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Hemagglutinin Displayed Baculovirus Protects Against Highly Pathogenic Influenza

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ABSTRACT

Baculovirus (BV) replicating in insect cells can express a foreign gene product as part of its genome. The influenza hemagglutinin (HA) can be expressed from BV and displayed on the surface of baculovirus (HA-DBV). In this study we first generated six recombinant baculoviruses that expressed chimeric HAs with segments of the BV glycoprotein (gp64). The signal peptide (SP) and cytoplasmic tail (CT) domains of gp64 can enhance the display of HA from A/PR8/34 on BV surface, while the transmembrane (TM) domain of gp64 impairs HA display. Different doses of either live or β-propiolactone (BPL)-inactivated HA-DBV were administered to BALB/c mice. Live HA-DBV elicited higher hemagglutination-inhibition (HAI) titers than BPL-inactivated HA-DBV, and provided sterilizing protection. A second generation recombinant BV simultaneously displaying four HAs derived from four subclades of H5N1 influenza viruses was constructed. This tetravalent H5N1 HA-DBV vaccine elicited HAI titers against all four homologous H5N1 viruses, significantly decreasing viral lung titers of challenged mice and providing 100% protection against lethal doses of homologous H5N1 viruses. Moreover, mice vaccinated with HA-DBV had high levels of IFNγ-secreting and HA-specific CD8+ T cells. Taken together, this study demonstrates that HA-DBV can stimulate strong humoral, as well as cellular immune responses, and is an effective vaccine candidate for influenza.

1. Introduction

Each year, seasonal influenza causes over 300,000 hospitalizations and 36,000 deaths in the US alone [1]. The emergence of the novel H1N1 influenza virus in 2009 demonstrated how quickly a new influenza pandemic can sweep across the world. The spread of highly pathogenic H5N1 viruses in birds and coincident infections in humans have raised the concerns that H5N1 viruses may cause a new pandemic in humans. Vaccination is an effective method to prevent influenza infection. There are two influenza vaccine approaches licensed in the US; the inactivated, split vaccine and the live-attenuated virus vaccine. Inactivated vaccines can efficiently induce humoral immune responses but generally only poor cellular immune responses.

Baculoviruses are a family of large rod-shaped enveloped viruses with a large circular double-stranded DNA genome (80-200 kb). Baculoviruses infect some insects, but not mammals [2]. *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) is the most well studied baculovirus and most extensively used for protein expression because the polyhedron (PH) and p10 promoters are efficient promoters [3]. Compared to other protein expression approaches, the baculovirus expression vector system (BEVS) produces abundant protein yields with appropriate eukaryotic glycosylation and other modifications. Recently, this system has also been used for virus-like particle (VLP) production for vaccines against HIV, HPV and influenza [4–6]. However, the BV-derived VLPs are always accompanied with BV contamination. Therefore, separating VLPs from contaminating BV is one of the obstacles that need to be overcome.

Due to its low cytotoxicity and absence of pre-existing antibodies [7,8], AcMNPV has emerged as a potent vaccine vector [9–13]. Foreign immunogens or peptides can be displayed on the envelope of AcMNPV by fusion with the baculovirus major envelope protein gp64 [14,15]. Based on the baculovirus display system, some efficient vaccines have been studied not only for viral diseases, but also for parasitic disease, such as, classical swine fever virus [16], influenza virus [12,17–19], avian reovirus [11], bovine herpesvirus [20], *Plasmodium berghei* [13,14], and *Plasmodium fal-*

Abbreviations: AcMNPV, *Autographa californica* multiple nucleopolyhedrovirus; BEVS, baculovirus expression vector system; BPL, β-propiolactone; CT, cytoplasmic tail; CTL, cytotoxic T lymphocytes; DBV, displayed baculovirus; HA, hemagglutinin or hemagglutination assay; HAI, hemagglutination inhibition; IFU, infectious unit; PFU, plaque form unit; SP, signal peptide; TM, transmembrane; VLP, virus-like particle.

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Table 1
Primers used for PR8-HA displaying constructs

Primer	Primer sequence (5'-3')	Primer annotation
A	CGCTGATCAGCCACC**ATG**CTACTGGTAAATCAGTCACAC	Forward primer for gp64 Signal peptide with *Bcl* I site
B	CGAGCTCGTCGACAGGCCTGAATTCGGATCCCGCAAAGGCAGAATGCGCC	Reverse primer for gp64 Signal peptide with multiple cloning sites
C	CAGGCCTGTCGACGAGCTCGCGGCCGCGTTCATGTTTGGTCATGTAG	Forward primer for gp64 TM-CT with multiple cloning sites
D	AAGCGGCCG**TTA**ATATTGTCTATTACGGTTTCTAATC	Reverse primer for gp64 TM-CT with *Eag* I site
E	CAAGTCGACGCCACC***ATG***AAGGCAAACCTACTGGTCC	Forward primer for HA of PR8 virus with *Sal* I site
F	CTCGCGGCCGC**TCA**GATGCATATTCTGCACTGC	Reverse primer for HA of PR8 virus with *Not* I site
G	GCGGGATCCGCAGACACAATATGTATAGGC	Forward primer for PR8 HA without SP (with *BamH* I)
H	AACGCGGCCGCAATCTGATAGATCCCCATTGATTC	Reverse primer for PR8 HA without TM, CT (with *Not* I)
I	GGCTCTAGA**TTA***ATATTGTCTATTACGGTTTCT*ACACATCCAGAAACTGATTGC	Reverse primer for PR8 HA with CT of gp64 (with Xba I)

Underlined sequences are restriction enzyme sites. Bolded sequences are start or stop codons.

ciparum [8]. Most BV display strategies rely on gp64 protein which is the major envelope protein of baculovirus. Both influenza HA and baculovirus gp64 are type I transmembrane glycoproteins comprised of an amino-terminal signal peptide domain, carboxy-proximal transmembrane domain and cytoplasmic tail domain. Both proteins mediate viral entry into the host cells and efficient virion budding [21,22]. HA and gp64 proteins get incorporated into the infected host cell membrane. During the budding process, the budding virons pick up the protein as the constituent viral envelope [19,23]. Therefore, influenza HA can be displayed on the surface of baculovirus [12,17,19,24]. In this study, we investigated how the SP, TM, CT domains of gp64 influenced HA incorporation into BV. HA-DBVs protected mice against virus challenge even with low doses of vaccine. Moreover, we constructed a recombinant baculovirus simultaneously displaying four HAs derived from four subclades of H5N1 influenza. Vaccination with the tetravalent HA-DBVs stimulated strong humoral and cellular immune responses and protected mice against lethal H5N1 influenza virus challenges.

2. Materials and methods

2.1. Cells and viruses

Spodoptera frugiperda (Sf9) cells were propagated at 28 °C in Sf-900II serum free medium. Cells were infected by each recombinant baculoviruses at a multiplicity of infection (MOI) of 0.1-1.0 and virus supernatants were collected 4 days post-infection. Influenza viruses used in this study included the subtype H1N1, A/Puerto Rico/8/1934 (PR8), or the PR8-reassortant H5N1 viruses representing A/Vietnam/1203/2004 (VN/04), A/Indonesia/5/05 (IN/05), A/Whooper Swan/244/Mongolia/05 (WS/05), and A/Anhui/1/05 HA (AH/05). Each reassortant virus expressed the HA and NA derived from H5N1 viruses and the internal protein genes came from A/PR/8/1934 donor virus. Each virus was used to infect mice as previously described [25].

2.2. Construction of plasmids and recombinant baculoviruses

The SP, TM, and CT domains of the gp64 gene were amplified from Bacmid DNA by PCR. The full-length or ectodomain of HA genes from mouse-adapted PR8 were amplified by PCR from one plasmid containing full-length HA of the PR8 virus. A series of plasmids encoding the SP, TM, CT regions of gp64 and various portions of HA were generated using the following strategy. Nine primers (A-I as shown in Table 1) were used to generate the chimeric HA-gp64 genes. Primers A and B were used to amplify the gene fragment encoding the gp64 SP. Primers C and D were used to amplify the gene fragment encoding gp64 TM and CT. Primers E and F were used to amplify full-length HA of PR8. Primers G and H were used to amplify the ectodomain of PR8 HA (without SP, TM and CT). Primers E and I were used to amplify PR8 HA, but the CT derived from gp64. Primers E and H were used to amplify PR8 HA without TM and CT. Primers G and F were used to amplify PR8 HA without SP. Primers G and I were used to amplify PR8 HA without SP, and CT deriving from gp64. Appropriate fragments were serially inserted into pFastBac transfer vector (Invitrogen, Carlsbad, CA) in frame. Thus, each construct expresses chimeric PR8 HA proteins (Fig. 1A and B). All recombinant baculoviruses were generated using the Bac-to-Bac system (Invitrogen, Carlsbad, CA) and designated as Bac-HA, Bac-spHA, Bac-spHAct, Bac-HAct, Bac-HAtmct, Bac-spHAtmct.

The recombinant virus selection and amplification were performed following standard protocols. The infectious titers of recombinant baculoviruses were determined by the BacPAK Baculovirus Rapid Titer Kit (Clontech, Mountain View, CA) and are expressed as infectious units per milliliter (ifu/ml).

2.3. Purification of HA-displayed baculovirus

The recombinant baculoviruses were produced by infecting Sf9 cells at an MOI of 0.1. Supernatants were collected 4 days after

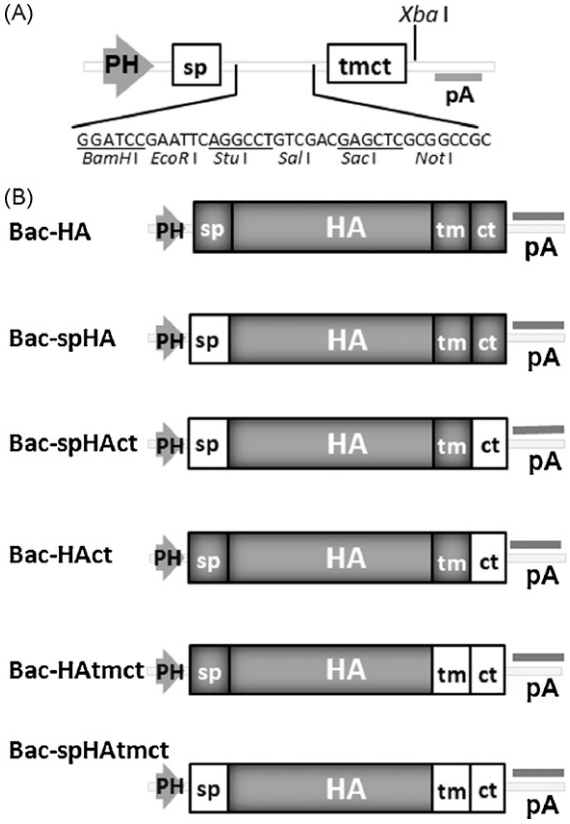

Fig. 1. Schematic illustration of the HA-pseudotyped baculovirus. (A) Modified transfer vector with SP, TM, CT domain sequences of gp64 and multiple cloning sites. (B) Schematic diagram of chimeric HA constructs. All components derived from HA are shown in dark gray while those from gp64 are shown in shadow.