Oral Presentation Abstracts

53

THE ISPOPRENYLATED LARGE ISOFORM OF HEPATITIS DELTA VIRUS PROTEIN ACTIVATES STAT-3 AND NF-KB, VIA OXIDATIVE STRESS

S. Brichler[1,2], V. Williams[1,2], E. Khan[1,2], P. Dény[3], D. Kremsdorf[2], E. Gordien[1,2]

[1]Laboratoire de Virologie, Hopital Avicenne, Bobigny, [2]INSERM U845, Faculté Paris Descartes, Paris, [3]INSERM U821, Lyon, France

Corresponding Author's E-mail: segolene.brichler@avc.aphp.fr

Hepatitis delta virus (HDV) requires co or superinfection with hepatitis B virus (HBV). HDV expresses two proteins, the small (p24) and the large (p27) delta antigen, which are identical except for an additional 19 residues at the C terminus of p27, which contains an isoprenylation site on a cysteine (C) residue, C211. Isoprenylation is critical for interaction with HBV envelope proteins for virus assembly and secretion of virions. Chronic HDV infection is often responsible for a more rapid progression to cirrhosis and hepatocarcinoma. The mechanisms for this more agressive liver disease, are poorly understood. Using a transient transfection cellular model, we show that p27 induces the activation of NF-κB and STAT-3 transcription factors via production of reactive oxygen species (ROS). Indeed, flow cytometric analysis of cellular ROS levels was significantly higher in cell expressing p27. Moreover, in the presence of antioxidants [pyrrolidine dithiocarbamate (PDTC), N-acetyl Lcysteine (NAC)] or Ca2+ inhibitors (BAPTA-AM, TMB-8 and Ruthenium Red), p27-induced activation of NF-κB and STAT-3 was dramatically reduced. Oligo in vitro pull down experiments show binding of STAT-3 and NF-κB on their cognate response element in p27 expressing cells, and, both p24 and p27 were also found in the protein-binding complex. Evidence is presented for involvement of an endoplasmic reticulum stress by the isoprenylated p27, as p24 and a mutated form of p27 where the C211 was replaced by a serine, show a very low production of ROS and a very weak or no activation of STAT-3 and NF-κB. These results provide an insight into the mechanisms by which p27 can alter intracellular events relevant to liver pathogenesis associated with the delta viral infection.

54

STUDY OF THE REPLICATION OF HEPATITIS DELTA VIRUS (HDV) CLADE 1, 6 AND 8 PROTOTYPES IN CELL CULTURE

V. Mom[1], B. Bloquel[1], N. Falah[2], J.-C. Cortay[2], D. Yu[1], C. Sureau[3], H. Olivier[1], A. Kay[1], E. Gordien[4], F. Le Gal[4], B. Lina[2], F. Zoulim[1], P. Dény[1,4]

[1]U871, INSERM, LYON-1, [2]FRE3011, CNRS, Lyon, [3]Molecular Virology, INTS, Paris, [4]Laboratoire Associé au CNR des Hépatites B, C et Delta, Université Paris 13, Bobigny, France

Corresponding Author's E-mail: paul.deny@inserm.fr

Background: HDV is a satellite of HBV that requires HBsAg for virion egress and penetration into hepatocytes. Dual HBV-HDV infections induce severe liver diseases: acute or chronic hepatitis, cirrhosis and hepatocellular carcinoma. Around 18 million people are infected with both viruses. Eight HBV and HDV genotypes are described, sometimes linked to specific geographical area, such as HDV5-HDV8 in Africa.

Material and methods: To explore HBV-HDV interactions. Our study compared HDV6 and HDV8 replication to HDV1 prototype, using transfection of tandem repeat-RNA expression vectors and HBsAg-coding plasmids in Huh7 cell and infection of Primary Human Hepatocytes.

Results: Our *in vitro* model indicated that HDV1, 6 and 8 behave differently. HDV1 replicated well, either without or with HBsAg from HBV genotype D or E, and produced infectious viral particles for human primary hepatocytes. HDV6 displayed very efficient replication. However, in the presence of HBsAg, only faint amount of extracellular viral RNA was detected, suggesting a defect in viral assembly. HDV8, neither maintained its RNA replication nor produced western blot-detectable HD proteins. However, intranuclear HDAg accumulation occurred for each construct. As S-HDAg is essential for HDV-RNA replication, we studied S-HDAg expression, using vectors expressing a double Strep-Tag-S-HDAg. This allowed to compare protein expression levels and to assess that S-HDAg was expressed at a lower level for HDV8 than for HDV1 and HDV6, suggesting a default in S-HDAg production or stability.

Conclusion: Each different clade prototype behaves differently. HDV1 had a complete replication cycle, including the efficient production of viral particles. HDV6, having the same level of RNA replication and editing than HDV1, had a defect in viral assembly with both HBV/D-E HBsAgs. Transcription initiation from HDV8 did not maintained in cell culture, due to a defect in HDV8-S-HDAg production. These differences might contribute to the wide viral spreading of HDV1 on Earth.

55

HUMAN LIVER CHIMERIC UPA MICE AS A NEW ANIMAL MODEL FOR THE STUDY OF HDV/HBV INFECTION

L.V. Mancke[1], M. Lütgehetmann[1,2], T. Volz[1], T. Bornscheuer[1], L. Allweiss[1], M. Ben M Barek[1], A.W. Lohse[1], J. Petersen[3], M. Dandri[1]

[1]Department of Internal Medicine, [2]Department of Medical Microbiology, Virology, Hygiene, University Medical Center Hamburg-Eppendorf, [3]Institute for Interdisciplinary Medicine at Asclepios Clinic St.Georg, Hamburg, Germany

Corresponding Author's E-mail: lida.mancke@googlemail.com

Background: Hepatitis delta virus (HDV) is a subviral agent which can cause severe liver disease in association with chronic hepatitis B virus (HBV) infection. Although HDV requires HBV envelope proteins for assembly and infectivity, little information is available about the pathophysiology of HDV infection and whether HDV can directly inhibit HBV replication in vivo. Aim of this study was to establish a model of HDV infection using uPA/SCID mice repopulated with primary human hepatocytes.

Methods: We performed HDV infection experiments using either naïve (N=13) or HBV-chronically infected (N=5) human liver chimeric mice. In both experimental settings, animals received HDV/HBV human infectious serum containing 10^6 HDV (genotype 1) and 10^8 HBV (genotype D) genome equivalents. Serological and intrahepatic HBV (rcDNA, cccDNA) and HDV-RNA viral levels were measured over time by quantitative RT-PCR. Presence of HBsAg, HBcAg and HDAg was ascertained by immunohistochemistry.

Results: De novo synthesis of genomic HDV-RNA was determined both in serum and liver of 5/5 HDV super-infected mice. HDV-RNA viremia rose up to 1x10^9/copies/ml within 8 weeks after HDV inoculation. Notably, a median 60% reduction of HBV levels was found in HDV super-infected animals as HDV replication increased, suggesting that HDV may hinder HBV replication in vivo. Establishment of HDV infection in co-infected mice was rapid and efficient (13/13 mice), with HDAg-positive staining already detectable after 3 weeks of infection. Remarkably, 6 weeks post infection mice developed high viral titres (both 10^8 copies/ml) with the majority of human hepatocytes staining HDAg-positive, although HBV infection was still detectable in a minority of human hepatocytes (0,2 cccDNA/cell), confirming that HDV can replicate in human hepatocytes in the absence of HBV.

Conclusions: We established a new and efficient model of chronic HDV/HBV infection for the study of HDV replication mechanisms, as well as for in vivo preclinical antiviral drug evaluations.

56

PROTECTION OF WOODCHUCKS FROM HEPATITIS D VIRUS INFECTION AFTER IMMUNIZATION WITH DNA IMMUNIZATION AND ADENOVIRAL BOOST

M. Fiedler[1], A. Schumann[1], A. Kosinska[1], M. Lu[1], L. Johrden[2], O. Wildner[2], M. Roggendorf[1]

[1]Institut für Virologie, Universitätsklinikum Essen, Essen, [2]Institut für Virologie, Universität Bochum, Bochum, Germany

Corresponding Author's E-mail: melanie.fiedler@uni-due.de

Hepatitis D virus (HDV) superinfection of hepatitis B virus (HBV) carriers causes a high rate of chronicity and severe liver disease. Therefore, the design of a vaccine protecting HBV-carriers form HDV-superinfection is eligible. In a previous study we showed that immunization of chronically woodchuck hepatitis virus (WHV) infected woodchucks with DNA expressing HDV-antigen (HDAg) administered by gene gun resulted in a modulation of the course of HDV-superinfection.

In the current study we immunized three naïve woodchucks in a prime/boost regimen to further enhance the immune response and to improve the outcome of HDV- infection. After immunization with DNA expressing HDAg (via gene gun and intra-muscularly) adenoviral vectors 5 and 35, both expressing HDAg, were administered sequentially. Subsequently, these animals and three naïve controls were challenged with 10^9 genome equivalents of WHV and 10^5 genome equivalents of HDV.

Virus-specific CTLs were determined by a flow cytometric degranulation assay based on staining for CD107a after stimulation of PBMC with a panel of twenty-six HDAg- peptides and previously characterized WHVcore- and WHVsurface-peptides. After the WHV/HDV challenge HDV-RNA and WHV-DNA were measured by qualitative and quantitative PCR and spot blot hybridization.

The immunization induced no measurable cellular or humoral immune response. After WHV/HDV challenge WHV-DNA was detected for up to 25 weeks in all woodchucks. HDV-RNA became positive in 3/3 controls, but 2/3 HDV-immunized woodchucks were protected from HDV infection. In the animal with the breakthrough HDV-RNA became detectable later than in the controls (week 7 vs. weeks 4 and 5). The WHV-specific immune response showed a typical course seen before in acute WHV-infection. An HDV-specific immune response was not measurable.

These promising data will be enlarged by additional immunization/challenge experiments in naïve and chronically WHV-infected woodchucks.

57

INTRAHEPATIC HDV RNA, HBV DNA AND HBV RNAS QUANTIFICATIONS REVEAL SUPPRESSION OF HBV PREGENOME RNA PRODUCTION WITH MAINTENANCE OF PRES/S TRANSCRIPTION IN HDV/HBV CO-INFECTED PATIENTS

T. Pollicino[1], G. Raffa[1], T. Santantonio[2], G. Gaeta[3], G. Iannello[4], A. Alibrandi[5], G. Squadrito[1], I. Cacciola[1], C. Calvi[6], G. Colucci[7], M. Levrero[8], G. Raimondo[1]

[1]Internal Medicine, University of Messina, Messina, [2]Clinic of Infectious Diseases, University of Foggia, Foggia, [3]Infectious Diseases, University of Naples, Naples, [4]Private Statistician, Roma, [5]SEFISAST, University of Messina, Messina, [6]Roche Diagnostics, Monza, Italy, [7]Roche Molecular Systems, Rotkreuz, Switzerland, [8]Internal Medicine, La Sapienza University of Rome, Rome, Italy

Corresponding Author's E-mail: tpollicino@unime.it

HDV and HBV interactions have been poorly investigated at intrahepatic level in humans. The aim of this study was to explore replicative and transcriptional activities of the two viruses in co-infected patients.

We examined both liver and serum samples from 21 HDV/HBV co-infected and 22 HBV mono-infected patients with chronic liver disease by applying real-time PCR assays to evaluate intrahepatic amounts of HBV DNA, covalently closed circular DNA (cccDNA), pregenomic RNA (pgRNA), preS/S RNAs and HDV RNA. In the sera, besides HBV DNA and HDV RNA levels, HBsAg concentrations were also determined. HBV sequencing was performed in all the isolates.

HDV co-infected cases showed significantly lower median levels of serum HBV DNA (10^5-fold less), intrahepatic rcDNA (10^2-fold less) and cccDNA (10^2-fold less) compared with HBV mono-infected cases. Moreover, both pgRNA and preS/S RNA amounts were significantly lower (both, 10-fold less) in HDV-positive patients. Serum HBsAg concentrations were comparable between the two patient groups. In fact, preS/S RNA and HBsAg amounts per cccDNA molecule were higher in HDV-positive (3- and 10-fold, respectively) than in HDV-negative patients, showing that HBV replication was strongly reduced whereas preS/S gene transcription was maintained. The ratio of cccDNA to intracellular total HBV DNA showed a higher proportion of cccDNA molecules in HDV-positive cases. In these patients intrahepatic and serum HDV RNA were associated with cccDNA but not with HBsAg or HBV DNA levels. Finally, HBV genomes with large deletions in the basal-core-promoter/precore region were detected in 5/21 HDV-positive but in no HDV-negative patients and were associated with the lower viremia levels.

Conclusions:

(1) The decrease of pgRNA transcription with maintenance of preS/S RNA transcription may explain the reduction of HBV replication without the parallel decrease of HBsAg synthesis commonly observed in HDV infection.

(2) HDV might favour the selection of HBV variants with low replicative capacity.