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.

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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.

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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.

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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