of pVIc, an 11-amino acid cofactor, via a series of contiguous structural changes occurring over a 54-amino acid long, bifurcated pathway. The other cofactor is the viral DNA. It has been a conundrum as to how 70 molecules of AVP-pVIc complexes can cleave multiple copies of six different virion precursor proteins at 3200 processing sites inside a nascent virion. Either the enzyme or its substrates must move, but these sequence independent DNA binding proteins cannot readily diffuse in three-dimensional space, because they remain bound to the highly concentrated (>500 g/L), tightly packed viral DNA. The conundrum may have been solved; AVP-pVIc complexes can slide along viral DNA via one-dimensional diffusion, thereby providing a way for AVP to locate and process the precursor proteins. AVP-pVIc complexes exhibited directionless sliding on viral DNA that could last more than one second and cover more than 20,000 base pairs via the largest one-dimensional diffusion coefficient observed for any protein moving along DNA, 21×10^6 bp^2/s. The ability of AVP via pVIc to exploit the DNA contour to guide it to its substrates may represent a new paradigm for virion maturation. Among potential therapeutic targets deduced from the activation mechanisms are: sites along the 54-amino acid long activation pathway, the DNA binding sites, the actin binding sites, the pVIc binding sites, as well as the active site. We determined crystal structures of these sites at high resolution (1.6 Å for AVP-pVIc and 0.98 Å for AVP). Structure-based drug design recently identified a compound predicted to bind to the pocket in which the N-terminus of pVIc binds and the active site; it has a K_i of 2.2 μM. Finally, because a number of sites on the enzyme interact with each other, a drug regimen may be designed that would prevent resistance to antiviral drugs from arising.

doi:10.1016/j.antiviral.2008.01.059

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Efficacy of T-1106 or T-705, Alone or in Combination with Ribavirin, in the Treatment of Hamsters Infected with Yellow Fever Virus

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Hamsters infected with an adapted Jimenez strain of yellow fever virus (YFV) have similar disease pathology to that seen in YFV-infected humans. The hamster model has been useful in the evaluation of antiviral compounds against YFV, including T-1106, which was shown to be effective in reducing disease parameters with a minimal effective dose between 10 and 32 mg/(kg d). The objective of the first study was to determine the efficacy of T-705, a fluorinated and non-ribosylated chemical similar to T-1106, in the treatment of YFV. Activity was observed in Vero cells with an EC90 of 418 ± 28 μM (SI > 9.6), which was lower than the EC90 for T-1106 of 677 μM (SI > 5.9). No significant improvement of disease parameters was seen with the oral administration 100 mg/(kg d) of T-705, although a trend towards improvement was observed. However, treatment of hamsters with 400 mg/(kg d) of T-705 was shown to be effective in significantly improving survival, serum ALT and AST levels, and weight change when treatment was started at 2 days post-virus inoculation (dpi). Significant improvement of survival was also seen with this dose of T-705, beginning as late as 3 dpi. The objective of the second study was to compare the activity of T-1106 and T-705 alone versus either of the two compounds in combination with ribavirin for the treatment of YFV disease. A synergistic effect was seen in cell culture when T-1106 or T-705 was combined with ribavirin. Treatment of hamsters with a combination of T-1106 or T-705 and ribavirin was superior to monotherapies. In summary, T-705 is efficacious in the treatment of YFV disease in hamsters, although a much higher dose (∼20-fold) is required as compared with T-1106. Superior activity was seen when T-1106 or T-705 was combined with ribavirin as compared with the administration of the monotherapies.

Acknowledgements: Supported by contracts NO1-AI-30048 and NO1-AI-15435 from the Virology Branch, NIAID, NIH.

doi:10.1016/j.antiviral.2008.01.060

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AlphaV Integrin-mediated Adhesion of Monocyte-derived Macrophages Influences HIV Infection

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Monocytes and macrophages are an important reservoir of human immunodeficiency virus (HIV) and may represent the largest reservoir of this virus in tissue. We have previously shown that an alphaV integrin blocking antibody inhibited HIV-1 infection in monocyte-derived macrophages (MDM), revealing an unexpected role of this integrin in HIV replication [Bosch et al., 2006. Antiviral Res.]. Integrins play a pivotal role in the interaction of cells with the extracellular matrix, with important implications for cell adhesion, migration and proliferation. To further characterize the role of alphaV integrin in HIV replication, MDM and HeLa-MAGI cells were infected using R5 or X4-tropic virus in the presence or not of a small heterocyclic non-peptide RGD mimetic (S36578-2) selective for avb3 and avb5 integrins. MDM are alphaV integrin positive cells. In MDM, the presence of S36578-2 inhibited HIV replication in a dose-dependent manner and in the absence of toxicity. Importantly, compounds from the same family showed an IC50 in correlation with in vitro measured affinity for avb3 and avb5, suggesting a strong specificity of its alphaV-dependent antiviral activity. Blockade of avb3 and avb5 integrins with S36578-2 also inhibited HIV replication in alphaV positive HeLa-MAGI cell line. In both cases, antiviral activity of S36578-2 is linked to a change in cellular morphology, thus giving further evidences of the integrin function's impairment. Supporting these data, S36578-2 antivi-

ral activity was lost when MDM were cultured in low-adhesive conditions. HIV-1 infection in MDM has been shown to be influenced by integrin function, as seen by the antagonist-dependent inhibition of viral replication. Thus, our data supports the idea that blocking avb3 and avb5 integrins interaction with its ligand compromise HIV replication in MDM.

doi:10.1016/j.antiviral.2008.01.061

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Use of the HCV Cell Culture (HCVcc) System for Antiviral Drug Testing

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Recently described HCV cell culture (HCVcc) systems have provided the opportunity to study the entire virus life cycle in vitro. We are developing protocols to maximize the usefulness of the HCVcc system for developing therapies, especially those directed against previously inaccessible steps in the viral replication cycle, such as entry, virion assembly and viral exit. Using a chimeric genotype 2a virus (J6/JFH-1/JC1), we have shown that virus titers remain relatively constant for at least 13 days post-electroporation in Huh7.5 cells even when media is harvested and replenished daily, making it possible to maximize yields. While developing virus yield assays, we found that after infection of Huh7.5 cells at low MOI, HCVcc titers were less than 3×10^2 TCID50/ml until d3 post-infection, resulting in a limited dynamic range for a virus yield assay. We therefore developed virus yield assays that focus on specific stages of the life cycle. In one protocol, we analyze intracellular HCV RNA levels in response to compound treatment after infection. In a second protocol, we analyze the effect of compound on the production of infectious particles after electroporation of infectious RNA. By design, both of these assays mimic treatment of acute infection. Therefore, we developed an assay that mimics chronic HCV infection. In this assay cells that are persistently producing high levels of infectious virus are treated with inhibitors to determine their effect on ongoing virus production. Our current efforts are focused on applying these acute and chronic models to an HCVcc system that expresses replication-dependent Renilla luciferase (Renilla J6/JFH-1/JC1). In initial studies we have found that cells infected with a low MOI of Renilla J6/JFH1/JC1 virus produce a robust Renilla chemiluminescence signal 48 h after infection. Ongoing efforts to further develop this system will provide simpler reporter-based virus yield assays that can be used to assess the effectiveness of antivirals that target virus entry, replication and egress.

doi:10.1016/j.antiviral.2008.01.062

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In Vitro Vascular Leak as a Model of Viral Hemorrhagic Fever

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Viral hemorrhagic fever (VHF) describes a group of diseases associated with infection by a number of genetically diverse, enveloped single-stranded RNA viruses including: (1) filoviruses, (2) arenaviruses, (3) flaviviruses, and (4) bunyaviruses. Although clinical presentations of VHF can vary by virus, a critical hallmark of human VHF infection is the loss of vascular barrier function resulting in changes in plasma volume and development of coagulation defects that can result in bleeding, pulmonary edema, and shock. Evidence suggests a role for innate and adaptive immune cells and mediators in the development of vascular leak in addition to direct infection of EC by virus. While the development of virus-specific antiviral therapies is critical to the treatment of VHF, development of therapeutics aimed at prevention of vascular leak may provide broad-spectrum treatment for a variety of infectious agents associated with VHF without a requirement for precise identification of the agent, often a challenge in VHF endemic regions of the developing world. Our laboratory has optimized an existing cell-based model of vascular leak that measures electrical resistance to allow screening of potential inhibitors of vascular leak in arenavirus-, bunyavirus-, and flavivirus-infected EC. Our results indicate that Pichinde' virus (arenavirus), Dengue virus (flavivirus), and Hantavirus (Bunyavirus) infection of EC induces a decrease in electrical resistance indicating and increase in vascular permeability that requires virus infection and/or stimulation with proinflammatory cytokines or chemokines. Using this model, a panel of small molecule inhibitors targeting with cell signaling pathways involved in EC structural or functional integrity have been screened with results supporting the hypothesis that host–cell targeting of EC may be useful in the treatment of VHF. Taken together, these data support the use of this assay as a screen for active compounds for viral cellular targets associated with VHF while also identifying additional therapeutic targets for drug discovery.

doi:10.1016/j.antiviral.2008.01.063

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Resistance to Pyrimidinedione HIV Inhibitors Requires Multiple Mutations in Reverse Transcriptase, Envelope and Core Proteins

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The pyrimidinediones are small molecule HIV inhibitors with two distinct mechanisms of action, inhibiting HIV-1 RT at subnanomolar concentrations through interaction at the hydrophobic NNRTI binding pocket and the entry of both HIV-1 and HIV-2 at low nanomolar concentrations by interaction