A Baculoviral Display System to Assay Viral Entry

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In this study, we evaluated a baculoviral display system for analysis of viral entry by using a recombinant adenovirus (Ad) carrying a luciferase gene and budded baculovirus (BV) that displays the adenoviral receptor, coxsackievirus and adenovirus receptor (CAR). CAR-expressing B16 cells (B16-CAR cells) were infected with luciferase-expressing Ad vector in the presence of BV that expressed or lacked CAR (CAR-BV and mock-BV, respectively). Treatment with mock-BV even at doses as high as 5 µg/mL failed to attenuate the luciferase activity of B16-CAR cells. In contrast, treatment with CAR-BV with doses as low as 0.5 µg/mL significantly decreased the luciferase activity of infected cells, which reached 65% reduction at 5 µg/mL. These findings suggest that a receptor-displaying BV system could be used to evaluate viral infection.

Key words  baculovirus; virus; infection; receptor

The process of viral infection involves entry of the virus into the cell, followed by replication of the viral genome and other viral components in the host cell. 1) Whereas the molecular mechanisms underlying viral replication have largely been elucidated, the key molecules for entry, the viral receptors on host cells, have never been fully identified. Most host receptors are integral membrane proteins, and it is difficult to prepare their recombinant proteins because of their hydrophobicity. Since recombinant proteins are needed to screen inhibitors for viral entry and to produce antibodies against host receptors, preparation of inhibitors, such as chemicals, peptides and antibodies, for viral entry has been delayed.

The baculoviral expression system in insect cells has been widely used for preparation of recombinant proteins. 2) Hamakubo and colleagues found that baculoviral particles are released from baculovirus-infected cells; the membranes of these budded baculovirus (BV) display host-cell-derived membrane proteins. 3) Interestingly, the activity and topology of these host-origin proteins remain intact in the baculoviral membrane. 4) Moreover, a baculoviral envelope protein gp64 transgenic mice were generated, and method to generate monoclonal antibodies against membrane proteins by immunization of gp64 transgenic mice with membrane protein-displayed baculovirus has been established. 5) These findings suggest that a baculoviral display system may be useful for assaying viral entry, leading to creation of monoclonal antibodies against host receptors.

In the present study, we investigated whether a baculoviral display system work as an assay system for viral entry using recombinant adenovirus (Ad) vector and a receptor for Ad, coxsackievirus and adenovirus receptor (CAR). 6)

MATERIALS AND METHODS

Cell Culture  Mouse melanoma B16-CAR cells 7) were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal calf serum (FCS) and 2 mg/mL G418. 293 cells were cultured in DMEM supplemented with 10% FCS. SF9 cells (Invitrogen, Gaithersburg, MD, U.S.A.) were cultured in Grace’s insect cell culture medium supplemented with 10% FCS.

Preparation of Recombinant Ad Vector  An improved in vitro ligation method 8) was used to generate a recombinant type 5 Ad vector that encoded a fusion protein comprising enhanced green fluorescence protein and firefly luciferase (EGFPLuc). The recombinant Ad vector (Ad-EGFPLuc) was purified from transfected cells by using CsCl gradient centrifugation. Viral titers were determined spectrophotometrically. 9)

Preparation of Recombinant Baculoviruses  Recombinant BVs were prepared by using the Bac-to-Bac Baculovirus Expression System (Invitrogen) according to the manufacturer’s protocol. SF9 cells were transduced with the CAR-encoding bacmid, recombinant CAR-BV were recovered by centrifugation of the conditioned medium, 10) and SF9 cells were infected with recombinant CAR-BV. At 72 h after infection, the culture supernatant of the infected SF9 cells was centrifuged to pellet recombinant CAR-BV, which were resuspended in Tris-buffered saline and stored at 4°C until use.

Western Blotting  Mock-BV, CAR-BV, and B16-CAR cells were lysed in lysis buffer (25 mM Tris–HCl [pH 7.5], 1% Triton X-100, 0.5% sodium deoxycholate, 150 mM NaCl, 5 mM ethylenediaminetetraacetic acid (EDTA)) containing protease inhibitors (Sigma, St. Louis, MO, U.S.A.). The protein content of the resulting lysates was measured by using the BCA protein assay kit (Pierce Chemical, Rockford, IL, U.S.A.). The protein content was determined using bovine serum albumin as the standard. Samples of cellular lysates (20 μg) and BV lysates (5 μg) underwent sodium dodecyl sulfate–polyacrylamide gel electrophoresis followed by blotting of proteins to a polyvinylidene difluoride membrane. The membrane was treated with 5% skim milk to inhibit nonspecific binding, incubated with an anti-goat CAR antibody (R&D Systems, Minneapolis, MN, U.S.A.), and then incubated with a peroxidase-labeled secondary antibody. Immuno-reactive bands were visualized by using chemiluminescence reagents (GE Healthcare, Buckinghamshire, U.K.).

Infection Assay  Aliquots of Ad-EGFPLuc vector (4 × 107 µg/mL) underwent sodium dodecyl sulfate–polyacrylamide gel electrophoresis followed by blotting of proteins to a polyvinylidene difluoride membrane. The membrane was treated with 5% skim milk to inhibit nonspecific binding, incubated with an anti-goat CAR antibody (R&D Systems, Minneapolis, MN, U.S.A.), and then incubated with a peroxidase-labeled secondary antibody. Immuno-reactive bands were visualized by using chemiluminescence reagents (GE Healthcare, Buckinghamshire, U.K.).
viral particles per mL) were incubated with mock-BV or CAR-BV (0.5 or 5 µg/mL) and an anti-BV gp64 antibody (0.065 or 0.65 µg/mL; AcV1, Santa Cruz Biotechnology, CA, U.S.A.) for 2 h at 37°C to prevent non-specific binding of gp64 to cells. B16-CAR cells were seeded onto 96-well plates (2 × 10^4 cells per well); 50 µL of the mixture of Ad vector and BVs was added to each well and incubated for 15 min, after which the medium was replaced with fresh growth medium. After an additional 24 h of culture, the luciferase activity in the lysates was measured by using a luminometer.

**Statistical Analysis** The data were analyzed for statistical significance by Student’s t-test.

RESULTS AND DISCUSSION

First, we prepared CAR-displaying BV. Lysates of CAR-B16 cells, a mouse myeloma line that expresses mouse CAR, yielded two bands, at 40 and 46 kDa (Fig. 1). In contrast, lysates of CAR-BV showed not only the 40-kDa form but also several bands lower and upper than 40 kDa (Fig. 1); these bands likely represent post-translational modifications. CAR contains two N-glycosylation sites and two disulfide-bonded loops in the extracellular domain. The putative molecular sizes of CAR are 40 and 46 kDa, in its non-glycosylated form and glycosylated forms, respectively. Protein folding and post-translational processing, particularly N-glycosylation, in insect cells differs markedly from that in mammal cells. For example, prolactin receptor expressed in insect cells was 29 kDa larger than that expressed in mammalian cells; this difference was attributed to N-glycosylation and ubiquitination.

To investigate whether CAR-BV inhibited adenoviral entry, B16-CAR cells were infected with Ad vector expressing luciferase in the presence of mock-BV or CAR-BV. Whereas treatment with mock-BV at doses as high as 5 µg/mL did not attenuate the luciferase activity of the infected B16-CAR cells, treatment with as little as 0.5 µg/mL CAR-BV significantly decreased their luciferase activity, which reached 65% reduction at 5 µg/mL (Fig. 2). These findings indicate that CAR-BV prevented the infection of cells by Ad vector. In support of our finding, recombinant prolactin receptor expressed in insect cells and prolactin receptor purified from rabbit mammary gland showed similar specificity and affinity to prolactin. Accordingly, the post-translational modification of CAR in insect cells may not hamper the ability of Ad vector to bind to its receptor.

Our current findings suggest that a baculoviral display system may be useful in the analysis of viral infection, which involves binding of the viral envelope to the viral receptor in the membrane of the host cell. Baculoviral display systems have also been used widely to generate monoclonal antibodies against the extracellular regions of membrane proteins. Future applications of baculoviral display systems might contribute the analysis of the mechanisms underlying the entry of pathogens into host cells and the generation of inhibitors of viral entry.

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