TITLE: Mechanisms of Mouse Hepatitis Virus Entry Into Cells

AUTHORS: Philip Koehler¹, Kristina Cade¹, Fiora D. Zoga¹, Susan T. Hingley¹,*

AFFILIATIONS: ¹Department of Pathology, Microbiology, Immunology & Forensic Medicine, *Center for Chronic Disorders of Aging, Philadelphia College of Osteopathic Medicine

ABSTRACT:

Background: Viruses can enter cells through several mechanisms, two common ones being clathrin-mediated and caveolin-mediated endocytosis. The clathrin pathway delivers viral particles to endosomes, with subsequent acidification of the endosome and endosome/lysosome fusion often a prerequisite for release of the viral genome into the cytoplasm. The caveolin-mediated pathway delivers virus initially into vesicles called caveosomes, which have a neutral pH, before viral uncoating occurs. Viral entry pathways can be examined by using various drugs to inhibit the different endocytosis pathways, as well as by siRNA technology to down-regulate expression of clathrin or caveolin proteins on the surface of host cells.

Methods: To evaluate the possible importance of clathrin- and caveolin-mediated entry pathways for mouse hepatitis virus (MHV) entry into cells, several inhibitors of clathrin-mediated endocytosis were used to inhibit infection with MHV. In addition, transfection with siRNA specific for clathrin or caveolin was used to monitor how down-regulation of these cellular proteins affect viral gene expression. The efficiency of siRNA transfection was determined by western blot analysis and real time reverse transcriptase polymerase chain reaction (RT²-PCR) using TaqMan gene expression assays. The abilities of recombinant virus expressing fusion proteins from two separate strains of MHV (MHV-A59 and MHV-2) to enter both mouse fibroblast cells (L2 cells) and brain-derived endothelial cells (bEnd cells) was evaluated by RT²-PCR for viral specific sequences generated within the first round of viral replication.

Results: We confirmed that surface expression of caveolin and clathrin can be inhibited by siRNAs specific for these proteins. Down-regulation of these two proteins had a similar affect on viral gene expression for both strains of MHV. However, viral entry, as reflected by viral-specific mRNA levels, was not diminished in siRNA transfected cells to the same extent as it was by the use of drugs specific for clathrin-mediated entry. Inhibitors of clathrin-mediated endocytosis demonstrated differing affects in the two cell lines, as well as differences between the two strains of MHV.

Conclusions: Data from cells treated with inhibitors of clathrin-mediated endocytosis, as well as from siRNA experiments, indicate differences in entry mechanisms utilized by MHV in the two cell lines.