

camelpox virus (CMLV) and cowpox virus (CPV) have raised questions about potential differences in VACV, CPV and CMLV replication cycles and the possible presence of other targets. Based on these data, we investigated the relative quantity of enveloped virus versus non-enveloped virus in the medium and cell lysates using density gradients performed on [methyl-<sup>3</sup>H]-thymidine labeled VACV, CPV and CMLV infected cells, either in the absence or presence of ST-246. We then characterized ST-246 resistant orthopoxviruses which were selected following increasing concentrations of ST-246 for approximately 17 passages. Analysis of cesium chloride gradient fractions indicated that most of the viruses produced during a productive VACV, CPV and CMLV infection were enveloped. The spread of VACV appeared to involve both intra- and extracellular enveloped forms. In contrast, CPV and CMLV produced few extracellular enveloped forms and seemed to propagate via intracellular infectious particles. These data indicated a difference in the way of propagation of CMLV/CPV and VACV. It was also clear that the antiviral activity of ST-246 was due to an interference with the formation of enveloped forms and that the discrepancy in the inhibitory effect of ST-246 on orthopoxvirus replication was more likely due to differences in orthopoxvirus spread. The IC<sub>50</sub>s determined for each ST-246 resistant virus mutant were at least 100–2000-fold higher than those of the wild-type viruses. Sequencing of the F13L genes of both resistant and wild-type VACV, CPV and CMLV strains is currently ongoing. We have demonstrated the antiviral potency of ST-246 on the production of enveloped orthopoxvirus particles and confirmed differences in VACV, CPV and CMLV replication cycles. ST-246 resistant viruses have been plaque purified and the determination of mutations induced by ST-246 in the F13L gene is currently under investigation.

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#### **Vaccinia DNA Polymerase is Profoundly Inhibited by Cidofovir and (S)-HPMPA Incorporated into the Template Strand**

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Cidofovir (CDV) and (S)-9-[3-hydroxy-(2-phosphonomethoxy)propyl]adenine (HPMPA) are analogs of dCMP and dAMP, respectively, and are effective inhibitors of poxvirus replication. However, the precise mechanism by which these drugs inhibit viral growth remains unclear. We have previously used vaccinia virus as a model system to show that the diphosphoryl metabolites of these drugs are substrates for vaccinia DNA polymerase, but once incorporated into the penultimate 3'-end of the primer strand inhibit both the polymerase and 3'-to-5' exonuclease activities. Interestingly, although HPMPA exhibits a much lower EC<sub>50</sub> than CDV, HPMPApp is not nearly as effective an inhibitor of primer extension and exonuclease activity in vitro compared to CDVpp. This suggests that other mechanism(s) must account for the differ-

ences in relative activities of the two drugs. To investigate these mechanisms, we examined the effects of CDV and HPMPA on vaccinia DNA polymerase activity when incorporated into the template strand. These templates were prepared using a two-step enzymatic method. First, oligonucleotide-primed templates were prepared containing a single dGMP or dTMP residue (to direct CDV or HPMPA incorporation, respectively) and multiple dUMP residues. Primer extension was used to incorporate each drug, and then the dUMP-containing strand was degraded using uracil DNA glycosylase. Labeled primers were then annealed to the newly extended strands and we tested whether vaccinia DNA polymerase could extend these primers back across the drug residue. We found that although the correct nucleotide could be incorporated opposite the drug lesion, further extension by the DNA polymerase was blocked. Control templates, containing either dCMP or dAMP at the same sites, did not show this block. These results suggest that although primer extension is slowed by the incorporation of these drugs, the profound effects on DNA replication are more likely caused by drug incorporated into the template strand. HPMPApp is a much better substrate for vaccinia DNA polymerase than is CDVpp, but this makes it the more effective drug because more HPMPA is then incorporated into the template strand.

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#### **Development of a Model for the Study of Antivirals Against Molluscum Contagiosum Virus (MCV)**

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Molluscum contagiosum virus (MCV) and variola virus (VARV) are the only two poxviruses that are specific for humans. VARV has been eradicated from the human population through extensive programs of vaccination. MCV is present worldwide and is directly passed by direct skin contact to produce cutaneous and, rarely, mucosal lesions. It occurs predominantly in preadolescent children, sexually active adults, and in individuals with impaired immunity. The study of MCV has been hampered by the lack of an in vitro system that allows virus replication. We describe here the growth of MCV in 2D (two dimensional) and 3D cultures of primary human keratinocytes (PHKs). PHKs were isolated from neonatal foreskins and used to prepare monolayer cultures and organotypic raft cultures, which are able to mimic fully differentiated skin. Fresh lesions obtained from preadolescent children were used to recover the MCV. Organotypic epithelial raft cultures were infected with different MCV fresh isolates and after a period of approximately 25 days were processed for histological examination. A typical cytopathic effect characterized by the appearance of huge infected cells, with internal organelles dislocated and obliterated by a large intracytoplasmic