Several neutralizing mouse monoclonal antibodies (MAbs) specific to glycoprotein E of TBE virus were tested in the model animal protection experiments using lethal doses of this virus. Mouse MAb demonstrated protective activity in the absence of antibody dependent enhancement was selected. Variable domains of this mouse Mab and constant domains of human immunoglobulin were used to construct humanized antibody. This humanized antibody produced by CHO cell line shown nanomolar affinity and ability to neutralize TBE infectivity in vitro. Moreover, this antibody demonstrated protective activity when administrated 1 2 3 days before infection and therapeutic activity when administrated 1 2 days post infection in TBE virus infected mice, and this activity was higher than therapeutic activity of commercially available specific immunoglobulin from donor blood.

REF O10

Characterization of human cytomegalovirus microRNA temporal expression profile and target prediction by dynamic expression analysis

Marta TREVISAN[1], Carlotta ALBONETTI[1], Enrico LAVEZZO[1], Tiziana SANAVIA[2], Alessandro SINIGAGLIA[1,3], Barbara DI CAMILLO[2], Giorgio PALÙ[1], Luisa BARZON[1]
[1]Department of Molecular Medicine, University of Padova, Padova, ITALY; [2]Department of Information Engineering, University of Padova, Padova, ITALY; [3]IOV Istituto Oncologico Veneto, Padova, ITALY
<marta.trevisan@unipd.it>

Targets for human cytomegalovirus (HCMV) miRNAs in host transcriptome are still largely unknown. Aim of this study was to characterize the effects of HCMV miRNAs on host human miRNA and mRNA temporal expression profiles after infection, identifying candidate gene targets. To this aim, tandem microarray analysis of miRNA and mRNA temporal expression was done in time series of MRC 5 cells infected with HCMV Towne strain. The majority of HCMV miRNAs was found to be expressed since the earliest stages or within 24 hours post infection and continued to accumulate over time. Differentially expressed mRNAs were selected according to the area of the region bounded by the expression profiles related to control and post infection cases. Identification of HCMV miRNAs targets was done by integrating the prediction of sequence based algorithms with Pearson correlation between viral miRNAs and cellular mRNAs temporal profile to detect significant negatively correlated target genes. This method, for instance, permitted to identify 55 significantly correlated target for HCMV miR US25 2 5p among the 1034 sequence based selected candidate genes. Experimental validation of some selected predicted targets was done by standard lucyferase activity assays and western blot analysis. In conclusion, an integrated analysis of viral miRNAs and host mRNA using a meta consensus approach based both on sequence prediction methods and on correlation analysis of dynamic expression data allowed the prediction and identification of host targets for HCMV miRNAs.

REF O11

Non liposomal delivery of anti rabies virus siRNAs counteracts viral growth and spread in vitro

Tobias HALBACH, Johannes HARDER, Konstantin SPARRER, Thomas CARELL, Karl Klaus CONZELMANN
LMU, Munich, GERMANY
<tobi.halbach@gmx.de>

Rabies virus (RABV) infection continues to be a threat throughout the world, with more than 55,000 human deaths each year. The majority of rabies cases occur in rural regions of Africa and South Asia, where RABV infection is endemic and access to rapid medical treatment is hindered. After the first symptoms of rabies emerge, post exposure treatment and vaccinations are not potent anymore and the outcome of the disease is almost exclusively fatal. Therefore, the need for an efficient antiviral therapy against rabies is still a major issue. RNA interference (RNAi), which silences expression of specific target genes could represent a promising tool for treating RABV infections in mammalian hosts. However, difficulties including delivery of siRNA/miRNA, short term efficiency, the emergence of resistant subpopulations and the resistance of viral RNPs against RNAi limit the potential of this method. Here, we developed a novel non liposomal siRNA delivery system that can counteract RABV growth and spread in vitro. Using siRNAs that were covalently linked to an arachidonoyl ethanol amide (anandamide) ligand via their 3'end we could specifically target cells expressing the receptor for the anandamide ligand (the cannabinoid receptor), which is predominantly present on neuronal and immune cells. Indeed, RABV infected human immune cells and mouse neuronal cells that were treated with the anandamide modified siRNAs showed a strong inhibitory effect against RABV infection. Adapting this system to an in vivo model, it might represent a promising tool to limit RABV infections in vivo.

REF O12

Inhibition of Pyridimine Biosynthesis Pathway Suppresses Viral Growth Through Innate Immunity

Pierre Olivier VIDALAIN[1,2], Marianne LUCAS HOURANI[1,2], Daniel DAUZONNE[3,4], Pierre JORDA[3,4], Gaëlle COUSIN[3,4], Alexandru LUPAN[5,6], Olivier HELYNCK[5,6], Grégory CAIGNARD[1,2], Geneviève JANVIER[1,2], Gwénaëlle ANDRÉ[7,8], Nicolas ESCRIOU[1,2], Philippe DESPRÈS[9], Yves JACOB[10,11], Hélène MUNIER LEHMANN[5,6], Frédéric TANGY[1,2]
[1]Unité Génomique Virale et Vaccination, Institut Pasteur, Paris, FRANCE; [2]CNRS, UMR 3569, Paris, FRANCE; [3]Institut Curie, Paris, FRANCE; [4]CNRS, UMR 176, Paris, FRANCE; [5]Unité de Chimie et Biocatalyse, Institut Pasteur, Paris, FRANCE; [6]CNRS, UMR 3523, Paris, FRANCE; [7]Unité de Biochimie Structurale, Institut Pasteur, Paris, FRANCE; [8]CNRS, URA 2185, Paris, FRANCE; [9]Unité Interactions moléculaires Flavivirus Hôtes, Institut Pasteur, Paris, FRANCE; [10]Unité de Génétique Moléculaire des Virus à ARN, Institut Pasteur, Paris, FRANCE; [11]Dana Farber Cancer Institute, Center for Cancer Systems Biology (CCSB) and Department of Cancer Biology, Boston, USA
<vidalain@pasteur.fr>

Searching for stimulators of the innate antiviral response is an appealing approach to develop novel therapeutics against viral infections. Here we established a cell based reporter assay to identify compounds stimulating expression of interferon inducible antiviral genes. We screened a total of 41,353 small molecules and selected DD264 for its immuno stimulatory and antiviral properties. While searching for its mode of action, we identified DD264 as an inhibitor of pyrimidine biosynthesis, establishing a yet unsuspected link between this pathway and the expression of antiviral genes. Furthermore, we found that antiviral activity of DD264 or brequinar, a well known inhibitor of pyrimidine biosynthesis pathway, is strictly dependent on cellular gene transcription and required Interferon Regulatory Factor 1 (IRF1). Altogether, our results better explain the antiviral property of pyrimidine biosynthesis inhibitors and unravel a novel pathway that induces cell resistance to RNA virus infections.