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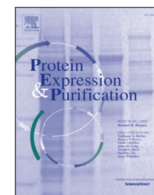

Expression and antigenicity of recombinant human respiratory syncytial virus glycoproteins having different affinity tags

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ABSTRACT

Human respiratory syncytial virus (HRSV) is a main cause of lower respiratory tract infections in infants and the elderly. Glycoprotein (G) is major antigen on the viral surface, and plays a key role for virus entry. Therefore, purification of the glycoprotein of HRSV is critical for the development of HRSV vaccine and serological diagnosis. In this study, we report the design and characterization of glycoprotein engineered rationally to enhance the protein solubility and to facilitate efficient purification. We permuted HRSV glycoproteins with two tags: (i) an immunoglobulin (Ig) M signal peptide and a protein A B domain tag to render HRSV glycoprotein secret into the culture media and (ii) a foldon and 6 × histidine tag with or without transmembrane domain. Three recombinant baculoviruses were constructed: (i) transmembrane-truncated HRSV glycoprotein (amino acid positions 66–298) inserted with the N-terminal IgM signal peptide and protein A B domain (MG-GΔTM), (ii) truncated HRSV glycoprotein (amino acid positions 66–298) fused with a C-terminal foldon and 6 × histidine tag (GΔTM-FH), and (iii) full-length HRSV glycoprotein (amino acid positions 1–298) fused with a C-terminal foldon and 6 × histidine tag (G-FH). Highly soluble recombinant MG-GΔTM protein was clearly purified using one-step affinity chromatography with IgG-sepharose resin, whereas the recombinant G-FH protein and truncated GΔTM-FH were purified partially using nickel-resin. Although, the antigenicity of GΔTM-FH was stronger than highly mannose-rich MG-GΔTM protein, MG-GΔTM induced neutralizing antibodies efficiently in the mice to protect from infectious HRSV.

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1. Introduction

Human respiratory syncytial virus (HRSV) is an enveloped, negative-strand, 15.2-kb, RNA virus of the family *Paramyxoviridae*, subfamily *Peunovirinae*. The HRSV genome consists of 11 genes in the order of nonstructural accessory proteins (NS1 and NS2), nucleoprotein (N), phosphoprotein (P), matrix protein (M), short transmembrane glycoprotein (SH), fusion protein (F), attachment glycoprotein (G), RNA synthesis factors (M2-1 and M2-2), and large RNA polymerase (L) [1].

HRSV is a major causative agent of severe respiratory illness in infants and elderly worldwide [2–4]. In addition, HRSV plays a role in severe eosinophilia, chronic obstructive pulmonary disease, and asthma [5]. Although researchers have attempted to develop HRSV vaccines, no effective and safe vaccines are yet available, particularly those evoking a protective immune response with neutralizing antibodies. In a clinical trial involving the use of a formalin-inactivated aluminum-precipitated HRSV (FI-RSV) vaccine candidate in the 1960s [6], major safety concerns were noted. Thus, safe vaccine candidates, such as subunit vaccines or virus-like particles (VLPs), are required urgently. To this end, both HRSV glycoproteins and fusion proteins have been investigated as potential antigenic viral proteins that may allow the induction of sufficient neutralizing antibodies and a protective immune response to HRSV infection [7–10].

HRSV attachment glycoproteins (HRSV-G) are highly diverse, however, they are also strongly antigenic. HRSV glycoproteins can be classified into subgroups A and B [11]. They consist of an N-terminal cytoplasmic domain (amino acid positions1–37), a hydrophobic signal/anchor (amino acid positions 38–63), and an ectodomain (amino acid positions 64–298) glycosylated heavily

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with N- and O-linked glycans as a type II transmembrane protein [12,13]. These glycoproteins support viral attachment and membrane fusion to the cell surface by interacting with a pivotal HRSV fusion protein [14–16]. In previous reports, VLPs bearing HRSV glycoproteins showed an effective vaccination in mouse animal models [9], and monoclonal anti-HRSV glycoprotein antibodies were shown to have potential applications in the prophylactic treatment of pulmonary inflammation caused by HRSV infection in animal models [17,18]. Therefore, HRSV glycoprotein is an attractive protein target for the development of vaccine candidates and passive immunotherapy. The critical factor for the development of HRSV vaccination is the design of an optimal recombinant HRSV glycoprotein and its purification system.

Recombinant HRSV glycoprotein has been cloned and expressed successfully in *Escherichia coli* [10], insect cells [7,19], yeasts [20], and mammalian cells [21,22]. However, these studies have shown that HRSV glycoprotein having a 6 × histidine tag was highly insoluble and that mammalian cell expression systems exhibit insufficient protein production [22].

In this study, we cloned three recombinant HRSV glycoproteins fused with two different affinity tags, (i) IgM signal peptide-*Staphylococcus aureus* protein A B domain (MG). It was hypothesized that an MG fusion would render the HRSV-G protein to be secreted into media, and would enhance the solubility of the HRSV G protein and purification by the IgG-sepharose resin. (ii) Foldon-6 × histidine (FH), which is also widely used for its convenient purification abilities using metal affinity chromatography and for the enhancement of solubility. Recombinant baculoviruses (rBVs) that overproduce HRSV A2-G were constructed to investigate protein expression efficiency and glycosylation patterns. Moreover, we evaluated the antigenicity of recombinant HRSV glycoproteins in a BALB/c mouse model.

2. Methods

2.1. HRSV preparation

The HRSV A2 strain (#VR-2450; ATCC, Manassas, VA, USA) was cultured in Hep-2 cells (#CCL-23; ATCC). On day 2 or 3 after infection at a multiplicity of infection (MOI) of 0.01, HRSV was harvested from the cell culture supernatant, and the virus titer was determined by the infection of Hep-2 cell monolayers followed by a low-melting agarose plaque assay. The virus was inactivated with 0.01% formalin, and gentle stirring for 72 h at 37 °C in an incubator. The formalin-fixed virus was collected by suspension with phosphate-buffered saline (PBS) after centrifugation as previously described [23] and used as the antigen of immunoblots and in enzyme-linked immunosorbent assays (ELISAs).

2.2. Insect cell cultures

Spodoptera frugiperda (Sf) 21 cells and HighFive cells (#B85502; Invitrogen, Carlsbad, CA, USA) were cultured in Sf-900 II SFM media (#10902088; Invitrogen) containing 10 μg/mL gentamycin at 28 °C in an incubator. Sf21 cells were used to produce rBVs, whereas the HighFive cells were used for the expression of the recombinant HRSV glycoproteins after infection with the rBVs at an MOI of 1.0.

2.3. Construction of rBVs containing full and truncated HRSV glycoproteins

To enhance the solubility of the HRSV glycoprotein, a thrombin cleavage site and FH tag were subcloned after the N-terminal transmembrane domain (TM; amino acid positions 1–65)-deleted glycoprotein gene. Briefly, long primers containing *Kpn*I and *Hind*III sites were annealed by cooling slowly after boiling for 10 min. These long primers for amino acids, i.e., TGRLVPRGSPGSGYIPEAPRDGQAYVRKDGEWVLLSTFLGHHHHHH, were subcloned into the *Kpn*I-*Hind*III sites of the pFastBac1 vector, yielding the pFastBac-FH vector. The sequences of these primers were 5′-AGGGTACCGGTCGTCTGGTGCCCCGTGGTTCCCCCGGTTCCGGTTACATCCCCGAGGCTCCCCGTGACGGTCAGGCTTACGTGCGTAAGGACGGTGAGTGGGTGCTGCTCAGCACCTTCCTGGTGCACCACCACCACCACCACTAATAAGCTTGT-3′ for the forward primer and 5′-ACAAGCTTATTAGTGGTGGTGGTGGTGGTGCACCAGGAAGGTGCTGAGCAGCACCCACTCACCGTCCTTACGCACGTAAGCCTGACCGTCACGGGGAGCCTCGGGGATGTAACCGGAACCGGGGGAACCACGGGGCACCAGACGACCGGTACCCT-3′ for the reverse primer. Then, their *BamH*I-*Kpn*I restricted fragments of the HRSV glycoprotein polymerase chain reaction (PCR) products were inserted into the *BamH*I-*Kpn*I sites of the pFastBac-FH vector. The sequences of the primers used for PCR of the glycoprotein were as follows: FH-HRSV-A-forward; 5′-AGGGATCCAACCACAAAGTCACACCAACAACTGC-3′, FH-HRSV-A-reverse; 5′-AAGGTACCCTGGCGTGGTGTGTTGGGTGG-3′ for the N-terminal TM-deleted HRSV glycoprotein gene, and FF-HRSV-A-forward; 5′-AGGGATCCATGTCCAAAAACAAGGACCAACGC-3′, FF-HRSV-A-reverse; 5′-AAGGTACCCTGGCGTGGTGTGTTGGGTGG-3′ for the full-length HRSV glycoprotein gene. The restriction enzyme sequences were underlined. The PCR products were sequenced using the polyhedrin promoter and SV40 poly A primers. The generation, preparation, and titration of the rBV vector were performed using a Bac-to-Bac baculovirus expression system (#10359-016; Invitrogen) according to the manufacturer's instructions. Recombinant bacmids were generated in DH10Bac cells (#10361-012; Invitrogen). These bacmids encoding truncated glycoproteins were transfected into Sf21 cells in 6-well plates using the Cellfectin transfection reagent (#10362-100; Invitrogen). Primary rBVs were collected on post-transfection day 3, and viral stocks were amplified by infection into new large-scale Sf21 cell cultures. rBV-GΔTM-FH was generated and expressed N-terminal transmembrane region-truncated HRSV A2-G fused with the FH tag.

The TM-truncated HRSVA2-G (amino acid positions 66–298) was amplified by PCR using an MG-HRSVA2-G forward primer bearing a thrombin cleavage site; 5′-ATGGGGATCCTGGTGCCCCGTGGTTCCGAATTCGGTCAAAACATCACTGAAGAATTTTAT-3′ and a reverse primer; 5′-AATGGTACCCTACATGATATTTGTGGTGGATTTACC-3′. After digestion with restriction enzymes, *EcoR*I and *Kpn*I, the PCR products were cloned into the corresponding enzyme sites of pFastBac-MGa (Sogang University, Seoul, Korea) [24]. The resulting recombinant transfer vector was named pFastBac-MG-GΔTM. rBV-MG-GΔTM was constructed by tagging the IgM signal peptide and the B domain of the *Staphylococcal* protein A (MG), which specifically binds to IgG, to the N-terminal of GΔTM.

2.4. Preparation of the crude extract of insect cells infected with rBVs

For the expression of rBVs, 3×10^7 HighFive cells were seeded in a T175 flask. After infection with the recombinant virus at an MOI of 1.0, the cells were grown at 28 °C for 72 h. Crude extracts of insect cells infected with the rBVs were collected after treating the cells for 10 min with radio-immunoprecipitation assay (RIPA) buffer (50 mM Tris-HCl, pH 8.0, 150 mM sodium chloride, 1.0% NP-40, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate; #R0278; Sigma-Aldrich, St. Louis, MO, USA) containing complete protease inhibitors (#04693124001; Roche, Indianapolis, IN, USA) and 2 U/mL Benzonase (#70746; Merck Millipore, Darmstadt, Germany) at room temperature. The extracts were sonicated for 2 min (12 cycles of 10 s sonication followed by 10s pause) on ice and centrifuged at

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