SI of 100. Time of addition studies demonstrated activity of this compound when added as late as 16 h after virus challenge of Vero cells with an EC_{90} of 8.9 μg/ml. Significant improvement in survival, serum levels of ALT, and virus titer in the liver was observed after bid treatment with 120 mg/kg/d of 2′-C-MeC for 7 days beginning just prior to virus challenge. A 4-day bid treatment regimen with this dose beginning 4 h prior to virus challenge was also effective in significantly improving survival. A lower dose of 80 mg/kg/d was also effective in significantly improving survival and serum ALT. Treatment with the 120 mg/kg/d dose initiated beginning 2 dpi was effective in significantly improving survival and serum ALT. Due to the severe gastrointestinal effects associated with long-term treatment, this compound and its derivatives may not be clinically viable for the treatment of chronic HCV. Alternatively, short-term treatment of an acute flaviviral disease like YFV would likely minimize or eliminate deleterious side effects associated with long-term treatment, potentially making the use of 2′-C-MeC and active derivatives a viable option for therapeutic intervention.

Acknowledgement: [Supported by N01-AI-30048, N01-AI-30063 (Southern Research Institute) from the Virology Branch, NIAID, NIH].

doi:10.1016/j.antiviral.2010.02.380

71

Immunosafety Assessment of CD4 MAB-based Bifunctional HIV Entry Inhibitor (CD4-BFFI) using *In Vitro* Immunoassays

Ford Kirschenbaum[1,*], Sandhya Bohini[1], Harald Kropshofer[2], Nick Cammack[1], Surya Sankuratri[1], Changhua Ji[1]

[1] *Roche Palo Alto, Palo Alto, USA;* [2] *Roche Basel, Basel, Switzerland*

We have previously described a CD4 monoclonal antibody (mAb)-based bifunctional HIV entry inhibitor (CD4-BFFI). CD4-BFFI demonstrated highly potent anti-HIV activities and excellent *in vivo* stability. Since CD4-BFFI binds to CD4 and CD4 is involved in $CD4^+$ T cell activation and other immunological functions, it is important to assess the potential immunological liabilities of CD4-BFFI before it enters human studies. We evaluated the direct effects of CD4-BFFI on $CD4^+$ T cells to see if it activates T cells via cross-linking CD4 molecules on cell surface. Our results showed that CD4-BFFI did not activate Jurkat cells or peripheral blood mononuclear cells (PBMC). There have been reports that some antibodies, especially those targeting blood cells, induced quick and marked cytokine release (cytokine storm) when dosed in humans, which may result in severe complications and even deaths. To assess the risk, an *in vitro* assay was performed using human whole blood from multiple donors. CD4-BFFI was incubated with human blood for 6 h, no cytokine secretion was observed, while the control anti-CD52 antibody (alemtuzumab) caused significant release of cytokine TNF-α and neutrophil activation (elevated CD11 expression) in 11 of the 12 donor blood samples. To investigate whether CD4-BFFI interferes with the co-receptor function of CD4 on T cells, an in vitro T cell activation assay was performed using Jurkat T cells and MACSiBeads that mimic antigen-presenting cells (APC). Significant activation of Jurkat cells was observed after stimulation with MACSiBeads. Co-incubation with CD4-BFFI showed no effects on Jurkat cell activation. Similar results were obtained using primary human PBMC cultures. An antigen-specific T cell activation assay was then developed for further evaluation. Human PBMC from cytomegalovirus (CMV)-infected donors was stimulated with CMV pp65 protein and significant activation of $CD4^+$ T cells (elevated CD69 and CD25 expression) was observed. Co-treatment with CD4-BFFI showed no effect on T cell activation. In summary, by using several in vitro immunoassays, we have demonstrated that CD4-BFFI did not activate human whole blood or T cells, and it did not interfere with the co-receptor function of CD4 in T lymphocytes in APC-mediated T cell activations.

doi:10.1016/j.antiviral.2010.02.381

72

Inhibition of Severe Acute Respiratory Syndrome Coronavirus Replication in a Lethal SARS-Cov Balb/C Mouse Model by Stinging Nettle Lectin, Urtica Dioica Agglutinin (UDA)

Yohichi Kumaki*, Miles K. Wandersee, Kevin W. Bailey, Aaron J. Smith, Craig W. Day, Jason R. Madson, Donald F. Smee, Dale L. Barnard

Institute for Antiviral Research, Utah State University, Logan, USA

Keywords: BALB/c mice; SARS-CoV; Urtica dioica agglutinin (UDA).

Urtica dioica agglutinin (UDA) was tested for efficacy in a lethal SARS-CoV-infected BALB/c mouse model. UDA is a small plant monomeric lectin, 8.7 kDa in size, with an N-acetylglucosamine specificity and inhibits viruses from Nidovirales in vitro. In the current study, groups of BALB/c mice were infected with 2 LD50 of virus and treated intranasally with UDA at the doses of 20, 10, 5 and 0 mg/kg/day for 4 days beginning at 4 h post virus exposure. Treatment with UDA at 5 mg/kg significantly protected mice against a lethal infection with mouse-adapted SARS-CoV ($p < 0.001$), but did not significantly reduce virus lung titers. All mice receiving UDA treatments were also significantly protected against weight loss due to the infection ($p < 0.001$). UDA also effectively reduced lung pathology scores. All mice receiving poly IC:LC, the positive control drug, survived the infection ($p < 0.001$). At day 6 after virus exposure, all groups of mice receiving UDA or poly IC:LC had much lower lung weights than did the placebo-treated mice. Our data suggest that UDA treatment of SARS infection in mice leads to a substantial therapeutic effect that protects mice against death and weight loss.

Acknowledgment: This work was supported by contracts NO1-A1-30048 and NO1-AI-15435 from the Virology Branch, National Institute of Allergic and Infectious Diseases, National Institutes of Health.

doi:10.1016/j.antiviral.2010.02.382

73

Viprolaxikine, a Novel Cytokine-like Protein from Insect Cell Cultures can Reduce Dengue-2 Virus Titres in Mammalian Cells

Chaowanee Laosutthipong*, Timothy Flegel

Mahiadol University, Bangkok, Thailand

Dengue virus (DEN) is an arthropod-born virus that causes dengue fever and dengue hemorrhagic fever in human hosts, but no disease in mosquito vectors. Viruses often persist in insects and other arthropods such as shrimp in either single, dual or multiple infections without gross signs of disease. From the supernatant solution of grossly normal C6/36 mosquito cell cultures persistently infected with DEN-2 virus, a novel antiviral agent was separated by ultrafiltration (5 kDa). Pre-incubation of mammalian (Vero) cell cultures with the ultrafiltrate reduced DEN-2 titres by up to 4 logs upon subsequent challenge. There was no reduction in titre for Vero cells pre-incubated with ultrafiltrate from uninfected C6/36 cells. Protease treatment of the protective ultrafiltrate removed its anti-DEN-2 activity while heating did not. Since 8-hr pre-incubation with the unltrafiltrate was required to obtain maximum protection against DEN-2, the active substance was called viprolaxikine,

a small, cytokine-like protein capable of inducing an anti-DEN-2 response in Vero cells.

doi:10.1016/j.antiviral.2010.02.383

74

The Susceptibility of Isolates of Pandemic 2009 H1N1 Influenza A Virus to Russian Domestic Antivirals

Irina Leneva[1,*], Alesya Romanovskaya[2], Elena Burtseva[3], Mike Eropkin[4], Alexander Shestopalov[2]

[1] *Centre of Chemistry of Drugs, Moscow, Russia;* [2] *Center of Virology and Biotechnology "Vector", Novosibirsk, Russia;* [3] *D.I. Ivanovsky Institute of Virology, Moscow, Russia;* [4] *Institute of Influenza, St.-Petersburg, Russia*

The antiviral drugs rimantadine, arbidol and ingavirin produced and widely used in Russia are licensed for the phrophylaxis and the treatment of influenza A and B. Ribivirin has been long recognized as a broad-spectrum antiviral agent with particularly distinct activity against orthomixoviruses (that is, influenza) and paramyxoviruses. The purpose of this study was to provide detailed information on Russian domestic drug susceptibility of the pandemic 2009 H1N1 influenza A virus. A/California/04/2009 and A/California/07/2009, that were obtained from CDC, and 4 viruses that were isolated in Russia from patients infected with 2009 H1N1 virus were used in this study. The results of experiments have shown that arbidol and ribavirin inhibited selectively the reproduction of studied viruses in MDCK cells. All 6 tested 2009 H1N1 viruses exhibited IC_{50} values characteristic of other laboratory and epidemic strains of influenza viruses. The IC_{50} for arbidol ranged from 4 to 8.5 μg/ml, whereas those for ribavirin ranged from 1.5 to 3 μg/ml. Rimantadine in nontoxic for cells concentrations does not affect the reproduction of all studied isolates of pandemic 2009 H1N1 influenza A virus. We revealed no significant antiviral activity of ingavirin in cell culture in nontoxic concentrations (up to 200 μg/ml) against all studied 2009 H1N1 influenza viruses. Sequencing of viruses with the further analysis on mutations which are responsible for resistance to anti-influenza drugs were conducted. The data obtained in cell culture have been confirmed by the results of genome analysis of all 6 studied viruses. It was shown previously that resistance to arbidol-resistant mutants generated in vitro has been due to substitutions in different positions of HA2 subunit of HA protein. The sequence of genes of all studied viruses did not reveal the replacements defining resistance to arbidol while both viruses contained a mutation in position 31 of M2-protein which is responsible for resistance to adamantanes.

doi:10.1016/j.antiviral.2010.02.384

75

Small Molecule Inhibitors of *De Novo* Cell-free Capsid Assembly Effective against *Flaviviridae* and *Togaviridae*

Vishwanath R. Lingappa[1,*], Juliane Gentzsch[2], Kiel Copeland[1], I.-ting Jaing[1], Michael Corpuz[1], Pamela Glass[3], Jaisri R. Lingappa[4], Brenna Kelley-Clarke[4], Debendranath Dey[1], Colm Kelleher[1], Andy Atuegbu[1], Amy Anderson[1], Josh Lehrer-Graiwer[1], Thomas Pietschmann[2], Clarence R. Hurt[1], William Hansen[1]

[1] *Prosetta Bioconformatics, Inc., San Francisco, USA;* [2] *Division of Experimental Virology, TWINCORE, Hannover, Germany;* [3] *Virology Division, USAMRIID, Ft Detrick, USA;* [4] *Department of Global Health, University of Washington, Seattle, USA*

We have established separate cell-free protein synthesis (CFPS)-based screens for small molecules that block any step in the pathways of host-catalyzed capsid assembly of Hepatitis C virus (HCV) and Venezuelan equine encephalitis virus (VEEV), members of the families *Flaviviridae* and *Togaviridae*, respectively. For HCV, over 80,000 small molecules were screened and approximately 400 initial hits were counter screened to exclude inhibitors of protein synthesis, thereby narrowing hits to approximately 90 molecules representing over 20 distinct pharmacophores with molecular weight <500 Da. Approximately 75% of these chemical classes (16 of 21) have been demonstrated to be active against infectious Hepatitis C virus. For VEEV, a screen of approximately 20,000 compounds yielded a large number of shared hits with the HCV screen as well as some with activity against VEEV but not against HCV. Several of the compounds active against live HCV in cell culture are active at similar doses against infectious Dengue virus in cell culture but are not active against VEEV. Thus it appears that a substantial subset of the novel small molecules that emerged from the HCV screen are relatively specific for flaviviruses, having activity against two different members of family *Flaviviridae* and no activity against a member of the *Togaviridae*. Data will be presented on a small molecule active against each of these two viral families. Together these findings suggest the potential for a new generation of broad-spectrum antiviral therapeutics active against whole families of viruses. Other data suggest that many of these drugs target host factors and thus, the breadth of their activity raises the possibility that they may be less susceptible to the development of virus resistance.

doi:10.1016/j.antiviral.2010.02.385