O185
Abstract (oral session)

Effects of plasma membrane-deforming lysolipids on virus-mediated syncytium formation

M. Ciechonska*, R. Duncan (Halifax, CA)

Objective: Virus-mediated cell-to-cell fusion is a hallmark cytopathic effect accompanying infection by several enveloped viruses. While virus-to-cell fusion during entry has been extensively studied, little is known about cellular pathways involved in the process of cell-to-cell fusion. The non-enveloped reovirus fusion-associated small transmembrane (FAST) proteins are the smallest known membrane fusion proteins. Unlike enveloped virus fusogens, FAST proteins are non-structural and are not involved in virus entry. Their sole function is to induce syncytium formation following infection, making them an ideal model for the study of cellular pathways involved in syncytiogenesis. In this study, we conducted a comparative, temporal analysis of FAST protein- and influenza hemagglutinin (HA)-mediated cell-to-cell fusion in order to determine whether viral proteins designed for entry and those designed for cell-to-cell fusion elicit a similar response during fusion pore expansion and syncytium formation. Methods: HA and p14-FAST were transfected and HA fusion was triggered by trypsinization and incubation of cells at pH4.8. Pore formation was monitored by transfer of a cytoplasmic fluor between quail fibroblasts and Vero epithelial cells. Syncytium formation, quantified by counting syncytial nuclei, indicated pore expansion. In addition, we developed a novel method to analyze pore expansion by adding the membrane curvature agent lysophosphatidylcholine (LPC) during our temporal analysis of syncytium formation. Results: We report that while LPC does not inhibit FAST protein mediated pore formation, it does inhibit pore expansion resulting in a reversible ‘stalled pore’ phenotype. In contrast, LPC inhibits both HA-mediated pore formation and pore expansion, this ‘stalled pore’ phenotype is also reversible. This is the first example of syncytium development arrest following a membrane fusion event. Conclusion: While the fusion reactions instigated by enveloped virus and non-enveloped virus cell-to-cell fusogens differ, the syncytium formation stage is likely similar, and is a cellular response to an assault on the plasma membrane. By stalling pore expansion, we isolated a cell-dependent stage of cell fusion and showed that the FAST model mimics the effects of envelope virus-induced syncytium formation. Using this system, we identified several cellular proteins involved in syncytium formation, which are currently under investigation in our laboratory.