced by addition of isopropyl-β-d-thiogalactoside (IPTG) at a final concentration of 1 mM.

2.4. Expression plasmid construction

Recombinant mDEC205-scFv-SPG-CP (msSC) encodes three proteins, including mDEC-205-scFv [17], SPG containing the IgG-binding domain (GenBank accession no.: CAA27638.1), and CP (RSQSRSRYYRQRQRSRRRRRRS) (Fig. 1). mDEC-205-scFv was fused to SPG-CP with the linker (GGGGS)3 to generate msSC. BamHI and SacI restriction sites were introduced at the 5′ and 3′ ends, respectively, of the fusion gene. Following *E. coli* codon optimization, the fused gene was synthesized by Genewiz Inc. (South Plainfield, NJ, USA) and cloned into pUC57-Amp vector. Correct insertion of the DNA fragment was confirmed by sequencing. The fragment with BamHI and SacI sites in pUC57-Amp was subcloned into the pET-28a expression vector to generate pET-28a-msSC.

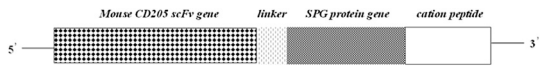

Fig. 1. Schematic illustration of the protocol used to generate msSC fusion protein.

2.5. Expression and purification of mDEC205-scFv-SPG-CP (msSC) protein

pET-28a-msSC was transformed into *E. coli* BL21(DE3) pLysS cells to generate the expression strain BL-pET-28a-msSC. A single colony was inoculated into 2 ml LB containing 50 μg/ml kanamycin at 37 °C for 12 h. Cells were then inoculated into 100 ml fresh LB with kanamycin until mid-log phase (absorbance at 600 nm of about 0.6–1.0) and induced by addition of IPTG. The cells were cultured for an additional 4–6 h and harvested by centrifugation at 8000 × g for 10 min. *E. coli* cells were re-suspended in 10 mM phosphate-buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, and 2 mM KH2PO4, pH 7.4) and lysed by sonication for 30 min, followed by centrifugation at 8000 × g for 10 min at 4 °C. The cell pellet was washed with PBS containing 2 M urea followed by one wash in 4 M urea. Cell debris was dissolved in 8 M urea and centrifuged at 12,000 × g for 3 min at 4 °C. The protein in the supernatant was dialyzed sequentially against 10 mM PBS containing 6, 4, 2, and 0 M urea at 4 °C. Optimal dilutions were determined to prevent aggregation during refolding. Protein concentration was determined by UV absorption at 280 and 260 nm (reference: 320 nm) on a spectrophotometer (2800 UV/VIS, Shanghai Sunny Hengping Scientific Instrument Co., Ltd., Shanghai, China). msSC was purified from BL-pET-28a-msSC cell inclusion bodies and detected by western blotting using a monoclonal Ab against His and visualized with HRP-conjugated goat anti-mouse secondary Ab and an enhanced chemiluminescence detection system.

2.6. Cytotoxicity assay

Cell viability was assessed using Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's instructions. HepG2 cells (5 × 103 per well) were seeded on 96-well plates with 100 μl media one day prior to adding mSC protein. The next day, cells were incubated with 15, 30, 60, or 120 μg/ml msSC protein, for 3 h at 37 °C and 5% CO_2. Water-soluble Tetrazolium Salt 8 reagent was then added, followed by a 3-h incubation. The number of viable cells was measured with a microplate reader (Bio-Rad, Hercules, CA, USA) at optical density (OD) 450. Data are expressed as mean ± standard deviation (SD) of three measurements.

2.7. Determination of msSC binding to human and mouse IgG by enzyme-linked immunosorbent assay (ELISA)

A microplate was coated with 100 μl of 10 mM PBS containing 10 μg/ml msSC fusion protein and incubated overnight at 4 °C. After blocking with 5% not-fat milk powder for 2 h at room temperature, serial dilutions of HRP-conjugated human or mouse IgG (1:10–1:5120) were added to the microplate, followed by a 1-h incubation at 37 °C. The solution was reacted by adding o-phenylenediamine dihydrochloride substrate (Sigma–Aldrich, St. Louis, MO, USA), and msSC binding activity was measured at OD_{450} with a