to the normal squamous epithelial tissues. Typical cytopathic changes identical to those found in the squamous epithelium in vivo were observed throughout the raft following infection with the different viruses. We have now assessed the feasibility of using mononuclear cells (MCs) as viral carriers to infect organotypic epithelial raft cultures. For this purpose, mononuclear cells were isolated from human umbilical cord blood by Ficoll-Hypaque density gradient centrifugation, stimulated with phytohemaglutinin and incubated in the presence of IL-2. MCs were infected with HSV-1, HSV-2 or VV at a multiplicity of infection of approximately 0.01, incubated overnight and then washed to remove the viral inoculum. The MCs were added on top of the raft cultures after the epithelial cells were allowed to differentiate for 6 days. MCs were able to transfer the virus to the epithelium and histological changes characteristic of HSV infection could be observed. When the anti-herpesvirus agents, acyclovir, penciclovir, HPMPC (cidofovir) or HPMP-5azaC were added to the culture media at the time the MCs carrying HSV were added on top of the rafts, the epithelium was protected from the virus-induced cytopathic effect in a dose-dependent manner. The antiviral activity was quantified by measuring viral titers by plaque assay. Similarly, HPMPC and HPMP-5-azaC were able to protect the epithelial cells from VV spread by the MCs. Our results show the possibility of using MCs as a transfer vehicle for HSV and VV infections of epithelial cells grown in 3D and suggest that MCs could be responsible for the spread of the virus infection.

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Structural Basis for the Expanded Substrate Specificity of Vaccinia Virus Thymidine Kinase: Insight from the Crystal Structure

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Vaccinia virus, like other orthopoxviruses, encodes an enzyme with thymidine kinase (TK) activity. This enzyme is closely related to the human cytosolic enzyme, TK1, and both belong to the type 2 family of TK homologs. Previous studies with *N*-methanocarbathymidine (*N*-MCT), a new antiviral agent with activity against cowpox virus replication, demonstrated that the compound was much less effective in TK negative strains of the virus and suggested that the enzyme could preferentially phosphorylate this compound. A similar TK dependence was also observed with a series of 5-substituted deoxyuridine analogs. These results were unanticipated since the viral TK and TK1 share 70% identity at the amino acid level and were predicted to exhibit very similar substrate specificities. This was investigated further by purifying both enzymes in bacteria and comparing their relative ability to phosphorylate a number of thymidine analogs. This analysis revealed that the viral enzyme exhibited a marked preference for a few compounds including *N*-MCT and fialuridine. The crystal structure of recombinant TK at 2.9 Å resolution explains the structural basis for these differences. A comparison of this structure with the previously published structure of human TK1 revealed significant differences in the catalytic site. In particular, the active site of the viral enzyme appeared to be more open than the human homolog. These results have important implications in the development of antiviral therapies to orthopoxvirus infections. Thymidine analogs, such as (*N*)-MCT that are selectively phosphorylated by this enzyme in infected cells could be developed as potent and highly selective inhibitors of orthopoxvirus infections.

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Inhibition of an Innate Antiviral Response by Human Cytomegalovirus UL97 Kinase is Antagonized by Maribavir

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Nuclear aggresomes are cellular structures that function to degrade highly ordered protein aggregates in the nucleus, such as viral structural proteins and are an important innate antiviral response. The formation of nuclear aggresomes occurs around PML oncogenic domains (PODs, ND10 sites). Many viruses including human cytomegalovirus disrupt PODs, presumably to protect against the sequestration and degradation of viral proteins in the nucleus. One previously described facet of the UL97 negative phenotype was the formation of large nuclear aggregates, which also formed when cells were infected with the wt virus in the presence of the UL97 kinase inhibitor, maribavir (MBV). Interestingly, the kinase was shown to inhibit the nuclear aggregation of pp65 when both proteins were expressed transiently in COS7 cells. Here, we report that the kinase also reduced the nuclear aggregation of the tegument protein pp71, as well as cellular protein that is a marker for nuclear aggresomes. Thus, it appeared that the inhibition of nuclear aggregations was more general than previously suspected. To investigate this further, we examined the effect of the kinase on PODs and observed that it disrupted their formation in a manner similar to IE1, which was used as a positive control. This effect did not occur either with a point mutant of UL97 that has no kinase activity, or if the kinase activity was inhibited by MBV. These results taken together suggest that one important function of UL97 is to inhibit the formation of PODs and the degradation of structural proteins by nuclear aggresomes. In the absence of UL97 kinase activity, at least 90% of the mass of viral structural proteins is sequestered in nuclear aggresomes resulting in a significant impairment of viral morphogenesis. We conclude that the inhibition of the kinase by MBV impairs the ability of the virus to inactivate this innate