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Occlusion-derived baculovirus: Interaction with human cells and evaluation of the envelope protein P74 as a surface display platform

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ABSTRACT

To develop complementary baculovirus-based tools for gene delivery and display technologies, the interaction of occlusion-derived baculovirus (ODV) with human cells, and the functionality of the P74 ODV envelope protein for display of the IgG-binding Z domains (ZZP74) were evaluated. The cellular binding of ODV was concentration-dependent and saturable. Only minority of the bound virions were internalized at both 37 and 4 °C, suggesting usage of direct membrane fusion as the entry mode. The intracellular transport of ODV was confined in vesicular structures peripheral to the plasma membrane, impeding subsequent nuclear entry and transgene expression. Transduction of ODV was not rescued by mimicking the preferred alkaline environment and lowered temperature of the ODV infective entry, or following treatment with the microtubule depolymerizing agent nocodazole or with the histone deacetylase inhibitor sodium butyrate. Similar to unmodified P74, the ZZP74 chimera localized in the intranuclear ring zone, and was enriched in virus-induced microvesicles. However, Western blotting of ODV and budded virions (BV), as well as viral envelope and nucleocapsid fractions combined with functional infection/transduction studies revealed incorporation of the ZZP74 fusion protein into viral nucleocapsids. The ZZP74 BV preserved normal infectivity, polypeptide profile, and morphology, but became incapable of entering and transducing human cells.

1. Introduction

Baculoviruses are arthropod-specific viruses and unique among other virus families by having two distinct viral phenotypes: occlusion-derived virus (ODV) and budded virus (BV). ODV and BV represent phenotypes of an identical viral genotype that are produced at different locations in the infected cell and at different times of the life cycle. Consequently, the two phenotypes vary in biological, biophysical as well as biochemical properties, and serve a specific biological function (Braunagel and Summers, 1994; Slack and Arif, 2007; Summers and Volkman, 1976). The preliminary infection is established by ODV within its larval lepidopteran host, whereas BV, produced by the ODV-infected midgut cells, is responsible for spreading the infection throughout the host. The infection ultimately leads to the death and liquefaction of the insect host, releasing millions of viral occlusions, i.e. polyhedra, in the environment (Engelhard et al., 1994; Monsma et al., 1996; Volkman et al., 1976; Washburn et al., 1995, 1999, 2003). Each viral phenotype executes its function under extremely distinct environmental conditions and targets different cell types. ODV has been assigned as a specialist since, in nature, it only infects the highly differentiated columnar epithelial cells within the alkaline conditions of the larval midgut. In contrast to ODV, BV is a generalist being highly infectious to the tissues of the host and to cultured cells (Braunagel and Summers, 1994; Slack and Arif, 2007; Volkman et al., 1976), and able to transduce numerous mammalian cell types with high efficiencies (Hu, 2006). BV is produced in the late phase of the infection cycle and derives its envelope from the modified plasma membrane of the host cell during budding. Production, nucleocapsid envelopment, and virion occlusion of ODV occur in the very late phase of infection in the nucleus (Blissard and Rohrmann, 1990; Slack and Arif, 2007).

Traditionally, baculoviruses have been applied as targeted biocontrol agents and for heterologous gene expression (Summers, 2006). The expression system was initially established in insect cells, and has now found versatile applications also in mammalian cells. In addition to delivery of therapeutic genes (Hu, 2006; Wang et al., 2006), baculovirus serves as a promising vector for the delivery of cDNA of infectious RNA viruses towards antisense therapy approaches, and RNA interference to silence specific target genes

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(Kaneko et al., 2006; Nicholson et al., 2005; Ong et al., 2005). Other applications include cell-based assays for drug screening (Condreay et al., 2006), generation of viral vectors (Buonaguro et al., 2006; Huang et al., 2007; Ward et al., 2007), immunotherapy (Kitajima and Takaku, 2008; Kitajima et al., 2007; Troadec et al., 2007), delivery of vaccine immunogens (Strauss et al., 2007; van Oers, 2006; Yang et al., 2007), and production of recombinant IgG antibodies (Liang et al., 2001). In addition, modification of the vector phenotype by baculovirus display technology has been adapted for studies on complex virus–host cell interactions, cell and tissue targeting, eukaryotic library development, antibody production, as well as vaccination (Makela and Oker-Blom, 2006, 2008).

The BV of *Autographa californica* multiple nucleopolyhedrovirus (*Ac*MNPV) has played a fundamental role in the evolution of baculovirus-based applications. The studies conducted with BV and the increasing understanding of baculovirology have generated optimism that the *Ac*MNPV ODV could become a complementary tool for analogous appliance (Makela and Oker-Blom, 2008). ODV envelope proteins have already provided unique tools to study the mechanisms of integral membrane protein trafficking from the ER to the outer and inner nuclear membranes and the formation of virus-induced, intranuclear membrane microvesicles (Braunagel and Summers, 2007). In addition to the development of new strategies for insect control, better understanding of the structure and function of ODV and polyhedra would allow more versatile appliance of these phenotypes in biotechnology.

Most of the established data on baculovirus–mammalian cell interactions and baculovirus display technology pertains to BV. In contrast to BV, ODV derives its envelope from intranuclear membranes, possesses no natural tropism towards mammalian cells, and would facilitate simultaneous introduction of several nucleocapsids and genetic payloads into target cells. In addition to large-scale vector production in occlusion bodies, refined ODV purification methods are available. The present study elucidated the interaction of *Ac*MNPV ODV with human cells, and analyzed the functionality of the ODV-specific P74 envelope protein as a platform for the display of heterologous peptides on the ODV surface.

2. Materials and methods

2.1. Antibodies and cells

The following baculovirus-specific primary antibodies were used: mouse monoclonal P10C6 VP39 (Whitt and Manning, 1988), B12D5 GP64 (Keddie et al., 1989), and N25-8C P74 (Faulkner et al., 1997), as well as rabbit polyclonal *Ac*MNPV BV (Braunagel and Summers, 1994), AcMNPV ODV (Volkman, 1983), E66 (Hong et al., 1994), and P10 (unpublished) antibodies. Other primary antibodies included rabbit IgG (Sigma–Aldrich, Saint Louis, MO), rabbit polyclonal NCL3 nucleolin (Christian et al., 2003), mouse monoclonal early endosome antigen-1 (EEA-1; Abcam, Cambridge, UK), and mouse monoclonal lysosome-associated membrane protein 2 (LAMP-2; Developmental Studies Hybridoma Bank, Iowa City, IA). Secondary antibodies used were fluorescent goat anti-mouse or anti-rabbit Alexa Fluor® 488, 555, and 546 conjugates (Molecular Probes, Eugene, OR) and alkaline phosphatase-conjugated secondary antibodies (Promega, Madison, WI). *Spodoptera frugiperda* (*Sf*9; GibcoBRL, Grand Island, NY), HepG2 human hepatocarcinoma (American Type Culture Collection, ATCC HB-8065), and A549 human carcinoma cell lines (ATCC, CCL-185) were cultured as previously described (Makela et al., 2006; Matilainen et al., 2006).

2.2. Generation of the recombinant baculoviruses

The sequence of CMV promoter/enhancer and EGFP coding region was PCR-amplified as previously described (Makela et al., 2008) and ligated into the corresponding KpnI site of the firefly luciferase-encoding pSV40-luc (Makela et al., 2006), resulting in an intermediate plasmid pSV40-luc/CMV-EGFP. The gene encoding polyhedrin was PCR-amplified with 5′-A AAA GAA TTC ATG CCG GAT TAT TCA TAC CGT C-3′ forward and 5′-AAAA CTG CAG TTA ATA CGC CGG ACC AGT GAA-3′ reverse primers containing EcoRI and PstI restriction sites (underlined), respectively, using wild-type baculovirus stock as a template. The product was subcloned under the polyhedrin promoter of pSV40-luc/CMV-EGFP. The resultant plasmid, pSV40-luc/CMV-EGFP/polh, was used to generate the recombinant baculovirus *Ac*WT.

The *ZZP74* fusion gene was constructed as follows: the 5′-AT ATC TCT AGA ATG GTA GAC AAC AAA TTC AAC AAC AAA GAA C-3′ forward and 5′-AA AAA CTG CAG CGC GTC TAC TTT CGG CG-3′ reverse primers (5′XbaI and 3′PstI sites underlined) were used to amplify the coding region of the IgG-binding ZZ domains of protein A by PCR from the plasmid pZZVSVgTM (Ojala et al., 2004), followed by ligation into a blank pFastBac Dual, resulting in a plasmid pZZ. The *P74* gene was amplified with 5′-AA AAA CTG CAG ATG GCG GTT TTA ACA GCC G-3′ forward and 5′-AA AAA AAG CTT TTA AAA TAA CAA ATC AAT TGT TTT ATA ATA TTC G-3′ reverse primers (5′PstI and 3′HindIII sites underlined) using purified baculovirus genome (bacmid; Invitrogen, Carlsbad, CA) as a template, and cloned into the pZZ in frame with the coding region of the ZZ domains. The *ZZP74* fusion gene, containing the SV40 polyadenylation signal from the pFastBac Dual, was then amplified using 5′-AT ATC CCC GGG ATG GTA GAC AAC AAA TTC AAC AAC AAA GAA C-3′ forward and 5′-A AAA CCC GGG GAT CCA GAC ATG ATA AGA TAC ATT GAT G-3′reverse primers (SmaI sites underlined). To facilitate the ligation reaction, 3′-overhanging A- and T-ends were generated (0.2 mM dATP/dTTP, 72 °C, 30 min) to the SmaI-digested PCR product and to the pSV40-luc/CMV-EGFP/polh, respectively. The resultant plasmid, pSV40-luc/CMV-EGFP/polh/ZZP74, was used to generate the recombinant baculovirus *Ac*ZZP74. *Ac*ZZVSVG is an expression marker gene-deficient derivative of *Ac*ZZVSVgTM-EGFP (Ojala et al., 2004).

The recombinant baculoviruses were produced using the Bac-to-Bac® Baculovirus Expression System (Invitrogen, Carlsbad, CA). Viral titers were determined by end-point dilution assays from non-concentrated baculovirus stocks using standard protocols.

2.3. Isolation of occlusion-derived virus from cell culture

*Sf*9 cells grown in 400 ml suspension culture (2×10^6 cells/ml) at 28 °C were infected with BV at an MOI (multiplicity of infection) of 1. The cells were collected at 5 days post infection (p.i.; $2000 \times g$, 10 min, room temperature, RT) and the pellet was resuspended in H_2O. To liberate polyhedra, cells were lysed by incubating with SDS (0.5% final concentration, rotating, 30 min, RT) followed by centrifugation ($2000 \times g$, 30 min, RT). The polyhedra-containing pellet was resuspended in 40% sucrose followed by washes with H_2O and centrifugations in between ($2000 \times g$, 30 min, RT), and finally suspended in 60% glycerol or H_2O. The subsequent liberation of ODV from polyhedra was essentially performed as described by Haas-Stapleton et al. (2004). The ODV preparations used were a mixture of ODVs containing single and multiple nucleocapsids per virion. Total protein concentrations of ODV were determined with NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE) at A280 nm using bovine serum albumin (BSA) as a reference.