Serodiagnosis of Murine Norovirus in Mice using Recombinant Virus-like Particles

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Introduction

Murine norovirus (MNV), a viral pathogen of mice first recognized in 2003, is prevalent in contemporary research mouse colonies, causes persistent enteric infections, and replicates in dendritic cells and macrophages. Thus, this viral infection has great potential to alter research data obtained from infected mice. As a result, inexpensive, high throughput, and effective serological tests are necessary to detect infected mice. Currently, in vitro propagated whole virus preparations are being used as antigens in multiplexed fluorescent immunoassays (MFIs) to detect serum antibodies to MNV in infected mice. However, generating sufficient amounts of in vitro propagated whole virus of some MNV strains is difficult because not all noroviruses grow well in cell culture.

Objective

Our goal is to clone open reading frames (ORFs), ORF2 and ORF3, and the 3' untranslated region (3' UTR) of MNV-2 and MNV-3 into a baculovirus (BV) expression system to produce MNV virus-like particles for use as antigens in serologic assays. ORF2 is known to code for the MNV capsid protein (VP1) and ORF3 codes for small structural basic protein (VP2). Studies with Norwalk virus, a norovirus that infects humans, have shown that Norwalk virus VLPs can be produced by only expressing ORF2. However, greater quantities of VLPs can be produced when both ORF2 and ORF3, and the 3' UTR are expressed as VP2 has been found to increase the expression of VP1. As a result, large quantities of MNV VLPs can be produced for use as diagnostic antigens.

Hypothesis

- A baculovirus expression system will be effective in generating large quantities of recombinant MNV VLPs.
  - Gene of interest is inserted into the BV genome and replaces the polyhedrin protein, a viral protein that is expressed at very high levels in insect cells infected with BV
  - Inserted gene located downstream from the polyhedrin protein promoter, which will result in high level expression of recombinant VLPs

- Recombinant MNV VLPs can serve as highly sensitive and specific antigens to detect MNV antibodies in infected mice sera

Materials and Methods

ORF2, ORF3, and the 3' UTR of the MNV-2 and MNV-3 genomes were cloned into an entry vector. Using the Bac-to-Bac Baculovirus Expression System, an expression clone was created by recombining the entry clone containing our gene of interest with a destination vector. The destination vector was then used to transform competent DH10BAC *Escherichia coli* cells. These competent cells contain a BV shuttle vector (bacmid) and a helper plasmid, which contains an antibiotic resistance gene and encodes transposase, an enzyme that allows for the transposition of our clone into the bacmid. Colonies with recombinant bacmids were identified by blue-white screening and antibiotic selection. In addition, the recombinant bacmid DNA was purified from the transformants and analyzed by PCR. Transfection of Sf9 insect cells with purified bacmid DNA generated a P1 recombinant BV stock. This stock was amplified to produce a high-titer P2 stock, which was PCR analyzed to determine if the virus being produced was recombinant BV containing our insert. In addition, plaque assays were prepared to determine the viral titer of the P1 stock in order to perform a multiplicity of infection (MOI) time course study using High Five insect cells infected with P2 virus. Infected cells were harvested at 2 time points (48 and 72 hours) and protein production analyzed by Western blot. Extracted protein was purified and analyzed under transmission electron microscopy (TEM).

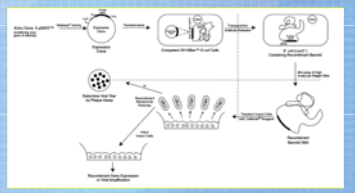

Figure 1. Overview of the Bac-to-Bac Baculovirus expression system.

Results

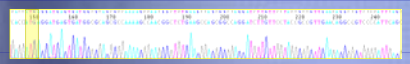

Figure 2. Excerpt of plasmid DNA sequence analysis. DNA sequencing of vectors used to create recombinant bacmid confirmed that MNV-2 and MNV-3 inserts were present in the plasmids. Yellow area indicates start codon for VP1 sequence. Sequences were 100% identical to MNV-2 and MNV-3 viral capsid protein sequences.

Figure 3. Blue-white screening of E. coli colonies with recombinant bacmids. White *E. coli* colonies represent colonies that contain the bacmid DNA in which the MNV genes have been inserted downstream of the polyhedrin promoter and disrupted the *lacZ* gene. Blue colonies represent *E. coli* colonies that contain bacmid DNA in which MNV genes have not been inserted. As the *lacZ* gene has not been disrupted, these *E. coli* can still metabolize X-Gal resulting in a blue color.

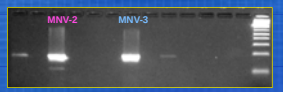

Figure 4. MNV PCR analysis. Purified DNA from P2 viral stocks confirmed that recombinant baculovirus was being produced.

Figure 5. Recombinant baculovirus viral plaques . Viral plaques are observed as disrupted areas in the insect cell monolayer that are surrounded by infected cells. Plaque assays of the MNV-2 and MNV-3 P2 viral stock indicated that viral titers were about 2 x 10^9 pfu/ml and 9 x10^8 pfu/ml, respectively.

Figure 6. Western blot analysis. Insect cell cultures were inoculated with MNV 2 or MNV 3 recombinant BV at a MOI of 0.1 and incubated for 48 or 72 h. Membranes were probed with sera from MNV infected mice. Analysis of protein production of infected cells confirmed the expression of recombinant MNV capsid proteins (red arrow at ~59 kD).

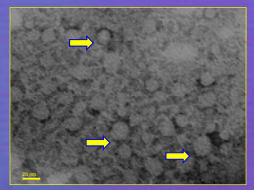

Figure 7. MNV virus-like particles. Transmission electron micrograph of negatively stained recombinant MNV virus-like particles (arrows) produced in a baculovirus expression system.

Conclusions

- Recombinant BV expressed MNV viral capsid proteins are immunogenic and form virus-like particles

Future Study

- Further evaluate recombinant MNV VLPs for potential use in diagnostic immunoassays
- Assess recombinant VLPs as effective antigens in high throughput serodiagnostic immunoassays
- Compare the diagnostic sensitivity and specificity of recombinant MNV VLPs to whole virus MNV antigens in diagnostic immunoassays

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