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Vaccination of mice with recombinant baculovirus expressing spike or nucleocapsid protein of SARS-like coronavirus generates humoral and cellular immune responses

Bingke Bai [a,b], Xinya Lu [a,b], Jin Meng [a,b], Qinxue Hu [a], Panyong Mao [c], Baojing Lu [a,b], Ze Chen [a], Zhiming Yuan [a], Hanzhong Wang [a,*]

[a] *State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, 430071 Hubei, PR China*
[b] *Graduate School of the Chinese Academy of Sciences, Beijing 100049, PR China*
[c] *Department of Virology, Institute of Infectious Disease, 302 Hospital, Beijing 100039, PR China*

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Abstract

Continuous efforts have been made to develop a prophylactic vaccine against severe acute respiratory syndrome coronavirus (SARS-CoV). In this study, two recombinant baculoviruses, vAc-N and vAc-S, were constructed, which contained the mammalian-cell activate promoter element, human elongation factor 1α-subunit (EF-1α), the human cytomegalovirus (CMV) immediate-early promoter, and the nucleocapsid (N) or spike (S) gene of bat SARS-like CoV (SL-CoV) under the control of the CMV promoter. Mice were subcutaneously and intraperitoneally injected with recombinant baculovirus, and both humoral and cellular immune responses were induced in the vaccinated groups. The secretion level of IFN-γ was much higher than that of IL-4 in vAc-N or vAc-S immunized groups, suggesting a strong Th1 bias towards cellular immune responses. Additionally, a marked increase of CD4 T cell immune responses and high levels of anti-SARS-CoV humoral responses were also detected in the vAc-N or vAc-S immunized groups. In contrast, there were significantly weaker cellular immune responses, as well as less antibody production than in the control groups. Our data demonstrates that the recombinant baculovirus can serve as an effective vaccine strategy. In addition, because effective SARS vaccines should act to not only prevent the reemergence of SARS-CoV, but also to provide cross-protection against SL-CoV, findings in this study may have implications for developing such cross-protective vaccines.

Keywords: Cellular immune response; Humoral immune response; Recombinant baculovirus; SARS-like coronavirus

1. Introduction

Baculovirus (autographa californica multiple nucleopolyhedrovirus, AcMNPV) expression systems have been widely used for producing recombinant proteins in insect cells (Hou et al., 2003; Li et al., 2003; Luckow and Summers, 1988; Miller, 1988; Ren et al., 2001), due to the high levels of expression and proper post-translational modification (Jones and Morikawa, 1996; Nakhai et al., 1991; Sridhar et al., 1994). In addition, baculoviruses can be efficiently taken up by mammalian cells without viral replication (Carbonell et al., 1985; Tjia et al., 1983; Volkman and Goldsmith, 1983). Modified AcMNPVs can express exogenous genes in mammalian cells (Boyce and Bucher, 1996; Delaney and Isom, 1998; Duisit et al., 1999; Hofmann et al., 1995; Shoji et al., 1997) when they contain promoters that are active in mammalian cells, such as Rous sarcoma virus (RSV) promoter and cytomegalovirus immediate-early (CMV-IE) promoter. For instance, AcMNPV-containing CMV-IE promoter can infect human hepatocytes and result in high-level expression of luciferase (Hofmann et al., 1995). Since it is safe, efficient, and gene delivery-specific, the use of baculoviruses may represent a novel vaccine strategy.

Severe acute respiratory syndrome coronavirus (SARS-CoV) is the causative agent of SARS (Drosten et al., 2003; Fouchier et al., 2003; Ksiazek et al., 2003; Peiris et al., 2003; Poutanen et al., 2003). To prevent another SARS epidemic, continuous

Abbreviations: N, nucleocapsid; S, spike; SARS-CoV, severe acute respiratory syndrome coronavirus; SL-CoV, SARS-like CoV.

* Corresponding author. Tel.: +86 27 87199239; fax: +86 27 87198072.
E-mail address: wanghz@wh.iov.cn (H. Wang).

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efforts have been made towards the development of a prophylactic vaccine. So far, vaccine studies have mainly focused on the two structural proteins, spike (S) and nucleocapsid (N), of SARS-CoV (Anton et al., 1996; Jackwood and Hilt, 1995). There is little knowledge regarding the immunogenicity of the N and S proteins of SARS-like coronavirus (SL-CoV) that is isolated from horseshoe bats, which are thought to be the possible animal reservoirs of SARS-CoV (Lau et al., 2005; Li et al., 2005; Ren et al., 2006). Since effective SARS vaccines should not only prevent the reemergence of SARS-CoV, but should also provide cross-protection against SL-CoV, we report the immunogenicity of N and S proteins of SL-CoV in mice using a modified baculovirus expression system.

2. Experimental

2.1. Materials and methods

2.1.1. Construction of recombinant baculoviruses

The EF-1α promoter sequence was amplified by PCR from vector pBudCE4.1 (Invitrogen, Carlsbad, CA) and the CMV promoter sequence was amplified from pcDNA3.1(+) (Invitrogen, Carlsbad, CA). The *gfp* gene was cloned into pFastBac DUAL vector under the control of the p10 promoter, designated pFB-GFP. The coding sequences of the N and S proteins of bat SL-CoV Rp3 strain (GenBank accession no. DQ071615) were amplified by RT-PCR and cloned into pFB-GFP under the control of the Polh promoter, respectively. The baculovirus promoters, p10 and Polh, were subsequently replaced by robust mammalian promoters, EF-1α and CMV, respectively. Recombinant viruses (vAc-N and vAc-S) were generated by transposition and transfection using the Bac-to-Bac system (Life Technologies). Virus was further amplified by propagation in Sf9 (*Spodoptera frugiperda*) cells, cultured in Grace's supplemented insect medium containing 10% (v/v) fetal bovine serum (HyClone). Virus titers were determined by plaque assay on Sf9 cells.

2.1.2. Transduction of BHK cells

Baby hamster kidney (BHK) cells were seeded in 35 mm plates for 12 h before transduction. Culture medium was replaced with virus inoculum and incubated at 37 °C for 2 h. After removal of virus, fresh medium was added and cultures were incubated at 37 °C for another 24 h before examining GFP expression under a fluorescence microscope (OLYMPUS IX51, Japan).

2.1.3. RT-PCR analysis

Total cellular RNAs from BHK cells, transduced with recombinant baculovirus, vAc-N or vAc-S, were isolated using TRIZOL® reagent (Invitrogen), and were further digested with RQ1 RNasefree DNase I (Promega) to remove residual DNA. cDNAs were synthesized with the MLV Reverse Transcriptase System, using random primers, according to the manufacture's instructions (Invitrogen). Subsequent PCR was performed, using primers specific for the S (nt 21,918–22,208) or N gene (nt 28,433–28,791) (GenBank accession no. AY278741). PCR primers were kindly provided by Dr. GuanYi, Hong Kong University. RT-PCR products were analyzed by 0.8% agarose gel. DNA maker DL2000 (D501A) was purchased form Takara.

2.1.4. Western blot analysis

After 24 h of transduction, BHK cells were harvested by centrifugation and raw proteins were extracted. These proteins were separated on 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes. Protein marker (SM0671) was purchased from Fermentas. The blots were blocked with 5% non-fat dry milk powder in TTBS (20 mM Tris–HCl, pH 8.0, 0.8% NaCl, 0.1% Tween-20) overnight at 4 °C, followed by 1 h incubation at room temperature with rabbit-derived antibody against SARS-CoV (kindly provided by Prof. L.F. Wang at the Australian Animal Health Laboratory). Following wash steps, the membrane was incubated with AP-conjugated anti-rabbit IgG (1:5000, Sigma) for 1 h and then developed in AP substrate for 5 min in the dark.

2.1.5. Immunization of mice

BALB/c mice ($n = 5$ per group) were subcutaneously and intraperitoneally immunized every 2 weeks with recombinant baculovirus, vAc-N or vAc-S, both at 7.5×10^6 pfu per dose. Mice were immunized four times in total for each condition. Ac-GFP (supernatants of Sf9 cells culture transfected with modified pFastBac DUAL, with *gfp* under the control of the Ef1α promoter) and PBS immunized groups were used as negative controls.

2.1.6. Enzyme-linked immunosorbent assay (ELISA)

Sera from immunized mice of different groups were collected before each immunization. The antibody titers for SARS-CoV were measured by enzyme-linked immunosorbent assay (ELISA). Briefly, 96-well microtiter plates (Costar) were coated with 200 ng per well inactivated SARS-CoV full antigens and incubated at 4 °C overnight. The plates were then blocked with PBS (pH 7.4) containing 1% BSA at room temperature for 2 h. Mouse sera, diluted 500-fold, were added to the wells and incubated at 37 °C for 45 min. Wells were rinsed five times with PBST (PBS containing 0.05% Tween-20), followed by a 30 min incubation with alkaline-phosphatase-conjugated goat-anti-mouse IgG (Sigma) (1:5000) at 37 °C. After five washings and a subsequent 15 min incubation with the substrate *para*-nitrophenyl phosphate (pNPP) solution at 37 °C in dark, reactions were terminated with a stop solution, and optical densities (ODs) were determined using a microplate reader set at 405 nm.

2.1.7. ELISPOT assay

Cellular immune responses to SARS-CoV were assessed by IFN-γ and IL-4 ELISPOT assays, using mouse splenocytes harvested 10 days after the final immunization. According to the instruction manual (U-CyTech, Netherlands), 96-well plates were coated overnight with 100 μl per well rat anti-mouse IFN-γ or rat anti-mouse IL-4 antibodies at 4 °C. Plates were washed