Oral Session 5: Respiratory Viruses, Emerging Viruses and Biodefense Chairs: Brian Gowen, Ph.D. and Graciela Andrei, Ph.D. 1:30–3:30 pm Sofia 1 and 2

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Important Role for Protein Kinase C-α in Combined Pneumolysin/Influenza A Virus-induced Pulmonary Endothelial Hyperpermeability

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Influenza viruses have posted an increasing threat of potential pandemics, as was shown in the past outbreaks involving H5N1 or H1N1 viruses. Influenza A virus (IAV) and *Streptococcus pneumoniae* represent important etiological agents of severe pneumonia, which is the main cause of death in children under 5 years of age worldwide. Mortality after influenza A infection has been suggested to be mainly due to secondary pneumoccocal infections. Death in pneumococcal-induced pneumonia can occur days after initiation of antibiotic therapy, when tissues are sterile and the pneumonia is clearing and correlates with the presence of virulence factors, the most important one of which is the pore-forming toxin pneumolysin (PLY). In this study, we report that co-treatment of monolayers of human microvascular endothelial cells (HL-MVEC) with both PLY (7.5 ng/ml) and UV-inactivated IAV (A/Wisconsin/33 (H1N1) strain, 1 IU/cell), induces a significant loss of barrier integrity (normalized transendothelial resistance drops from 1.0 to 0.4), as measured by means of using the electrical cell substrate impedance sensing technique (ECIS 1600R, Applied Biophysics, Troy, NY), whereas each treatment independently fails to do so. Since Protein Kinase C has been demonstrated to be involved in regulating endothelial permeability, we have therefore assessed its potential implication in the observed effects of IAV/PLY. As such, we could detect that both PLY and IAV induce PKC-α activation in HL-MVEC within 1 h. Moreover, a specific PKC-a inhibitor Ro-32-4032 (10 nM) significantly blunts the permeability-increasing effect of the combined IAV/PLY treatment in HL-MVEC by about 50%. In conclusion, these results indicate that PKC-α may represent an important therapeutic target in IAV infection-associated pulmonary endothelial hyperpermeability.

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2′- and 4′-Modified Ribonucleoside Analogs Can Inhibit All Four Serotypes of Dengue Virus in Human Primary Dendritic Cells as Competitive Inhibitors and Non-obligatory Chain Terminators

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Dengue virus (DENV), an emerging pathogen from the Flaviviridae family with neither vaccine nor antiviral treatment available, causes a serious worldwide public health threat. The RNA-dependent RNA polymerase NS5 of DENV is structurally related to NS5B of Hepatitis C Virus (HCV). We tested a number of 2′ and 4′-modified nucleoside analogs as inhibitors of either HCV or DENV, using hepatic and human primary dendritic cells as representative target cells, respectively. We identified a number of active nucleosides from these series, which inhibited all 4 serotypes of DENV in human transformed hepatocytes, as well as human primary dendritic cells. The biochemical profile of potent inhibitors of DENV replication was consistent with base-specific competitive inhibition of natural nucleoside substrate incorporation, and incorporation of 2′- or 4′-modified nucleosides into the nascent RNA was associated with immediate inhibition of RNA chain extension. Phosphoramidates could successfully circumvent a phosphorylation block of certain inactive nucleosides from the series in primary human dendritic cells. Among 4′-substituted nucleosides, Balapiravir (4′-azido-cytidine) was identified as a novel inhibitor of DENV replication. Based on these results, a randomized, double-blind, multiple-dose, placebo-controlled study was initiated to evaluate the safety, tolerability and efficacy of 5-day treatment with balapiravir in adult male patients with confirmed DENV infection and whose symptoms began within 48 h preceding the first administration of balapiravir. Further improvement in antiviral potency could be achieved with additional nucleoside modifications.

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2009 Pandemic Influenza Virus: What Special for its HA and NA?

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The 2009 pandemic influenza seemingly spreads extremely quickly with worrisome mortalities and resembles some characteristics of the previous three pandemics (1918 Spanish-flu, 1957 Asian-flu and 1968 Hong Kong-flu). The virus was recognized as a new swine-origin H1N1 influenza A virus (S-OIV). Functional and structural characterization of both the haemagglutinin (HA) (09H1) and the neuraminidase (NA) (09N1) might give us some clues about its pathogenesis and directs the drug application. In our group both the 09H1 and the 09N1 were prepared in a baculovirus-based system and the 09N1 enzymatic activity was verified *in vitro*. The 09N1 crystal structure has been solved (1.9 Å) and the structure surprisingly shows a Group 2 active cavity, different from other known N1 structures which are all categorized into Group 1. The 09N1 structures in complex with substrate sialic acid, Oseltamivir (Tamiflu) or Zanamivir (Relanza) have also been solved at 1.8 Å, 1.7 Å and 1.9 Å respectively, showing typical binding modes and revealing the structural basis of the effectiveness of the NA-targeted drugs against the 2009 pandemic. More importantly, the newly defined Group-1 150-loop cavity proposed as a drug target should be reconsidered as it is not as common as we thought. This is the first solved NA structure derived from swine and the first complex structure with sialic acid for Group 1 members.

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