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MALADIES INFECTIEUSES ÉMERGENTES

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Philippe Roingeard, Inserm U1259, Tours
Hélène Strick-Marchand, Institut Pasteur, Paris
Mirjam Zeisel, CRCL - Centre de Recherche en Cancérologie de Lyon

SOMMAIRE

PROGRAMME

Mardi 15 juin 2021

13h00-13h15 Introduction par François-Loïc Cosset, Président de l'AC42 de l'ANRS-Maladies infectieuses émergentes

13h15-14h00 CONFÉRENCIER INVITÉ
Philippe Roingeard, Inserm U1259, Tours
A hepatitis C vaccine: why and how?

14h00-15h30 **Session : TRAITEMENTS, PRÉVENTION & SUIVIS CLINIQUES; PATHOGÉNÈSE** Modérateurs : Amandine Verga-Gérard & Pierre Nahon

- Dimitri LOUREIRO, Université de Paris, Centre de recherche sur l'inflammation, Inserm U1149, CNRS ERL8252, Department of Hepatology, AP-HP, Hôpital Beaujon, Paris
  Étude de la réponse virologique précoce chez six patients atteint de l'hépatite chronique Delta avec fibrose avancée ou cirrhose compensée traités par Bulevirtide dans la vraie vie

- Elsa GOMEZ-ESCOBAR, Inserm U1259 MAVIVH, Université de Tours et CHRU de Tours
  Incorporation de l'apolipoprotéine E dans les particules sous-virales d'enveloppe chimères VHB-VHC : implications en vue de l'amélioration d'une stratégie vaccinale contre les hépatites

- Frank JUEHLING, Université de Strasbourg, Inserm, U1110, Institut de Recherche sur les Maladies Virales et Hépatiques, Strasbourg
  Perturbation persistante de l'horloge circadienne par l'infection chronique par le virus de l'hépatite C après élimination par des antiviraux à action directe

- Alessia VIRZI, Inserm, Institut de recherche sur les maladies virales et hépatiques, université de Strasbourg (IVH), Strasbourg
  L'analyse protéomique d'hépatocytes infectée par le VHB révèle le collagène VI comme moteur de la fibrose du foie

Session flash (présentation de 4mn)

- Doohyun KIM, Inserm U1052, CNRS UMR-5286, CRCL, Lyon
  Caractérisation des ARNs circulants du virus de l'hépatite B chez les patients atteints d'hépatite B chronique

- Dimitri LOUREIRO, Université de Paris, Centre de recherche sur l'inflammation, Inserm U1149, CNRS ERL8252, Department of Hepatology, AP-HP, Hôpital Beaujon, Paris
  Dysfonctionnements mitochondriaux chez les patients atteints de l'hépatite B chronique avec une Fibrose hépatique importante
- Issam TOUT, Université de Paris, Centre de recherche sur l'inflammation, Inserm U1149, CNRS ERL8252, Department of Hepatology, AP-HP, Hôpital Beaujon, Paris
  Importance des cellules B dans la perte de l'AgHBs au cours de l'hépatite B chronique

- Rayana TOYE, Inserm U1052, CRCL, Lyon et Diamniadio, Sénégal
  Variabilité génétique du virus de l'hépatite B en Gambie et au Sénégal : élargissement de la diversité des sous-génotypes du génotype A

- Discussion 15mn

15h30-16h00 Pause

16h00-18h00 **Session : INTERACTION VIRUS-CELLULE**

Modérateurs : Laurence Cocquerel & Mirjam Zeisel

- Cyrine BENTALEB, University of Lille, CNRS, Inserm, CHU Lille, Pasteur Institute of Lille, U1019-UMR9017-CIIL, Lille
  On the Host Membrane Rearrangement side of the Hepatitis E Virus Lifecycle

- Bertrand BOSON, CIRI – Centre International de Recherche en Infectiologie, Team EVIR, Univ Lyon, Université Claude Bernard Lyon 1, Inserm, U1111, CNRS, UMR5308, ENS Lyon,
  La protéine core du virus de l'hépatite C recrute les ARN génomique viraux via leur interaction avec Nup98 au sein des Annulate Lamellae pour promouvoir l'assemblage des particules virales

- Delphine BOUSQUET, Inserm U1052, CRCL et Institut de Recherche en Santé, de Surveillance Epidémiologique et de Formation (Iressef), Lyon
  Les ARNs sécrétés par le Virus de l'Hepatite B (VHB) sont transportés par les vésicules extracellulaires dans le surnageant des hépatocytes infectés.

- Sonia FIEULAINE, Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), Gif-sur-Yvette
  Pas de protéase en vue : Etude structure-fonction de la polyprotéine du virus de l'hépatite E et de son domaine de type domaine de liaison aux acides gras

- Margaux HEUSCHKEL, Institut de Recherche sur les Maladies Virales et Hépatiques, UMRS1110 Inserm, Université de Strasbourg
  Caractérisation du rôle proviral de JAK1 dans l'infection de cellules par le virus de l'hépatite D

- Julie LUCIFORA, CIRI – Centre International de Recherche en Infectiologie, Team EVIR, Univ Lyon, Université Claude Bernard Lyon 1, Inserm, U1111, CNRS, UMR5308, ENS Lyon
  Un nouveau modèle cellulaire permettant la réplication des virus des hépatites B, C, D et E et l'étude des gènes stimulés par l'interféron responsables des effets antiviraux

Session flash (présentation de 4mn)

- Zakaria BOULAHTOUF, Inserm, Institut de recherche sur les maladies virales et hépatiques, Université de Strasbourg (IVH)
  La phosphorylation de l'histone linker, un mécanisme potentiel dans le maintien de l'infection par le VHB

- Julie LUCIFORA, CIRI – Centre International de Recherche en Infectiologie, Team EVIR, Univ Lyon, Université Claude Bernard Lyon 1, Inserm, U1111, CNRS, UMR5308, ENS Lyon
  La fixation des protéines HDV à la boucle epsilon des ARNs HBV accélère leur dégradation entrainant une interférence virale d'HDV sur HBV

- Esther MARTIN de FOURCHAMBAULT, CIIL, Inserm U1019-CNRS UMR9017, Université de Lille
  Etude de l'interaction entre le virus de l'hépatite C et les peroxysomes.

- Karoline METZGER, CIIL, Inserm U1019-CNRS UMR9017, Institut Pasteur de Lille, Université de Lille
  Caractérisation de la replicase du virus de l'hépatite E

- Discussion 15mn

18h00-18h15 **Intervention de Yazdan Yazdanpanah, Directeur de l'ANRS-Maladies infectieuses émergentes**

18h15-19h00 **REMISE DES PRIX DE THÈSE**
Intervention de Philippe Roingeard, Coordonnateur pour le Prix de thèse de l'AC42
Présentation du Premier Prix
Présentation du Deuxième Prix

Mercredi 16 juin 2021

9h00-9h45 CONFÉRENCIER INVITÉ
Sébastien Pfeffer, Université de Strasbourg
Modulation of RNA-based immunity in viral infections

9h45-10h15 **Session : INTERACTION VIRUS-CELLULE**
Modérateurs : Hélène Strick-Marchand & Philippe Roingeard

Session flash (présentation de 4mn)

- Maika DEFFIEU, Institut de Recherche en infectiologie de Montpellier (IRIM), CNRS, Montpellier
  Développement d'outils d'imagerie permettant l'étude de la dynamique d'entrée du virus de l'hépatite B.

- Anne-Flore LEGRAND, CIRI, Team EVIR, Univ Lyon, Université Claude Bernard Lyon 1, Inserm, U1111, CNRS, UMR5308, ENS Lyon
  Les ligands du récepteur nucléaire FXR inhibent la réplication du virus de l'hépatite delta et l'infectivité des virions dans des modèles de culture cellulaire

- Natalia FREITAS, CIRI, Team EVIR, Univ Lyon, Université Claude Bernard Lyon 1, Inserm, U1111, CNRS, UMR5308, ENS Lyon
  Un peptide de fusion dans preS1 et la protéine-disulfure isomérase ERp57 sont impliqués dans le processus de fusion membranaire du virus de l'hépatite B

- Maxime VILLARET, EA4474 « Virologie de l'Hépatite C », Université Paris Descartes, Paris
  Rôle des apolipoprotéines dans la morphogenèse du virus de l'hépatite C : réévaluation en hépatocytes humains primaires

- Discussion 15mn

10h15-10h45 Pause

10h45-12h15 **Session : INTERACTION VIRUS-CELLULE**

Modérateurs : Massimo Levrero & Joachim Lupberger

- Guillaume GIRAUD, Inserm U1052- Cancer Research Center of Lyon (CRCL), Lyon
  Les hélicases DDX5 et DDX17 régulent la fidélité transcriptionnelle et la maturation des ARN du virus de l'Hépatite B dans des hépatocytes primaires humains infectés

- Danai MOSCHIDI, CNRS ERL9002 - BSI - Integrative Structural Biology, Lille
  Étude structurale et fonctionnelle de la protéine ORF3 du VHE par RMN en solution

- Virginie DOCEUL, UMR 1161 Virologie, INRAE, ANSES, Ecole Nationale Vétérinaire d'Alfort, Université Paris-Est, Maisons-Alfort
  Caractérisation d'un système d'infection persistante par le virus de l'hépatite E dans les cellules hépatiques humaines HepaRG

- Marie-Laure FOGERON, Molecular Microbiology and Structural Biochemistry, Labex Ecofect, UMR5086 CNRS-Université de Lyon
  Vers la caractérisation structurale de la ribonucléoprotéine du virus de l'hépatite D

Session flash (présentation de 4mn)

- Mathilde BRIDAY, Molecular Microbiology and Structural Biochemistry (MMSB), Université de Lyon, CNRS UMR5086, Lyon
  Caractérisation des interactions hétérologues de la protéine core du virus de l'hépatite B par RMN et ITC

- Jean-Charles CARVAILLO, Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), Gif-sur-Yvette
  Modélisation moléculaire du domaine C-terminal de la protéine Core du virus de l'hépatite B : Implications pour le trafic intracellulaire de la nucléocapside

- Kalouna KRA, Institut de Biologie Intégrative de la Cellule (I2BC), CNRS, Université Paris-Saclay, CEA, Saclay
  Influence des modulateurs d'assemblage sur la structure et la cinétique d'assemblage de la capside du virus de l'hépatite B

- Lauriane LECOQ, Molecular Microbiology and Structural Biochemistry (MMSB), UMR5086 CNRS, Université de Lyon
  Un changement conformationnel induit par un ligand dans la poche hydrophobe de la capside du virus de l'hépatite B

- Discussion 15mn

12h15- 12h30 **Clôture**

RÉSUMÉS DES INTERVENTIONS ORALES

Étude de la réponse virologique précoce chez six patients atteint de l'hépatite chronique Delta avec fibrose avancée ou cirrhose compensée traités par Bulevirtide dans la vraie vie

Early virological response in six patients with hepatitis D virus infection and advanced fibrosis orcompensated cirrhosis treated with Bulevirtide in real-life

Tarik Asselah[1,2], Dimitri Loureiro[1,2], Fréderic Le Gal[3], Stéphanie Narguet[1,2], Segolène Brichler[3], Valérie Bouton[4], Malek Abazid[4], Nathalie Boyer[1,2], Nathalie Giuly[1,2], Athenais Gerber[3], Issam Tout[1,2], Sarah Maylin[5], Cheikh Mohamed Bed[1,2], Patrick Marcellin[1,2],Corinne Castelnau[1,2], Emmanuel Gordien[3], Abdellah Mansouri[1,2]

[1]Université de Paris, Centre de recherche sur l'inflammation, Inserm U1149, CNRS ERL8252, F- 75018 Paris, France. [2]Department of Hepatology, AP-HP, Hôpital Beaujon, F-92110 Clichy,France. [3]Université de Paris 13, Laboratoire de microbiologie Clinique, Centre National de référence des Hépatites B, C et Delta, Inserm U955, AP-HP, Hôpital Avicenne, F-93000 Bobigny,France. [4]Université de Paris, Service de Pharmacie, AP-HP, Hôpital Beaujon, F-92110 Clichy, France. [5]Université de Paris, Laboratoire de Microbiologie, AP-HP, Hôpital Saint-Louis, F-75010 Paris, France.

Background and Aims: Hepatitis Delta Virus (HDV) infection is the most severe form of viralhepatitis. Bulevirtide (BLV, Hepcludex®) is an HDV/HBV entry inhibitor recently approved in the European Union for adult patients with CHD compensated liver disease and positive HDVRNA viral load (HDV-VL). This real-life preliminary report described early virological efficacy and safety of BLV in 6 patients with CHD and advanced fibrosis/compensated liver disease.

Method: Six patients were included: 4/6 patients were treated with the combination ofpegylated interferon 2a 180 µg/week (pIFN) and BLV 2 mg/day in sub-cutaneous injection and2/6 patients were treated with BLV monotherapy; during 12 to up 68 weeks (*Fig. 1*).

Results: Regarding on-treatment response for the 4 patients treated with BLV combined withpIFN, 4/4 had a decline of a minimum of 1-log10 of HDV-VL and 3/3 of 2-log10 12 and 24 weeks respectively. One patient among four had stopped the treatment at 12th week because of thrombocytopenia and an HDV-VL relapse was notified 24 weeks after treatment cessation. 1/4 had undetectable HDV-VL during the therapy (< 100 IU/mL). Regarding on-treatment response for the 2 patients treated with BLV monotherapy, 1/2 patient had a decline of HDV- VL by 1-log10 at 8 weeks and 1/1 by 2-log10 at 28th week. Regarding biochemicals response, 2/4 patients with combined therapy had ALT levels with normal values reached at 4 and 56 weeks. 1/2 patients with BLV monotherapy achieve ALT normalization at 4 weeks on-treatment. HBsAg levels were not affected. An elevation of total bile acids was observed in 3/6patients without pruritus.

Conclusion: The goal regarding efficacy is to achieve a sustained virological and biochemicalresponses which are the main surrogate markers associated with favorable outcome. In our study, on treatment 5/6 patients achieved this goal, however a patient had a relapse 24 weeksafter treatment cessation. BLV was well-tolerated in all 6 patients and no significant side effects, except a thrombocytopenia, were reported. Bile acids increase was observed in 3/6 patients without pruritus. These early data confirmed the interest in this new treatment. Final results will be important to demonstrate long-term clinical benefit (fibrosis reversibility and reduction in HCC).

Loureiro Dimitri : Dimitri.loureiro@inserm.fr

Incorporation of apolipoprotein E into HBV-HCV subviral envelopeparticles: implications for an improved hepatitis vaccine strategy

Incorporation de l'apolipoprotéine E dans les particules sous-virales d'enveloppe chimères VHB-VHC : implications en vue de l'amélioration d'unestratégie vaccinale contre les hépatites

Elsa Gomez-Escobar[1], Julien Burlaud-Gaillard[1,2], Clara Visdeloup[1], AdelineRibeiro E Silva[1], Pauline Coutant[1], Philippe Roingeard[1,2], Elodie Beaumont[1]

[1]Inserm U1259 MAVIVH, Université de Tours and CHRU de Tours, Tours, France
[2]Plate-Forme IBiSA des Microscopies, PPF ASB, Université de Tours and CHRU de Tours,Tours, France
These authors jointly supervised this work

With 71 million chronic carriers, hepatitis C is a major threat to public health. Although the introduction of direct-acting antivirals has improved the treatment of the hepatitis C virus (HCV)infection, they do not protect from reinfection or the progression to severe liver diseases. TheWorld Health Organization aims to eradicate viral hepatitis by 2030, but recent prospective studies have shown that this would only be possible in few countries if the current measures do not evolve. Therefore, the development of a prophylactic HCV vaccine is still a priority, along with the implementation of screening and treatment initiatives. In line with this, we developed a vaccine based on full-length genotype 1a HCV envelope proteins (E1 or E2) fused to the heterologous hepatitis B virus (HBV) small (S) envelope protein that self-assemble intohighly immunogenic, noninfectious and secreted subviral envelope particles (SVPs), resembling the HBV vaccine. Immunization of rabbits with these chimeric particles induced theproduction of antibodies capable to neutralize HCV *in vitro*.

We aimed to improve the neutralizing potential of these antibodies by considering the role of the apolipoprotein E (apoE) in the folding of the HCV envelope proteins, and therefore in the modulation of the sensitivity to antibody-mediated neutralization. To mimic the epitopes at theapoE-HCV envelope proteins interface, we generated new chimeric HBV-HCV SVPs bearing envelope proteins complexed with apoE. SVPs were produced by Chinese hamster ovary cellclones stably expressing proteins of interest, and then purified and characterized. New Zealand rabbits were immunized with SVPs of interest, and humoral responses (anti-S and anti-E1/-E2 antibodies) were evaluated through immunoassays. The neutralizing potential of the anti-E1/-E2 antibodies was determined by neutralization assays using genotype 1a and 2aHCV in cell culture.

We found that apoE interacted with both HCV envelope proteins (E1 and E2), both chimeric HBV-HCV proteins (E1-S and E2-S), and also with the wild-type HBV S protein. In purified SVPs, apoE was detected on their surface and shown to improve the folding of the HCV envelope proteins; however, the incorporation of the E2-S protein was lower in particles bearing apoE. Titers of anti-S antibodies were similar for all immunized animals, whereas anti-E1/-E2 antibody titers varied according to the presence or absence of apoE. The neutralizing potential of anti-E2 antibodies was estimated higher in rabbits immunized with apoE-bearing particles against both evaluated HCV genotypes. In conclusion, the association of apoE with HCV envelope proteins may be a good strategy for improving HCV vaccines based on viral envelope proteins.

Elsa Gomez Escobar : elsa.gomez@univ-tours.fr

Perturbation persistante de l'horloge circadienne par l'infection chronique par le virus de l'hépatite C après élimination par des antiviraux à action directe

Persistent perturbation of the circadian clock by chronic hepatitis C virus infection following cure with direct-acting antivirals

Frank Jühling[1,2*], Atish Mukherji[1,2*], Laurent Mailly[1,2], Carla Eller[1,2], Clara Ponsolles[1,2], Katharina Herzog[1,2], Nourdine Hamdane[1,2], Xiaodong Zhuang[3], Hiroshi Aikata[4], Michio Imamura[4], Jacinta Holmes[5], Shu-Chi Wang6, Ming-Lung Yu[7,8,9], Raymond Chung[10], Catherine Schuster[1,2], Emanuele Felli[1,2,11], Patrick Pessaux[1,2,11], Jane McKeating[3], Kazuaki Chayama[12,13,14], Thomas F. Baumert[1,2,11,15]

[1] Université de Strasbourg, Strasbourg, France; [2] Inserm, U1110, Institut de Recherche sur les Maladies Virales et Hépatiques, Strasbourg, France; [3] Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom; [4] Department of Gastroenterology and Metabolism, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan; [5] University of Melbourne, St Vincent's Hospital, Melbourne, Australia; [6] Department of Medical Laboratory Science and Biotechnology; Center for Cancer Research and Liquid Biopsy, Kaohsiung Medical University, Kaohsiung, Taiwan; [7] Hepatobiliary Division, Department of Internal Medicine and Hepatitis Center, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; [8] Hepatitis Research Center, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; [9] Center for Liquid Biopsy and Cohort Research, Kaohsiung Medical University, Kaohsiung, Taiwan; [10] Liver Center, Gastroenterology Division, Massachusetts General Hospital and Harvard Medical School, Boston, United States; [11] Institut Hospitalo-Universitaire, Pôle Hépato-digestif, Nouvel Hôpital Civil, Strasbourg, France; [12] Collaborative Research Laboratory of Medical Innovation, Hiroshima University, Hiroshima, Japan; [13] Research Center for Hepatology and Gastroenterology, Hiroshima University, Hiroshima, Japan; [14] RIKEN Center for Integrative Medical Sciences, Yokohama, Japan; [15] Institut Universitaire de France, Paris, France

Chronic HCV infection is a major cause of HCC. While viral cure by direct-acting anti-viral (DAA) agents decreases the overall HCC risk, large cohort studies revealed that HCC risk persists following cure especially in advanced fibrosis. The absence of reliable biomarkers to robustly predict HCC risk is a challenge for patient management. Despite significant research efforts, the molecular basis of HCV-induced HCC is still incompletely understood. The circadian clock (CC) is a well-known regulator of liver physiology and disease biology, while its clinical impact for HCV infection and hepatocarcinogenesis is unknown. Here, we aimed to investigate the role of the circadian clock in HCV-associated HCC risk. To study the perturbation of the CC in patients, we performed ChIP-seq and RNA-seq from liver tissue of patients with chronic HCV-infection, cured patients and non-infected control patients and measured gene expressions and transcription factor recruitments of CC components at different stages of disease pre- and post-cure. In addition, we performed perturbation studies in a human liver chimeric mouse model, primary human hepatocytes and HCV-permissive differentiated human hepatocellular carcinoma cells to unravel the regulation of affected CC- components in detail. We show that chronic HCV infection, a major cause of chronic liver disease (CLD) and HCC, results in marked epigenetic and transcriptional perturbations of CC-controlled pathways driving the progression of CLD and HCC in patients. Our data show that HCV-induced epigenetic and transcriptional perturbations of CC-controlled pathways persist upon viral cure in patients with advanced fibrosis. The downstream network of disturbed genes, including many oncogenes as well as tumor suppressor genes, are associated with the hallmarks of cancer, and correlate with the outcome and risk to develop HCC. Mechanistically, virus-induced cytokine signaling impairs the CC-function by altering the expression of key CC components and downstream signaling. Our results demonstrate that a human virus targets the CC and thereby drives fibrosis, a chronic inflammation-related disease, which is strongly linked with the development of cancer in advanced stages. Our findings provide novel opportunities for the discovery of urgently needed biomarkers and strategies for HCC surveillance and chemoprevention, and for HCC risk assessment in HCV- infected and cured patients.

Frank Jühling: juehling@unistra.fr

L'analyse protéomique d'hépatocytes infectée par le VHB révèle lecollagène VI comme moteur de la fibrose du foie

HBV proteomic analysis on hepatocytes identifies collagen VI asdriver for liver fibrosis

Alessia Virzì[1,2], Laura Heydmann[1,2], Evelyn Ramberger[3,4], Oliver Popp[3,4], Philipp Mertins[3,4], Eloi R. Verrier[1,2], Thomas F. Baumert[1,2,5,6] and Joachim Lupberger[1,2].

[1]Institut national de la santé et de la recherche médicale, institut de recherche sur les maladiesvirales et hépatiques, université de Strasbourg (IVH), Strasbourg, France; [2]Université deStrasbourg, Strasbourg, France; [3]Proteomics Platform, Max Delbrück Center for MolecularMedicine in the Helmholtz Society, Berlin, Germany; [4]Berlin Institute of Health, Berlin,Germany; [5]Pôle Hépato-digestif, Institut Hopitalo-Universitaire, Strasbourg, France; [5]Institutuniversitaire de France (IUF), Paris, France.

Background and aims: Chronic hepatitis B virus (HBV) pathological course are characterizedby the dysregulation of profibrogenic and oncogenic signaling within the host. Although controlof chronic infection by antivirals hampers liver disease progression, a reversal of liver fibrosisrequires long-term treatments, and the permanent viral eradication is hard to achieve. Considering the lack of efficient anti-fibrotic therapies, the understating of signaling pathwaysinvolved in fibrosis progression is urgently needed. Combining transcriptomics, proteomics andphospho-proteomics, we aim to identify the most relevant HBV-induced signaling pathways associated with this pathological progression.

Methods: We analyzed proteomic- and phospho-proteomic changes induced by HBV infectionof HepG2-NTCP cells using liquid chromatography-mass spectrometry (LC-MS), by tandem mass tag (TMT) labels. HBV-infected PHHs have been analyzed using RNA-seq data (GSE69590). Pathways dysregulations were studied using Gene Set Enrichment Analysis (GSEA) and validated both in primary human hepatocytes (PHHs) and cell line and using perturbation studies.

Results: Our analysis of HBV-infected hepatocytes revealed a significantly modulated signaling pathways involved in extra-cellular matrix (ECM) remodeling, both at proteomic andtranscriptomic level in HepG2-NTCP and PHHs, respectively. Among the ECM-relevant genesand proteins driving the enrichment, collagen VI was significantly upregulated upon HBV infection in hepatocytes, suggesting a relevance for HBV-associated liver fibrosis. In liver diseases, hepatic stellate cells account for the majority of collagen I and VI deposed into the extracellular matrix building up scar tissue. Incubation of stellate cell line LX-2 with collagen VIsignificantly induced the gene expression of LX-2 activation markers, like collagen I, comparedto LX-2 incubated with collagen I, suggesting a regulatory role of collagen VI for liver fibrosis. HBV proteins notably induce Akt phosphorylation. By screening the impact of disease-relevantsignaling pathways on collagen VI expression, we observed a correlation between a PI3K-Akttranscriptional signature and collagen VI expression in non-tumor liver tissue of HCC patientswith HBV.

Conclusions: HBV-infected hepatocytes significantly contribute to ECM remodeling by activation of pro-fibrotic stellate cells via collagen type VI. Our data highlight a functional role of HBV-induced Akt signaling in collagen VI production and liver fibrosis. Understanding of collagen VI-relevant signaling in hepatocytes thus may serve as potential antifibrotic targets inthe liver with potential impact on other liver disease etiologies.

Alessia Virzì: virzi@unistra.fr

Caractérisation des ARNs circulants du virus de l'hépatite B chezles patient atteints d'hépatite B chronique

Characterization of circulating Hepatitis B virus RNAs in chronichepatitis B patients

Doohyun Kim[1], Delphine Bousquet[1,2], Marie-Laure Plissonnier[1], Alexia Paturel[1,2], Hyoseon Tak[1], Françoise Berby[1], Isabelle Bordes[1], Aaron Hamilton[3], Marintha Heil[3], Massimo Levrero[1,2,4], Barbara Testoni[1] and Fabien Zoulim[1,2,5]

[1] INSERM U1052, CNRS UMR-5286, Cancer Research Center of Lyon (CRCL), Lyon, 69008,France; [2] University of Lyon, UMR_S1052, CRCL, 69008 Lyon, France; [3] Roche Molecular Diagnostics, Pleasanton CA; [4] Department of Internal Medicine - DMISM and the IIT Center for Life Nanoscience (CLNS), Sapienza University, Rome, Italy; [5] Department of Hepatology, Croix Rousse hospital, Hospices Civils de Lyon, France

Circulating HBV RNA (cirB-RNA) is emerging as a promising non-invasive biomarker for cccDNA transcriptional activity. However, the molecular characteristics and circulating particles containing cirB-RNA in vivo remain to be fully defined. A protocol base on a Iodixanol/Sucrose density gradient ultracentrifugation was set up to separate components of human serum according to their buoyant density. Each density fraction was analyzed for HBVDNA/RNA by qPCR and specific droplet digital (dd)PCR. Viral and extracellular vescicles (EVs)-associated proteins were analyzed by ELISA and Western Blotting. Nanoparticle tracking analysis (NTA) allowed to detect and quantify EVs content. 5'RACE PCR was used to discriminate cirB-RNA species in patients' serum. Sera from 7 untreated [4 HBeAg(+) and 3 HBeAg(-)] and 1 HBeAg(+) entecavir-treated CHB patients were subjected to density ultracentrifugation. Among the 10 fractions obtained, specific distribution of Dane particles, non-enveloped nucleocapsids and subviral particles was determined by ELISA and Western Blotting for viral proteins, together with quantification of HBV DNA. cirB-RNA was mainly detected in core-associated virion-like particles, but in 2 log10 less amount than HBV DNA. However, cirB-RNA was the predominant species in lighter density fractions (1.17-1.18 g/ml) deprived of viral proteins. The enrichment for EVs in these fractions was confirmed by detection of CD9 and CD81 EVs markers by western blotting and NTA. Distribution of cirB- RNA did not differ significantly in HBeAg(+) vs HBeAg(-) patients and in the entecavir-treatedpatient. Interestingly, in a serum with low HBsAg expression (570 IU/ml), cirB-RNA was mainlydetected in the EVs-enriched fractions. Lastly, 5' RACE analysis identified pgRNA, spliced pgRNA and HBx transcripts as the three main categories of cirB-RNA in patients' serum. Ourresults indicate that, in CHB patients' serum, EVs-enriched compartment contributes to the circulation of HBV-RNAs besides virion-like particles. Moreover, different HBV-RNA transcriptsin addition to pgRNA can be detected in vivo. Altogether, these data could significantly contribute to the characterization of cirB-RNAs as new viral biomarker.

Doohyun Kim : doohyun.kim@inserm.fr

Dysfonctionnements mitochondriaux chez les patients atteints de l'hépatite B chronique avec une Fibrose hépatique importante

Mitochondrial Stress in Patients With Chronic Hepatitis B Infection and Advanced Fibrosis

Dimitri Loureiro[1,2], Issam Tout[1,2], Ahmad Sleiman[1,2], Morgane Roinard[1,2], Nathalie Boyer[1,2], Stéphanie Narguet[1,2], Nathalie Pons-Kerjean[3], Corinne Castelnau[1,2], Nathalie Giuly[1,2], Vassili Soumelis[4,5], Jamel El Benna[1], Patrick Soussan[6], Richard Moreau[1,2], Valérie Paradis[1,2], Abdellah Mansouri[1,2&§], Tarik Asselah[1,2&§]

[1]Université de Paris, Centre de recherche sur l'inflammation, Inserm U1149, CNRS ERL8252, F- 75018 Paris, France. [2]Assistance Publique-Hôpitaux de Paris (AP-HP), Department of Hepatology, Hôpital Beaujon, F-92110 Clichy, France. [3]Assistance Publique-Hôpitaux de Paris (AP-HP), Service de Pharmacie, Hôpital Beaujon, F-92110 Clichy, France. [4]Université de Paris, Inserm U976 HIPI Unit, F-75006, Paris, France. [5]Assistance Publique-Hôpitaux de Paris (AP-HP), Laboratoire d'Immunologie et Histocompatibilité, Hôpital Saint-Louis, F-75010, Paris, France. [6]Université de Paris, Inserm U1135, Paris-75012, France. & Equal contribution

Background and Aims: hepatitis B virus (HBV) increase hepatic stress. Mitochondrial DNA is particularly sensitive to oxidative stress. We postulated that chronic HBV infection (CHB) may cause oxidative damage to mitochondrial DNA, resulting in mitochondrial dysfunction which might account for fibrosis progression in CHB.

Method: 136 treatment-naïve CHB mono-infected patients addressed to our center enrolled in this study. All demographics, biological and virological data were recorded. Liver mitochondrial DNA (mtDNA) damage was screened by long PCR and sequencing and levels by Slot blot. The expression of the main genes of cytochrome c oxidase subunits, mitophagy, mitochondrial peptidases and chaperonins, TNFα and IL6 were investigated by RT-qPCR and Western-blotting. Patients with advanced fibrosis (F3-F4 Metavir score; n=41) were compared to patients with mild-moderate fibrosis (F0-F2; n=86).

Results: Patients with F3-F4 fibrosis were older than patients with F0-F2, had comparable ALT, blood HBV DNA and HBsAg (*Table 1*). Whereas 100% of patients with F3-F4 fibrosis exhibited multiple mtDNA deletions, 50% of those with F0-F2 ($\chi c2=6.8$; $p<.001$) carried a single mtDNA deletion. Significant decreases were observed in F3-F4 compared to F0-F2 for the mRNAs of MT-CO1 (0.55±0.36 and 1.20±0.75, $p<.001$), HSPA9 (0.70±0.28 and 1.06±0.37, $p<.001$), HSPD1 (0.83±0.36 and 1.10±0.44, $p<.05$), Lon Peptidase 1, LONP1 (0.83±0.22 and 1.06±0.33, $p<.05$), Parkinson-juvenile disease protein 2, PRKN (0.45±0.26 and 1.12±0.57, $p<.0001$), and Phosphatase Tensin-Induced Putative Kinase-1, PINK1 (0.59±0.17 and 1.06±0.26,$p<.0001$). Liver TNFα mRNA was 1.72±0.2 and 0.99±0.2 ($p<.05$) and IL6 mRNA was 7.82±0.90 and 1.14±0.26 ($p<.05$) in F3-4 and F0-F2, respectively. Protein levels significantly decreased in F3-F4 compared to F0-F2 for MT-CO1, LONP1, PRKN and PINK1.

Conclusion: Diverse mtDNA damages were associated with alterations in mitochondrial function, mitochondrial unfolded protein response, mitophagy and inflammation in patients with CHB and advanced fibrosis. In addition to the damage caused by reactive species; decreased levels of mitochondrial transcripts lead to mitochondrial dysfunction. Modulating mitochondrial function is therefore an attractive therapeutic strategy to block the progression of fibrosis and prevents cirrhosis.

Loureiro Dimitri : Dimitri.loureiro@inserm.fr

Importance des cellules B dans la perte de l'AgHBs au cours de l'hépatite B chronique

B cells involvement in HBsAg seroclearance during chronic Hepatitis B

Issam Tout[1,2], Dimitri Loureiro[1,2], Nassima Boumghar[1,2], Stéphanie Narguet[1,2], Simplice Dzamitika[1,2], Nathalie Giuly[1,2], Nathalie Boyer[1,2], Corinne Castelnau[1,2], Vassili Soumelis3[,4], Valérie Paradis[5], Abdellah Mansouri[1,2], Tarik Asselah[1,2]

[1]Université de Paris, Centre de recherche sur l'inflammation, Inserm U1149, CNRS ERL8252, F-75018 Paris, France. [2]Department of Hepatology, AP-HP, Hôpital Beaujon, F-92110 Clichy, France. [3]Université de Paris, Inserm U976 HIPI Unit, F-75006, Paris, France. [4]Laboratoire d'Immunologie et Histocompatibilité, AP-HP, Hôpital Saint-Louis, F-75010, Paris, France. [5]Department of Pathology, AP-HP, Hôpital Beaujon, F-92110 Clichy, France.

Background: Hepatitis B virus (HBV) infection is a major risk factor for cirrhosis, and hepatocellular carcinoma (HCC). Chronic hepatis B (CHB) causes exhaustion of virus-specific T cells, but its impact on humoral immunity has been less studied. The evolution of B cell responses during CHB towards the HBsAg seroclearance phase and the associated mechanisms are poorly understood although. B cells are increasingly recognized as playing important roles in CHB. The development of antibodies against HBV surface antigen (anti-HBs) is a hallmark for the resolution of the infection and a therapeutic goal for CHB. CHB causes exhaustion of virus- specific T cells, but its impact on humoral immunity has been less studied. The aim of this study was to evaluate B cells involvement in HBsAg seroclearance during CHB.

Patients and methods: 165 CHB mono-infected patients were evaluated. Among them, n=95 were non-treated, n=55 were under treatment (tenofovir or entecavir monotherapy) and n=15 were non-treated and had lost HBsAg. We investigated by flow cytometry the phenotype, and function/functionality of global B cells and of HBsAg-specific B cells in peripheral blood mononuclear cells (PBMCs) from the aforementioned groups compared to a healthy controls group (n=15). We measured the plasma cytokine levels in these patients to understand pro- and anti-inflammatory trends in HBsAg-negative patients. We studied the expression (mRNA and proteins) of key genes involved in the immune functions in these different B cell subsets to identify the transcriptional or post-transcriptional regulations that would occur in the HBsAg seroclearance phase.

Preliminary results: Preliminary results were obtained in n=40 non-treated CHB patients group and in n=10 of HBsAg-negative patients and healthy controls groups. Significant increases were observed in HBsAg-negative patients compared to HBsAg-positive CHB patients for the percentage of CD27hi CD38hi plasma B cells (0,93±0,37 and 0,35±0,24, $p<0,001$) as well as a decrease of the percentage of CD21lo/CD27−/CD10− atypical memory B cells (0,069±0,043 and 0,25±0,067, $p<0,001$). We identified by Western blot and RT-QPCR that TLR9 (1,38±0,21 and 0,57±0,32, $p<0,05$) and BCL-2 (0,97±0,29 and 0,47±0,26, $p<0,05$) expressions were restored in HBsAg-negative patients compared to HBsAg-positive CHB patients. On the other hand, plasma IL-6 (12,6±4,51 and 47,2±11,42, $p<0,05$) and TNF-α (5,7±2,17 and 36,9±8,42, $p<0,05$) levels were decreased in HBsAg-negative patients compared to HBsAg-positive CHB patients.

Conclusion: With these preliminary data, significant modulations in B cells subsets were seen in HBsAg- negative patients compared to HBsAg-positive CHB patients. The identification of TLR9 and BCL-2 as important players in B cell response mechanisms throughout CHB and their restoration upon HBsAg loss may unravel novel immune mechanisms of HBsAg seroconversion and help develop therapeutic strategies to cure HBV infection. Full data will be important to confirm these observations and will presented at the time of the meeting.

Issam Tout : issam.tout@inserm.fr

Variabilité génétique du virus de l'hépatite B en Gambie et au Sénégal : élargissement de la diversité des sous-génotypes dugénotype A

Hepatitis B virus genetic variability in The Gambia and Senegal: expanding subgenotype diversity of genotype A

Rayana Maryse Toyé[1,2,†], Damien Cohen [1,†], Flor Helene Pujol[3], Amina Sow-Sall[2], Gora Lô[2], Kunikazu Hoshino[4], Masashi Mizokami[5], Fabien Zoulim[1], Maud Lemoine [6], Coumba Touré-Kane[2] and Isabelle Chemin[1]

[1] Institut National de la Santé et de la Recherche Médicale (Inserm) U1052, CRCL, Lyon, France; [2] Institut de Recherche en Santé, de Surveillance Epidémiologique et de Formation (Iressef), Diamniadio, Senegal; [3] Laboratorio de Virología Molecular, CMBC, Instituto Venezolano de Investigaciones Científicas (IVIC), Caracas, Venezuela; [4] Department of Infectious, Respiratory, and Digestive Medicine, Graduate School of Medicine, University of the Ryukyus, Okinawa, Japan; [5] Research Institute, Genome Medical Sciences Project, National Center for Global Health and Medicine, Ichikawa,Japan; [6] Division of Digestive Diseases, St Mary's Hospital, Imperial College London, London, UK; † Equal contribution.

Hepatitis B virus (HBV) classification comprises up to 10 genotypes with specific geographical distribution worldwide, further subdivided into 40 subgenotypes, which have different impacts on liver disease outcome. Though extensively studied, the classification of subgenotype A sequences remains ambiguous. This study aimed to characterize HBV isolates from West African patients and propose a more advanced classification of subgenotype A. Fourteen HBV full-length genome sequences isolated from patients from The Gambia and Senegal were obtained and phylogenetically analyzed. Phylogenetic analysis of HBV genotype A sequences isolated from Senegalese and Gambian patients exhibited separate clusters from the other known and confirmed subgenotypes A (A1, A2, A6). Most of the sequences (10/14) clustered with an isolate from Cuba, reported as subgenotype A4 (supported by maximal bootstrap value). Four isolates from The Gambia and Senegal clustered separately from all other subgenotypes and samples sequenced inthe study. Three of which from The Gambia, designated as an expanding clade of subgenotype A4, exhibited a mean inter-subgenotypic nucleotide divergence over the entire genome sequence higher than 4% in comparison with the other subgenotypes and the other isolates sequenced in the study, except with subgenotype A4 isolates (3.9%), and this was supported by a maximal bootstrap value. The last one from Senegal seemed to be an expanding subgenotype close to the new clade of A4. Amino acid analysis unveiled a novel motif specific to these isolates. This study revealed an expanding evolution of HBV subgenotype A and novel amino acid motifs. It also highlighted the need for a consensus regarding the analysis and classification of HBV sequences.

Rayana Toyé : rayanatoye@gmail.com

On the Host Membrane Rearrangement side of the Hepatitis E VirusLifecycle

Cyrine Bentaleb[a], Kévin Hervouet[a], Claire Montpellier[a], Charline Camuzet[a], Julien Burlaud- Gaillard[b], Martin Ferrié[a], Karoline Metzger[a], Anne Goffard[a], Jean Dubuisson[a], Philippe Roingeard[b], Cécile-Marie Aliouat-Denis[a] and Laurence Cocquerel[a]

[a] University of Lille, CNRS, INSERM, CHU Lille, Pasteur Institute of Lille, U1019-UMR 9017- CIIL- Center for Infection and Immunity of Lille, F-59000 Lille, France.
[b] Inserm U1259. Morphogénèse et Antigénicité du VIH et des Virus des Hépatites (MAVIVH), Université de Tours and CHRU de Tours, 37032 Tours, France.

Positive sense single-stranded RNA viruses induce host cell membrane rearrangements to facilitate their viral genome transcription, replication, viral particle release and to counteract the innate immune response. Membrane rearrangements have been well-characterized thanksto the use of the electron microscopy and there is a wide range of complexity between the hostmembrane remodeling and types and numbers of viral proteins implicated in these arrangements. Nevertheless, knowledge concerning viral factories induced by hepatitis E virus(HEV) has not been investigated yet. HEV is a single stranded positive-sense RNA virus that expresses three proteins named ORF1 (the viral replicase), ORF2 (the capsid protein) and ORF3 (a small protein involved in virion egress). Previously, we showed that HEV produces different forms of ORF2 capsid protein during its lifecycle. Notably, the infectious/intracellular ORF2 (ORF2i) is the ORF2 form associated with viral particles (Montpellier et *al*., 2018).

In our study, we analyzed and characterized the membrane rearrangements induced by HEVand sought to identify which viral protein is involved in this process. We used a cell culture system that combines the p6 HEV infectious strain and a subclone of PLC/PRF-5 cells. We generated antibodies that recognize the different forms of the ORF2 protein and used them inimmunogold labeling technique to address the specific localization of the capsid protein.

Our results show that HEV induces some host cell membrane rearrangements and notably twotypes of membranous structures. ORF2-specific immunogold labeling revealed tubular and vesicular structures in HEV-infected cells. In addition, double immunolabeling experiments withan anti-ORF3 antibody showed a co-distribution of ORF3 with the capsid protein inside the membranous structures. Further understanding of the formation of rearranged membranes was determined by the use of several viral mutants.

Overall, the study of membrane rearrangements induced by HEV still has many unanswered questions such as the contribution of host cell factors which we intend to also explore in this project. Further study of these likely critical structures and developing understanding of their nature seem essential to provide full comprehension of the HEV lifecycle.

Cyrine Bentaleb, cyrine.bentaleb@ibl.cnrs.fr

La protéine core du virus de l'hépatite C recrute les ARN génomique viraux via leur interaction avec Nup98 au sein des *Annulate Lamellae* pour promouvoir l'assemblage des particulesvirales

The hepatitis C virus capsid protein recruits viral genomic RNAsthrough their interaction with Nup98 in *Annulate Lamellae* to promote assembly of viral particles

Boson Bertrand, Mialon Chloé, Schichl Konstanze, Denolly Solène, CossetFrançois-Loïc

CIRI – Centre International de Recherche en Infectiologie, Team EVIR, Univ Lyon, UniversitéClaude Bernard Lyon 1, Inserm, U1111, CNRS, UMR5308, ENS Lyon, 46 allée d'Italie, F- 69007, Lyon, France.

Nup98 is an essential component of the nuclear pore but also participates to *Annulate Lamellae* structures localized in the cytosol. Nup98 has been shown to be involved in HCV assembly, since its down-regulation alters the production of infectious HCVcc particles; yet, itsrole remains undefined. Here we combined functional analysis with confocal microscopy and biochemical assays to study the interplay between Nup98, HCV core (capsid protein), NS5A (non-structural protein) and the viral genome.

Our results show that in HCV-infected cells, core is necessary and sufficient to induce re- localization of cytosolic Nup98 from *Annulate Lamellae* to lipid droplet-apposed areas in whichcore/NS5A and HCV (+)RNA are clustered in viral assembly sites. Furthermore, Nup98 silencing did not prevent the clustering of structural and non-structural proteins to assembly sites, suggesting that Nup98 is not a factor that is required for the morphogenesis of assemblysites. We also demonstrated that Nup98 interacts with viral genome with a strong apposition within infected cells. Interestingly, upon Nup98 down-regulation, we found that the viral (+)RNAgenome was specifically excluded from areas that contain active translating ribosomes and the core and NS5A proteins, suggesting a role of Nup98 in retention of viral RNA close to replication/translation/assembly sites.

Altogether, these results indicate that Nup98 is recruited by HCV core, from *Annulate Lamellae* to viral assembly sites, to locally increase the concentration of (+)RNA genome, which may favors its encapsidation into nascent virions.

Bertrand Boson: bertrand.boson@ens-lyon.fr

Les ARNs sécrétés par le Virus de l'Hepatite B (VHB) sont transportés par lesvésicules extracellulaires dans le surnageant des hépatocytes infectés.

Secreted hepatitis B virus (HBV) RNA associates to extracellular vesicles insupernatant of infected hepatocytes

Delphine Bousquet[1,2], Doohyun Kim[1], Annie Adrait[3], Yohann Couté[3], Maria Guadalupe Martinez[1], Alexia Paturel[1,2], Aaron Hamilton[4], Marintha Heil[4], Massimo Levrero[1,2,5], Barbara Testoni[1] & Fabien Zoulim[1,2,6]

[1]INSERM U1052- Cancer Research Center of Lyon (CRCL), Lyon, France
[2]University of Lyon, UMR_S1052, CRCL, 69008 Lyon, France
[3]Univ. Grenoble Alpes, CEA, INSERM, IRIG, BGE, Grenoble, France
[4]Roche Molecular Diagnostics, Pleasanton, CA
[5]Department of Internal Medicine - DMISM and the IIT Center for Life Nanoscience (CLNS), SapienzaUniversity, Rome, Italy
[6]Department of Hepatology, Croix Rousse hospital, Hospices Civils de Lyon, France

Background and aims

Chronic Hepatitis B virus (HBV) infection remains a global health burden, sincecurrent antiviral strategies are unable to eliminate the virus from infected hepatocytes and, thus, achievea complete cure. Non-invasive biomarkers are necessary to improve patients' management and the evaluation of new therapies. This study aims at characterizing the compartments containing extracellularHBV RNAs, which were recently proposed as a new surrogate marker of cccDNA transcriptional activity.

Methods

Supernatants from HBV-infected HepG2-NTCP cells and Primary Human Hepatocytes treatedor not with lamivudine were collected and processed through sucrose/iodixanol gradient separation, to allow physical separation of extracellular vesicles (EVs) and viral particles according to their buoyant density. Viral and EVs-associated proteins were analyzed by Western Blotting and ELISA, while HBV RNAs were detected by specific droplet digital (dd)PCR and correlated with intracellular viral replicative parameters (HBV-DNA, cccDNA, 3.5kb HBV-RNA). Nanoparticle tracking analysis (NTA) and mass spectrometry (MS)-based proteomics were used to further characterize the viral and non-viral components.

Results

ELISA and western blotting assays for HBs and HBc proteins, together with HBV-DNAquantification after gradient separation showed that virions were found in fractions corresponding to a density of 1,17-1,20 g/ml. CD9 expression, marker of exosomes/EVs, was detected only in lower density fractions (1,07-1,15 g/ml), which were deprived of HBc. ddPCR analysis using assays spanning the 5' region of 3.5Kb RNA or the HBx region suggested that 3.5 Kb RNA is the predominant but not the only viral RNA specie in cell supernatants. HBV-RNAs distribution across gradient fractions showed no significant differences between Lamivudine-treated vs untreated samples. Interestingly, HBV-RNAs weredetected not only in virion-like particles and non-enveloped capsids, but also in lighter fractions,suggesting that EVs could also carry secreted HBV-RNA. NTA analysis confirmed that these fractions were indeed containing EVs in size spanning from 30 to 150 nm and MS analysis revealed a significant enrichment for exosomal proteins (CD63, CD9, TSG101 or HSC70).

Conclusion

Our results show evidence for EV containing HBV RNAs and will help understanding the molecular biology of serum HBV RNA secretion. This knowledge will aid the development and interpretation of serum HBV RNAs as a novel biomarker for chronic HBV infection.

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Pas de protéase en vue : Etude structure-fonction de la polyprotéine du virus de l'hépatite E et de son domaine de typedomaine de liaison aux acides gras

No protease in sight: Structure-function study of hepatitis E virusreplication polyprotein and of its fatty-acid binding-like domain

Sonia Fieulaine[1], Virginie Gervais[1], Stéphane Bressanelli[1]

[1]*Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), 91198, Gif-sur-Yvette, France*

Hepatitis E virus (HEV) is one of the main causative agents of acute hepatitis and jaundice, affecting both developed and developing countries. At least 20 million people are infected eachyear worldwide. HEV usually causes a minor and asymptomatic infection that resolves on its own but around 70,000 people die per year after HEV infection. In addition, the virus can leadto acute hepatitis or chronic hepatitis that may progress to cirrhosis. Extrahepatic manifestations such as neurological complications are also observed. However, although its severity, its prevalence and its impact in health systems, HEV is poorly characterized and many questions remain unanswered about its functioning, especially at the structural level. The main reason is the lack of efficient HEV cell culture systems.

Like most single-stranded, positive-sense RNA (RNA+) viruses, HEV encodes a multi-domainpolyprotein, called ORF1, which is essential for viral replication. The main function of ORF1 isthe synthesis of new genomes that will be encapsidated before propagation to other cells. In contrast to other RNA+ virus polyproteins that are proteolytically processed by at least one viral protease, it is still not clear if HEV ORF1 is processed into discrete functional units or not(LeDesma *et al*, Viruses 2019). Moreover, the nature and the exact role of the putative HEV protease domain is currently highly debated (LeDesma *et al*, Viruses 2019). Interestingly, a recent study (Proudfoot *et al*, J. Virol. 2019) presents the 3D structure of a domain of HEV ORF1 that overlaps the C-terminal sequence of what was tentatively annotated as a possiblecysteine protease catalytic site (Koonin *et al*, PNAS 1992) and has been referred to as 'the HEV protease' ever since. The structure shows that the new, globular domain is actually not aprotease at all but folds as a fatty acid binding domain (FABD). We conclude that this work invalidates the existence of a protease domain in HEV polyprotein ORF1 and questions a polyprotein maturation process in HEV. In contrast, this striking result opens a new research area to understand the function of this newly identified domain in HEV replication.

In order to better characterize the architecture and the fate of the HEV polyprotein, we have started a structure/function study. First, using a wheat-germ cell-free expression system, we have expressed the HEV polyprotein. In our experimental conditions, the polyprotein mostly remains uncleaved, regardless of the genotype (GT1 and GT3). This result indicates that thereis no active protease in this system, *i.e.* neither in the wheat-germ extract nor in the polyproteinitself. Then we have developed a protocol to purify the detergent-solubilized uncleaved HEV- GT1 polyprotein. We now have enough pure soluble polyprotein to start a structural study by cryo-electron microscopy. Second, we have initiated a screening approach to identify ligands of the FABD-like domain. Hit ligands will be further characterized by determining their affinity constants and their binding mode to the protein. Of note, we have produced rabbit polyclonal antibodies specifically directed against HEV (GT1 and GT3) FABD-like domain. Antibodies recognize the recombinant purified FABD-like domain but also the FABD-like domain embedded in the polyprotein produced in our *in vitro* system. These antibodies should providea powerful tool to specifically detect the HEV polyprotein in infectious systems.

Sonia Fieulaine : sonia.fieulaine@i2bc.paris-saclay.fr

Caractérisation du rôle proviral de JAK1 dans l'infection de cellulespar le virus de l'hépatite D

Characterization of the proviral role of JAK1 in Hepatitis Delta Virusinfection

Margaux J Heuschkel[1], Charlotte Bach[1], Catherine Schuster[1], Thomas F Baumert[1,2], Eloi R Verrier[1]

[1] Institut de Recherche sur les Maladies Virales et Hépatiques, UMR_S1110 INSERM,Université de Strasbourg, France
[2] Institut Hospitalo-Universitaire, Pôle Hépato-digestif, Nouvel Hôpital Civil, Strasbourg, France

Chronic hepatitis D is one of the most severe forms of chronic viral hepatitis. It is caused by the hepatitis delta virus (HDV), a small satellite virus of the hepatitis B virus, which is responsible for an aggravation and accelerated liver disease in patients with chronic hepatitisB, as well as an increased risk of developing hepatocellular carcinoma. To date, no treatmentallows virus elimination in chronically infected patients. While the viral cycle is well described,the interactions between HDV and host factors are still poorly understood, although they represent relevant targets for the development of new therapeutic targets. In this context, we previously applied a dual approach of functional and chemical genomic screening to HDV- infected Huh7-NTCP cells and identified new cellular factors involved in the HDV viral cycle, including the triple enzyme CAD playing a key role in pyrimidine biosynthesis (Verrier *et al*, Gut 2020). Among the other hits, validation experiments identified 33 candidate genespotentially important for the viral cycle, including Janus kinase 1 (JAK1). JAK1 is known to bea major actor in the innate antiviral response. However, the results of the primary screen showed that a decreased expression of JAK1 is associated with a reduction of HDV infection in the cells, suggesting a proviral effect of the kinase. To investigate the functional role of JAK1in HDV infection, we first validated the phenotype by loss-of-function experiments using different sequences of siRNAs to rule out off-target effects. Validation in HepG2-NTCP cells and primary human hepatocytes confirmed a decreased HDV infection following JAK1 silencing, eliminating the specificity of the cell line used in the primary screen. For further functional analyses, we produced Huh7-NTCP-derived cell lines KO for JAK1 using CRISPR-Cas9 technology and observed a marked decrease in HDV infection in JAK1-KO cells compared to control cells. Finally, a specific JAK1 inhibitor, oclacitinib, exhibits a dose- dependent antiviral effect in both NTCP-expressing hepatoma cells and PHH, confirming the involvement of JAK1 in the HDV replication cycle. Next, to identify the signaling pathway by which JAK1 promotes HDV infection, we treated hepatoma cells with different activators of theJAK/STAT pathways, including IL-6 and IFN-α. While HDV infection is dose-dependently sensitive to IFN-α, our first results suggest increased HDV infection levels upon IL-6 treatment.This implies that HDV hijacks IL-6/JAK1-mediated innate immunity signaling pathways to promote virus infection. In this context, we aim to analyze the impact of JAK1 on the expressionof ISGs such as MOV10, a helicase for which we have previously confirmed the interaction with the delta antigen. In conclusion, we validated the proviral activity of JAK1 in HDV infection.Characterization of JAK1-HDV interaction will pave the way for a better understanding of HDVlink to innate immune responses and IFN-α treatment.

Margaux Heuschkel : margaux.heuschkel@etu.unistra.fr

Un nouveau modèle cellulaire permettant la réplication des virus des hépatites B, C, Det E et l'étude des gènes stimulés par l'interféron responsables des effets antiviraux

A new *in vitro* cell model allowing an efficient replication of hepatitis B/C/D/E viruses & the study of ISGs involved in broadantiviral effect

Julie Lucifora[1,2], Eloi R. Verrier[3], Maud Michelet[2], Caroline Pons[1,2], Thomas F. Baumert[3], Anna Salvetti[1,2], David Durantel[1,2]

[1] INSERM, U1111, Centre International de Recherche en Infectiologie (CIRI), Lyon, France ;
[2] INSERM, U1052, Cancer Research Center of Lyon (CRCL), University of Lyon (UCBL1), CNRS UMR_5286, Centre Léon Bérard, Lyon, France (past affiliation when [#] indicated)
[3] INSERM, Université de Strasbourg, Institut de Recherche sur les Maladies Virales et Hépatiques, UMR_S1110, Strasbourg, France

Type-I interferons have marked the medical practice to treat viral hepatitis in the last 40 years.Even if not used any more in the case of HCV, less used in the case of HBV, IFN-alpha (i.e. under its pegylated form) remains a backbone component for HDV infection treatment and anoccasional component to treat chronic HEV infections. However, the mode of action of this antiviral cytokine and reasons of the lack of efficacy in some patients remain largely unknown.In particular, it remains i) to determine what are the subsets of IFN-stimulated gene(s) (ISGs)necessary to achieve a strong antiviral effect against a given hepatovirus; ii) to identify ISGs (or set of ISGs) with broad antiviral activities; and iii) to investigate the precise mode of actionof ISGs (or set of ISGs) physiologically involved in antiviral effects.

In the frame of projects supported by the ANRS on co-infections with different hepatitis viruses,we engineered an HuH7.5-NTCP cells, which is able to efficiently replicate HCV, HBV, HDV and HEV, as evidenced by the specific detection of viral RNAs (RTqPCR) and proteins (WB and/or IF) in kinetic experiments (up to 3 weeks). To allow this potent replication, HuH7.5- NTCP cells needed to be partially re-differentiated by one week 2% DMSO treatment prior to infection. As expected, IFN-α treatment following virus inoculation strongly decreased the levels of intracellular HDV RNAs and completely abrogated HCV and HEV RNA amplification.Interestingly, in contrast to what observed in dHepaRG cells or primary human hepatocytes, IFN-α had no effect on the levels of HBV RNAs. These data suggested that antiviral ISG(s) efficient against HBV are not expressed/induced in dHuH7.5-NTCP. To get insights on this hypothesis, dHuh7.5-NTCP and dHepaRG cells were stimulated with IFN-α for 8h and gene expression analyzed by RNAseq. Computational analyses of differentially expressed genes (DEGs) highlighted expected similarities but also marked differences in the response to IFN-αtreatment in both cell lines. Notably, 179 dHepaRG-specific DEGs were identified, providing alist of putative restriction factors against HBV. This list included MX2, TRIM14 and ADAR1 thatwere previously described as antiviral effectors against HBV. On the other side, since IFN-α treatment led to decreased levels of intracellular HDV RNAs in both dHepaRG and dHuH7.5-NTCP cells, we hypothesize that ISGs efficient against this virus are among the 150 genes that were commonly upregulated in both cell lines. After an extensive review of the literature associated to each candidate, we selected 78 candidate ISG that will be investigated for theirpotential effect on HBV and HDV. To conclude, we set up a new *in vitro* model allowing replication of HBV, HDV, HCV and HEV and generated a data set that will allow to identify common "sets of ISGs" involved in the broad antiviral effect of IFN-α against these viruses.

Julie Lucifora ; julie.lucifora@inserm.fr

La phosphorylation de l'histone linker, un mécanisme potentiel dans lemaintien de l'infection par le VHB

HBV-induced linker histone phosphorylation, is required formaintenance of the viral life cycle

Zakaria Boulahtouf[1,2], Alessia Virzì[1,2], Laura Heydmann[1,2], Evelyn Ramberger[3,4], OliverPopp[3,4], Philipp Mertins[3,4], Eloi R. Verrier[1,2], Thomas F. Baumert[1,2,5,6] and Joachim Lupberger[1,2]

[1]Institut national de la santé et de la recherche médicale, institut de recherche sur les maladies viraleset hépatiques, université de Strasbourg (IVH), Strasbourg, France; [2]Université de Strasbourg,Strasbourg, France; [3]Proteomics Platform, Max Delbrück Center for Molecular Medicine in the Helmholtz Society, Berlin, Germany; [4]Berlin Institute of Health, Berlin, Germany; [5]Pôle Hépato- digestif, Institut Hopitalo-Universitaire, Strasbourg, France; [5]Institut universitaire de France (IUF), Paris, France.

Background and aims

Hepatitis B virus (HBV) is a main cause of liver failure and hepatocellular carcinoma (HCC) worldwide. Dysregulated signaling is a hallmark of liver disease and cancer progression. During infection, HBV disrupts several signaling pathways by hijacking host factors. Thiscontributes to liver disease progression and to the establishment of an oncogenic environment withinthe liver. Current antiviral therapies can only control, but not cure chronic HBV infection due to cccDNA, a persistent epigenetically controlled viral mini-chromosome. By combining phospho-proteomic analysis and experimental approach, we aim to identify HBV-induced signaling relevant forHBV life cycle and pathogenesis.

Methods

Phospho-proteomic and proteomic changes in HBV-infected HepG2-NTCP cells were analyzed by mass-spectroscopy to reveal the global landscape of signaling pathways phosphorylationderegulated by HBV infection. RNAi, cell viability assays and qPCR were instrumental to validate therelevance of host factors for HBV replication.

Results

Linker histones of the H1 family were among the most frequent differentially phosphorylatedproteins identified in our screen. The role of H1 is the compaction of chromatin regulating the accessibility of DNA for transcription. In non-infected cells, H1 proteins are exclusively phosphorylatedin M/S phase of the cell cycle leading to a detachment of H1 from the chromatin. We previously demonstrated that HBV infection predominantly replicates in G1 phase of the cell cycle replication (Eller, C et al. Nat Commun. 2020), where H1 should not be phosphorylated and thus chromatin should remain compacted. Our working hypothesis is that HBV actively phosphorylates H1 to maintainHBV transcription from cccDNA or promoting the expression of pro-viral factors. To validate an impact of H1 phosphorylation on HBV replication we mimicked H1 phosphorylation and detachment by RNA interference two days prior infection with HBV. Indeed, we demonstrated that silencing of H1 in HepG2-NTCP was well tolerated by the cells and correlated with a significant increase of HBV transcripts relative to cellular markers. This suggests a virus-induced gateway for HBV transcription in infected, quiescent cells.

Conclusions

Our phospho-proteomic atlas of HBV infection represents a resource for the identification of drivers of HBV life cycle and liver disease progression. Our data suggest a key role of H1 phosphorylation during HBV infection with a potential impact on cccDNA maintenance. Next steps will involve the identification of viral proteins linked to H1 phosphorylation and the validation using phospho-negative H1 mutants and their capability to bind cccDNA and inhibit HBV replication.We suggest that a specific suppression of H1 phosphorylation by targeting upstream signaling may represent a potential therapeutic target.

Zakaria Boulahtouf: zakaria.boulahtouf@etu.unistra.fr

La fixation des protéines HDV à la boucle epsilon des ARNs HBV accélère leurdégradation entrainant une interférence virale d'HDV sur HBV

Binding of HDV proteins to the epsilon stem loop in HBV RNAs accelerates their degradation and mediates the viral interference ofHDV on HBV

Julie Lucifora[1,2#], Dulce Alfaiate[2#], Maud Michelet[2], Caroline Pons[1,2#], Floriane Fusil[1], Fouzia Amirache[1], Axel Rossi[3], Anne-Flore Legrand[1], Serena Vegna[2#], Rayan Fahrat[2#], Michel Rivoire[4], Barbara Testoni[2], Fabien Zoulim[2], Stephane Daffis[6], Simon Fletcher[6], François-Loïc Cosset[1], Hildegard Büning[3], Anna Salvetti[1,2#*], and David Durantel[1,2#*]

[1]INSERM, U1111, Centre International de Recherche en Infectiologie (CIRI), Lyon, France 2 INSERM, U1052, Cancer Research Center of Lyon (CRCL), University of Lyon (UCBL1),CNRS UMR_5286, Centre Léon Bérard, Lyon, France (past affiliation when # indicated)
[2]Centre International de Recherche en Infectiologie (CIRI), INSERM, U111, Lyon, France
[3]Institute of Experimental Hematology, Hannover Medical School, Hannover, Germany
[4]Centre Léon Bérard (CLB), INSERM, U1032, Lyon, France
[5]Department of Hepatology, Croix-Rousse Hospital, Hospices Civils de Lyon, Lyon, France
[6] Gilead Sciences, Inc., Foster City, CA, USA
**equal contribution*

Chronic co-infection with Hepatitis B Virus (HBV) and Hepatitis Delta Virus (HDV) leads to oneof the most aggressive chronic form of viral hepatitis. In most patients, this co-infection is characterized by a negative interference of HDV on HBV. In this study, we aimed at elucidatingthe molecular mechanisms involved in this phenotype. Using relevant models, based on eitherprimary human hepatocytes (PHH), differentiated HepaRG cells and chimeric humanized mouse model (HuHep), we confirmed a negative impact of HDV on HBV (from different genotypes) replication, as evidenced by a decrease of all HBV transcripts and secreted HBV antigens. Concomitantly to HDV RNA replication, numerous interferon (IFN)-stimulated genes were switched-on in a MDA5-dependent manner, but not when the HDV antigens (HDAg) were expressed alone, suggesting that HDV RNA amplification was required to induce a strong IFNresponse. Interestingly, inhibition of the hepatocyte IFN response (using biochemical or CRISPR-Cas9 approaches) allowed only a partial rescue of HBV replication suggesting that HDV-induced IFN response was not the sole mechanism involved in the interference phenotype. Immunoprecipitation followed by qPCR assays (ChIP) neither showed an association of HDAg to cccDNA (i.e. HBV mini-chromosome), nor a modification of the acetylation status of histone H3 associated to cccDNA, which could have been responsible fora transcriptional repression. In contrast, RNAs stability analyses revealed that HDV infection or expression of HDAg alone induced an accelerated decay of HBV RNAs. Immunoprecipitation of HDAg followed by RT-qPCR analyses (RIP) indicated that HDAg are associated with HBV RNAs. Further RNA filtration and RNA mobility shift assays (REMSA) demonstrated a direct binding of HDAg to the epsilon steam loop of HBV RNAs. Altogether, these results indicate that the interference of HDV on HBV replication relies on both IFN- dependent and IFN-independent mechanisms, the latter occurring through direct binding of HDAg to HBV RNAs. A better knowledge of the interplay between HDV, HBV and hepatocyteinnate immunity, could allow a better understanding of the increased pathogenesis caused byHBV/HDV co-infections, as well as to develop novel and innovative immune therapeutic strategies.

Julie Lucifora ; julie.lucifora@inserm.fr

Etude de l'interaction entre le virus de l'hépatite C et les peroxysomes

Study of the interaction between hepatitis C virus and peroxisomes

Esther Martin de Fourchambault[1], Nathalie Callens[1], Yasmine Sebti[2], Jean Dubuisson[1], Yves Rouillé[1]

[1] Center for Infection and Immunity of Lille, INSERM U1019-CNRS UMR9017, Université LilleNord de France, Lille ; [2] INSERM UMR1011, Université Lille Nord de France, CHU Lille, InstitutPasteur de Lille.

Hepatitis C is still a global health issue since 71 million people are still infected by hepatitis C virus (HCV) according to WHO estimations. HCV is an enveloped virus with a 9,6 kb single stranded RNA genome. Its replication occurs in the cytoplasm of human hepatocytes, within double-membrane vesicles called membranous webs. To identify new host proteins recruitedto HCV replication sites, we performed a screen based on a proximity-biotinylation assay. ACBD5, a peroxisomal membrane protein involved in the formation of contact sites between peroxisomes and the endoplasmic reticulum, was one of the proteins that emerged from this experiment. Peroxisomes are small versatile organelles involved among other functions in lipidmetabolism and ROS regulation. Their metabolism and turn-over are altered by several viral infections such as herpesviruses (Beltrand *et al.* 2018), West Nile virus or dengue virus (You *et al.* 2015). In order to investigate a potential association between ACBD5 and HCV replicationcomplexes, we performed ACBD5 and NS5A (viral protein used as replication complexes marker) immunofluorescent staining. We found that ACBD5 is indeed partially recruited near replication complexes while still associated with other peroxisomal markers, indicating a recruitment of peroxisomes, and not only ACBD5, to HCV replication complexes. Furthermore,in a fraction of infected cells (20-35% regarding genotype), peroxisomes morphology is altered: while they appear as small spherical organelles in control cells, they seem to aggregate in bigger structures or ribbon-like forms in infected cells. We characterized these morphological alterations using confocal microscopy and 3D-reconstitution in naïve and infected cells over time. The kinetics of alteration up to 16 days post-infection was studied using two different HCV genotypes (2A and 3A). The average volume of a peroxisome increased from 0,15 μm^3 (+/- 0,01) in naïve Huh-7 cells to 0,35 μm^3 (+/- 0,02) at 16 days post-infection with HCV gt3A,while the average number of peroxisomes per cell dropped from 547 (+/- 94) in naïve cells to 242 (+/- 59) in infected cells in the same time. Then, we investigated the importance of peroxisomes in the life cycle of HCV. To do so, we generated peroxisome-deficient cells usingCRISPR-Cas9 technology, by targeting the Pex3 gene (Pex3-KO Huh-7 cells). Since peroxisomes are partially recruited near replication complexes, we first studied the impact of the absence of peroxisomes on HCV replication using luciferase-expressing replicons of threedifferent genotypes (1B, 2A and 3A). We monitored their replication during 4 days and found no impact of the absence of peroxisomes on HCV replication. We also titered infectious particles secreted at 3 to 4 days post-infection and again found that the absence of peroxisomes had no impact on HCV titers. These results indicate that peroxisomes are not required for HCV to establish an infection. We are currently studying peroxisomes' recruitmentmechanism near replication complexes and the impact of HCV infection on peroxisomes' metabolism.

Esther Martin de Fourchambault: esther.martin-de-fourchambault@ibl.cnrs.fr

Caractérisation de la replicase du virus de l'hépatite E

Characterization of the Hepatitis E Virus Replicase

Karoline Metzger, Cyrine Bentaleb, Claire Montpellier, Jean-Michel Saliou, Virginie Alexandre, Charline Camuzet, Maliki Ankavay, Jean Dubuisson, Laurence Cocquerel,Cécile-Marie Aliouat-Denis

Center for Infection and Immunity of Lille, INSERM U1019-CNRS UMR9017, Institut Pasteurde Lille, Université de Lille, Lille

Hepatitis E virus (HEV) is the major cause of acute hepatitis worldwide. Currently, no effective anti-viral treatment against HEV is available and an HEV vaccine is restricted to China.HEV is a positive-sense RNA virus expressing 3 open reading frames (ORFs). ORF1 encodes the ORF1 non-structural polyprotein, the viral replicase which transcribes the full-length genome and a subgenomic RNA that encodes the structural ORF2 and ORF3 proteins. Our aim is to better characterize ORF1: to determine whether it is processed during theHEV lifecycle and to identify the compartment of replication. As no commercial antibody recognizes ORF1 in HEV-replicating cells, we aimed at inserting epitope tags within the ORF1 sequence of the cell-culture selected genotype 3 p6 strain. First, a HEV replicon where the *Gaussia* luciferase reporter gene lies under the controlof ORF1 was used to assess the replication efficiency of the tagged genome by luminometry.The insertion of the 2 V5 tags in the hypervariable region did not significantly impact the replication of genomic and production of subgenomic RNA, whereas the insertion of an HA tagat the C-terminus of the RNA-dependent RNA polymerase reduced the production of subgenomic RNA.

Tags were next inserted both into the infectious full-length p6 HEV genome and into ORF1 heterologously expressed. Western-blot and immunoprecipitation analyses of tagged ORF1 expressed in the 3 systems showed a high molecular weight protein (>180kDa) that likely corresponds to the unprocessed form of ORF1 and that can be detected up to 25 days after electroporation of the p6 infectious RNA in PLC3 cells. Additionally, lower molecular weight proteins (90-180kDa) were detected in lower abundance, suggesting that ORF1 mightbe processed.

Mass spectrometry analyses were performed to identify the sequence of the potential cleavage products observed by western blot and colloidal blue staining. It appears that the N-terminus of all V5-tagged proteins of lower molecular weight is stable and corresponds to theN-terminus of the full length ORF1 protein. However, the C-terminus was not yet identified.

Interestingly, western blot analysis of subcellular fractions as well as confocal microscopy revealed cytoplasmic and nuclear localization of the tagged ORF1, indicating thatthe ORF1 might pass through the nucleus during infection.

Finally, we used the RNAscope technique to visualize the intracellular HEV RNA. Probes were designed against positive and negative stranded HEV RNA. Genomic and subgenomic RNA were found in close proximity to each other and to viral proteins as well.
These results will contribute to a better understanding of the HEV replication step.

Karoline Metzger, karoline.metzger.etu@univ-lille.fr

Développement d'outils d'imagerie permettant l'étude de la dynamique d'entrée du virus de l'hépatite B.

Engineering live imaging tools to study the dynamic of HBV entry inside hepatic cells

Maika S. Deffieu[1,2], Cristina M. Dorobantu[1,2], Srigokul Upadhyayula[3], Vincent Lucansky[1,2], Thomas F. Baumert[4], Raphael Gaudin[1,2]

[1]Institut de Recherche en infectiologie de Montpellier (IRIM) CNRS, Montpellier, France ;
[2] INSERM, Institut de Recherche sur les Maladies Virales et Hépatiques, Strasbourg, France ;
[3] Advanced Bioimaging Center, Department of Molecular & Cell Biology, University of California, Berkeley, CA ;
[4] INSERM U11110, Univ Strasbourg, Pole Hépato-digestif, Institut Hospitalo- universitaire, Strasbourg, France

Hepatitis B virus is a partially double stranded DNA virus responsible for the development of Hepatitis B disease. Although a vaccine exists, no cure is available to treat infected patients. Nucleoside analogues (Tenofovir, Entecavir), Myrcludex-B or pegylated interferon alpha treatments are under active clinical trials for HBV treatment. However, patients could develop drug resistances which emphasize the need for the search of new drug targets. Understanding the trafficking pathways used by HBV during its course of infection appears as an interesting strategy to identify new potential targets. Indeed, many aspects of the HBV entry life cycle is still unclear.

HBV is known to attach to the cell surface through heparan sulfate proteoglycan (Schulze A. et al Hepatology 2007) and interacts with the sodium taurocholate co-transporting polypeptide receptor (NTCP) and its co-receptor epithelial growth factor (EGFR) for internalization of the virus inside hepatic cells (Iwamoto M. et al PNAS 2019). Although, the binding site between NTCP and HBV envelop was previously described, the dynamics of NTCP-HBV interactions at the cell surface, as well as the process triggering EGFR recruitment to the NTCP-HBV complex was never directly observed. Following internalization by clathrin-mediated endocytosis, the virus is transported through the early and late endosomes. This endosomal transport is important as it favors the penetration of the virus inside the cell, but to avoid lysosomal degradation, viruses must operate specific mechanisms to prepare their escape from the endosome and release their particle content into the cytosol. HBV does not require an acidic pH for its release from the endosomes (Macovei A. et al. J. Virol. 2013), and so far, no mechanism was established to describe HBV viral transport and export from these compartments.

Trafficking pathways used by viruses are difficult to track by fixed imaging as they are mediated by quick and transient processes. Live imaging is then considered as a powerful tool to observe HBV entry in a spatiotemporal manner. Due to the specific physical properties of HBV, it has been a long withstanding effort in the field to develop tools to visualize the mechanistic events of entry. Indeed, HBV contains a compact genome with overlapping sequences, limiting the possibility to introduce fluorescent proteins sequences in the viral genome. In addition, HBV infectious particles are not easily distinguishable as they represent a weak proportion of the secreted viruses and are mostly present as subviral particles containing an envelope without capsid and genomes.

Using the HepAD38 cell line, commonly used for HBV production, we obtained stable cells expressing in "trans", HBV core proteins fused to the fluorescent proteins mRuby3 (HBV HBc- mRuby3) or mNeonGreen (HBV HBc-mNG). Characterization of the fluorescent viruses by single molecule studies, indicated that the fluorescent HBc-mNG and HBc-Ruby3 cores are packaged with HBV wild type core subunits to form the HBV capsid. Internalization assays as well as live imaging studies indicated that these fluorescent HBV viruses can enter inside hepatic cells and be transported inside endosomal vesicles. To follow the mobility of HBV fluorescent viruses related to its NTCP receptor, we engineered NTCP receptors fused to mNG or mRuby3 in HepG2 cells. The addition of a fluorescent tag to NTCP renders the cells permissive to HBV in a similar extend to wildtype NTCP-expressing HepG2 cells.

Our data support the use of these tools to study the kinetic of attachment, internalization, and endosomal transport of HBV inside hepatic cells. A better understanding of the spatiotemporal dynamics of virus entry is important to design novel HBV countermeasures.

Deffieu Maika: maika.deffieu@irim.cnrs.fr

Les ligands du récepteur nucléaire FXR inhibent la réplication du virus de l'hépatite delta et l'infectivité des virions dans des modèlesde culture cellulaire

Farnesoid X receptor alpha ligands inhibit hepatitis delta virusreplication and virion infectivity in cell culture models

Anne-Flore Legrand[1,2], Julie Lucifora[3], Benoît Lacombe[1], Camille Ménard[1], Maud Michelet[3], Adrien Foca[1], Pauline Abrial[1], Anna Salvetti[3], Michel Rivoire[4],Raphaël Darteil[5], Vincent Lotteau[1], David Durantel[3], Patrice André[1,2], and Christophe Ramière[1,2,7]

[1]CIRI, Centre International de Recherche en Infectiologie, Univ Lyon, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, 21 Avenue Tony Garnier, Lyon, F-69007, France ; [2]University of Lyon, Université Claude Bernard Lyon, Villeurbanne, France ; [3]INSERM, U1052, Cancer Research Center of Lyon (CRCL), University of Lyon (UCBL1), CNRS UMR_5286, Centre Léon Bérard, Lyon, France ; [4]INSERM U1032, Centre Léon Bérard (CLB), Lyon, France ; [5]EnyoPharma, Lyon, France ;[6]Virology Laboratory, Hospices Civils de Lyon, Hôpital de la Croix-Rousse, Lyon, France ;

Hepatitis delta virus (HDV) is a small defective RNA virus that requires the hepatitis B virus (HBV) for its propagation by bearing HBV envelop proteins at its surface. HDV super-infectionof chronically Hepatitis B Virus (HBV)-infected patients is considered to be the most aggressiveform of chronic viral hepatitis affecting approximately 25 million people worldwide. Co-infectionby both viruses is associated with a rapid progression towards fibrosis. Currently, only interferon (IFN)-alpha-based therapies are recommended by international guidelines for the treatment of HDV. However, these therapies do not show satisfactory results and are associated with side effects and relapses. Moreover, available direct anti-HBV drugs are not effective against HDV. Thus, new therapies are needed. Among them, Myrcludex, an entry inhibitor of both viruses, is currently evaluated. Previously, we showed that some ligands of the farnesoid-X-receptor alpha (FXR), the main nuclear receptor of bile acids (BA), are inhibitors of HBV replication. Except for the role of NTCP, the liver transporter of bile acids used by both viruses to infect hepatocytes, the link between BA metabolism and HDV life cyclehas not yet been explored. We thus wanted to determine the role of FXR and BA metabolismin HDV infection. For this, HDV mono-infections or HBV/HDV co-infections and super- infections were established in differentiated HepaRG (dHepaRG) cell line and primary humanhepatocytes (PHH). Using these relevant models, we analyzed the effect of several FXR ligands on HDV infection. We found that the FXR ligand GW4064 significantly decreased HDVRNAs in dHepaRG and PHH by 44% and 60% respectively. The amount of delta antigens wasalso reduced. The decrease of these intracellular markers of HDV replication was associated with a 60 % reduction of HDV virions secretion. Moreover, the specific infectivity of HDV particles was reduced by more than 95%, indicating that infectious properties of secreted particles were severely altered by treatment with FXR ligands. The antiviral activity of FXR ligands was confirmed to be FXR dependent. In conclusion, FXR ligands can inhibit HDV replication in a FXR-dependent manner, as well as virion secretion and specific infectivity. Theantiviral effect was superior to that obtained with IFN-α, suggesting that targeting FXR is a potent therapeutic approach for the treatment of HDV-infected patients.

Anne-Flore Legrand : anne-flore.legrand@inserm.fr

Un peptide de fusion dans preS1 et la protéine-disulfure isomérase ERp57 sont impliqués dans le processus de fusion membranaire du virusde l'hépatite B

A fusion peptide in preS1 and the protein-disulfide isomerase ERp57 are involved in hepatitis B virus membrane fusion process

Jimena Pérez-Vargas[1*], Elin Teppa[2,3*], Fouzia Amirache[1], Bertrand Boson[1], Rémi Pereira de Oliveira[1], Christophe Combet[4], Anja Böckmann[5], Floriane Fusil[1], Natalia Freitas[1], Alessandra Carbone[2**] and François-Loïc Cosset[1**$]**

[1]CIRI – Centre International de Recherche en Infectiologie, Team EVIR, Univ Lyon, Université Claude Bernard Lyon 1, Inserm, U1111, CNRS, UMR5308, ENS Lyon, 46 allée d'Italie, F-69007, Lyon, France ; [2]Sorbonne Université, CNRS, IBPS, Laboratoire de Biologie Computationnelle et Quantitative (LCQB) - UMR 7238, 4 place Jussieu, 75005 Paris, France. [3]Sorbonne Université, Institut des Sciences du Calcul et des Données (ISCD), 4 place Jussieu, 75005 Paris, France ; [4]Cancer Research Center of Lyon (CRCL), UMR Inserm 1052 - CNRS 5286 mixte CLB – UCBL1, F- 69008 Lyon, France ; [5]Molecular Microbiology and Structural Biochemistry, UMR5086 CNRS-Université Lyon 1, 7 passage du Vercors, 69367 Lyon, France.
**These authors contributed equally to the work.*
***These senior authors contributed equally to the work.*

Cell entry of enveloped viruses relies on the fusion between the viral and plasma or endosomal membranes, through a mechanism that is triggered by a cellular signal. Here we used a combination of computational and experimental approaches to unravel the main determinants of hepatitis B virus (HBV) membrane fusion process. We discovered that ERp57 is a host factor critically involved in triggering HBV fusion and infection. Then, throughmodelling approaches, we uncovered a putative allosteric cross-strand disulfide (CSD) bond in the HBV S glycoprotein and we demonstrate that its stabilization could prevent membrane fusion. Finally, we identified and characterized a potential fusion peptide in the preS1 domainof the HBV L glycoprotein. These results underscore a membrane fusion mechanism that could be triggered by ERp57, allowing a thiol/disulfide exchange reaction to occur and regulate isomerization of a critical CSD, which ultimately leads to the exposition of the fusion peptide.

Natalia Freitas: natalia.bezerra-de-freitas@ens-lyon.fr

Rôle des apolipoprotéines dans la morphogenèse du virus del'hépatite C : réévaluation en hépatocytes humains primaires

Role of hepatic apolipoproteins in HCV morphogenesis: areassessment in primary human hepatocytes

Maxime Villaret[1], Matthieu Lemasson[1], Lynda Aoudjehane[2], Filoména Conti[2], Véronique Pène[1], Arielle R. Rosenberg[1]

[1] EA4474 « Virologie de l'Hépatite C », Université Paris Descartes;
[2] UMRS 938 « CDR Saint-Antoine », INSERM, Paris, France

In HCV-infected patients, infectivity is mainly supported by virions associated with triglyceride-rich lipoproteins containing apolipoprotein B (ApoB) and exchangeable Apos, resembling very-low-density lipoproteins (VLDL). The formation of these lipo-viro-particles remains poorly understood. It could occur inside or outside the hepatocyte, which is both the primaryreplication site of HCV and the cell type specialized in VLDL biogenesis. Studies based on cellline culture have generated conflicting results on the role of VLDL biogenesis in HCVmorphogenesis; however, ApoE is believed to play a key role in infectious HCV production, although it may be substituted by virtually all other hepatic apolipoproteins acting in aseemingly redundant manner. Here we have reassessed the role of hepatic apolipoproteins inHCV morphogenesis using RNA interference in primary human adult hepatocytes (PHH), which, contrary to cell lines used in previous studies, produce authentic VLDL and lipo-viro- particles. ApoB depletion had no effect on HCV infectious cycle even though it expectedly inhibited VLDL biogenesis. ApoE depletion also had no effect on HCV production or infectivity in PHH whereas it almost completely abolished HCV production in Huh-7.5.1 cells. ApoCI depletion spared the production of HCV particles but reduced their infectivity unless the PHH preparations expressed a basal low level of this Apo, in which cases HCV infectivity was reduced by the depletion of ApoAI instead of ApoCI. Finally, depletion of ApoAII (a constituentof high-density lipoproteins, not of VLDL) reduced the production of HCV particles irrespectiveof their infectivity. These data in physiologically relevant human hepatocytes suggest a complex interplay of HCV with lipoproteins, where the virus hijacks not the VLDL biogenesis itself but apolipoproteins acting nonredundantly in at least two distinct steps of HCV morphogenesis: ApoAII in the virus assembly occurring necessarily intracellularly, and ApoCI,or contingently ApoAI, in the acquisition or enhancement of its infectivity.

Maxime Villaret : maxime.villaret@parisdescartes.fr

Les hélicases DDX5 et DDX17 régulent la fidélité transcriptionnelle et la maturationdes ARN du virus de l'Hépatite B dans des hépatocytes primaires humains infectés

Helicases DDX5 and DDX17 regulate hepatitis B virus (HBV) transcriptional fidelity and RNA processing in infected humanprimary hepatocytes

Fleur Chapus[1,2], Guillaume Giraud[1], Caroline Charre[1,2,3], Chloé Goldsmith[1,2], Maria-Guadalupe Martinez[1,2], Judith Fresquet[1], Audrey Diederichs[1], 2, Maëlle Locatelli[1,2], Hélène Polvèche[4,5], Caroline Scholtès[6], Hector Hernandez Vargas[2], Michel Rivoire[7], Cyril Bourgeois[2,5], Fabien Zoulim[1,2,3] and Barbara Testoni[1]

[1]INSERM U1052- Cancer Research Center of Lyon (CRCL), 69008 Lyon, France
[2]University of Lyon, UMR_S1052, CRCL, 69008 Lyon,
[3]Department of Hepatology, Croix Rousse hospital, Hospices Civils de Lyon, France
[4]CECS, I-Stem, Corbeil-Essonnes, 91100, France.
[5] Ecole Normale Supérieure de Lyon, INSERM U1210, CNRS-UMR 5239, 69007, Lyon,France,
[6]Department of Virology, Croix Rousse Hospital, Hospices Civils de Lyon, Lyon, France
[7]Centre Léon Bérard (CLB), INSERM U1032, 69008, Lyon, France

Background and Aims: Unaffected by current antiviral therapies, covalently closed circular DNA (cccDNA) is responsible for Hepatitis B virus (HBV) persistence in infected hepatocytes and represents the key molecule for viral life cycle. cccDNA is a stable chromatinized episomeand the template for the six viral mRNAs. Transcription from cccDNA has several peculiar features, among which the fact that all viral RNAs use a common polyadenylation signal (PAS).Mechanisms and factors determining HBV RNA 3' end processing are still poorly defined. Thisstudy, therefore, aimed to precisely map the 3' end of HBV transcripts and to decipher the molecular events regulating HBV mRNA transcriptional fidelity.

Method: RNA extracted from HBV-infected HepG2-NTCP cells and primary human hepatocytes (PHHs) was subjected to HBV-adapted 3' RACE followed by MinION Nanopore single molecule sequencing. These experiments were combined with RT-qPCR and Northernblot analysis of HBV transcripts, together with chromatin- and RNA-immunoprecipitation (ChIP, CLIP) and functional invalidation of candidate proteins by RNA interference (RNAi).

Results: Single molecule sequencing analysis precisely indicated that the majority of HBV mRNAs ended 13 nucleotides downstream of the PAS in both infected PHH and HepG2-NTCPcells. However, in HepG2-NTCP cells, a significant proportion of HBV transcripts displayed a transcriptional readthrough of hundreds of base pairs downstream of this PAS. This readthrough was correlated with a higher expression of the DDX5 and DDX17 RNA helicasesin HepG2-NTCP cells compared to PHHs. Accordingly, RNAi for both helicases triggered an increase in electromobility of HBV RNAs in Northern Blotting without any significant effects onglobal RNAs levels suggesting a shortening of viral transcripts that was confirmed by the disappearance of the transcriptional readthrough after MinION sequencing. Combination of ChIP and CLIP-qPCR experiments showed that both DDX5 and DDX17 were recruited to cccDNA and RNAs and were required for the recruitment of the termination complex components CPSF6 and NUDT21, known to favor transcriptional readthrough. Finally, repression of DDX5 and DDX17 increased intracellular encapsidated DNA levels suggesting an increased HBV replication.

Conclusion: Altogether, our data suggest that optimal viral replication requires a fine tune regulation of the 3' end processing of HBV mRNAs and point to a pivotal role of DDX5/17 helicases and the terminator complex components NUDT21 and CPSF6 in the HBV transcription termination process.

Guillaume Giraud : guillaume.giraud@inserm.fr

Étude structurale et fonctionnelle de la protéine ORF3 du VHE parRMN en solution

Structural and functional study of HEV ORF3 protein by NMRSpectroscopy

Danai Moschidi[1,2], Elian Dupré[1,2], Emmanuelle Boll[1,2], François-Xavier Cantrelle[1,2], Zoe Lens[1,2], Xavier Hanoulle[1,2]

[1]CNRS ERL9002 - BSI - Integrative Structural Biology, F-59000 Lille, France;
[2]Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1167 - RID-AGE - Risk Factors and Molecular Determinantsof Aging-Related Diseases, F-59000 Lille, France

Hepatitis E Virus (HEV) is the most common cause of acute viral hepatitis worldwide with over 22 million infections and around 44,000 deaths recorded annually. In developing countries, HEV is transmitted via the fecal-oral route through contaminated drinking water, whereas in developed countries, the transmission occurs by consumption of uncooked or undercooked meat from infected animals. There is no specific treatment for HEV infection, apart from a vaccine which is available only in China. Therefore, HEV represents a public health problem which is constantly growing around the world. HEV causes liver infection with broad range of clinical manifestations ranging from asymptomatic cases to acute liver failure patients. HEV is small, icosahedral virus with 27 to 34nm diameter that belongs to *Hepeviridae* family. It is a quasi-enveloped virus with non-enveloped virions found in the faeces and bile and enveloped virions by host-cell-derived membranes found in bloodstream. It contains a ~7.2kb positive-sense, single-stranded RNA genome. The viral genome contains three open reading frames: ORF1, ORF2 and ORF3. ORF1encodes a non-structural polyprotein that includes multiple functional domains responsible for the replication of the viral genome. ORF2 encodes the viral capsid protein that assembles to make the viral particles. ORF3 encodes a small regulatory multifunctional protein which is poorly characterized. Previous studies have shown that ORF3 is involved in the release of the infectiousviral particles and interacts with other viral and host proteins inside the cell. For this process, ORF3 interacts with UEV domain of Tsg101 protein from ESCRT-I machinery. It is also reportedORF3 to be localized associated with the intracellular membranes and the plasma membrane. There are two different proposed modes of its membrane anchoring. The first one is the oligomerization of ORF3 with a transmembrane insertion that forms an ion channel like a viroporin, and the second one is the membrane-association of ORF3 via palmitoylation of its N- terminal Cysteine-rich region and the presence of N- and C-terminus in the cytoplasm. In this study, we use NMR spectroscopy as well other biophysical techniques to get a detailed molecularcharacterization of the ORF3 protein in order to decipher its functional role(s) during the HEV lifecycle. First aim of this research is the expression and purification of HEV ORF3 protein and its structural characterization by solution NMR spectroscopy. Secondly, its interaction with human Tsg101 UEV protein is studied by diverse biophysical techniques as NMR spectroscopy, Isothermal Titration Calorimetry (ITC) and X-ray Crystallography. Finally, we want to further investigate the membrane anchoring of ORF3 and therefore we use Nanodiscs that mimic the membrane while they are lipid bilayers stabilized by two copies of membrane-scaffolding proteins(MSPs) to attach the protein and characterize it using NMR Spectroscopy. The detailed molecularcharacterization of HEV ORF3 will help to better understand its biological function(s) and can ultimately be used to design antiviral compounds.

Danai Moschidi : danai.moschidi@gmail.com

Caractérisation d'un système d'infection persistante par le virus del'hépatite E dans les cellules hépatiques humaines HepaRG

Characterization of a cell culture system of persistent hepatitis Evirus infection in the human HepaRG hepatic cell line

Marie Pellerin[1], Edouard Hirchaud[2], Yannick Blanchard[2], Nicole Pavio[1] et Virginie Doceul[1]

[1] UMR 1161 Virologie, INRAE, ANSES, Ecole Nationale Vétérinaire d'Alfort, UniversitéParis-Est, Maisons-Alfort.
[2] Agence National de Sécurité Sanitaire, de l'environnement et du travail (ANSES), laboratoire de Ploufragan-Plouzané-Niort, Unité génétique virale et biosécurité (GVB).

Hepatitis E virus (HEV) is considered as an emerging global health problem. The pathogen isresponsible for more than 2 000 cases of acute hepatitis in France every year resulting in majority from zoonotic infections associated with the consumption of raw or undercooked pork.In most cases, hepatitis E is a self-limiting disease and the virus is cleared spontaneously without the need of antiviral therapy. However, immunocompromised individuals can developchronic infection and liver fibrosis that can progress rapidly to cirrhosis and liver failure. For decades, the lack of efficient and relevant cell culture system and animal models has limited our understanding of the biology of HEV and the development of effective drugs for chronic cases. In the present study, we developed a model of persistent HEV infection in human hepatocytes in which HEV replicates efficiently. This HEV cell culture system is based on differentiated HepaRG cells infected with an isolate of HEV-3 derived from a patient sufferingfrom acute hepatitis E. Efficient replication was maintained for several weeks to several monthsas well as after seven successive passages on HepaRG naïve cells. Moreover, after six passages onto HepaRG, the virus was still infectious after oral inoculation into pigs. We also showed that ribavirin inhibited HEV replication in HepaRG cells. Using whole genome sequencing, 25 mutations including 8 non-synonymous mutations were detected in thegenome of the virus recovered after 6 passages into HepaRG. In conclusion, this system represents a relevant and efficient in vitro model of HEV replication that could be useful to identify host-virus interactions and putative mutations within the viral genome than can occur*in vitro* in the context of prolonged hepatitis E infection and to test antiviral drugs against chronic HEV infection.

Virginie Doceul: virginie.doceul@vet-alfort.fr

Vers la caractérisation structurale de la ribonucléoprotéine du virusde l'hépatite D

Towards the structural characterization of the Hepatitis D virusribonucleoprotein complex

Yang Yang[1], Marie-Laure Fogeron[1], Morgane Callon[2], Alexander Malär[2], LaurianeLecoq[1], Loïc Delcourte[1], Yi Ni[3], Stephan Urban[3], Beat H. Meier[2], Anja Böckmann[1]

[1]Molecular Microbiology and Structural Biochemistry, Labex Ecofect, UMR 5086 CNRS-Université de Lyon, 69367 Lyon, France
[2]Physical Chemistry, ETH Zurich, 8093 Zurich, Switzerland
[3]Department of Infectious Diseases, Centre for Integrative Infectious Disease Research,University Hospital Heidelberg, 69120 Heidelberg, Germany

The Hepatitis D virus is a defective virus lacking envelope proteins, and thus relying on co- infection, notably with the Hepatitis B virus, to complete its replication cycle. Its genome encodes for only two isoforms of the delta antigen, S- and L-HDAg, which constitute a ribonucleoprotein (RNP) with the viral RNA. The structure of the RNP remains undetermined.Here we report initial steps towards such an analysis using wheat germ cell-free protein synthesis (WG-CFPS) combined with solid- and solution-state NMR spectroscopy.

We synthesized using WG-CFPS both full-length S-HDAg and L-HDAg, as well as S-HDAg lacking the first 60 amino acids (SΔ60), in milligram amounts compatible with structural studies.On the one hand, S- and L-HDAg were isolated on density gradient and targeted to solid-stateNMR spectroscopy after complex sedimentation. Both isoforms formed a complex with RNA as shown by ^{31}P spectra. On the other hand, SΔ60 was fully soluble and targeted to solution- state NMR spectroscopy. A comparison of solid-state NMR spectra of S- and L-HDAg revealssignificant differences in the flexible parts, with L-HDAg showing less mobility. Spectrarecorded on SΔ60 coincide for the disordered part with the solid-state NMR spectra of S-HDAg, indicating no substantial impact of the assembly domain (1-60) on this part. In addition, a comparison of NMR signals of the folded part of S-HDAg and SΔ60 do not coincide, allowing to attribute the peaks in the S-HDAg/RNA complex to the assembly domain. Sequential assignments of SΔ60 are under way, and will reveal secondary structures as a function of amino-acid position for this protein.

Marie-Laure Fogeron : marie-laure.fogeron@ibcp.fr

Un changement conformationnel induit par un ligand dans la poche hydrophobe de la capside du virus de l'hépatite B

A pocket-factor-triggered conformational switch in the hepatitis B virus capsid

Lauriane Lecoq[1], Shishan Wang[1], Marie Dujardin[1], Peter Zimmermann[2], Leonard Schuster[2], Marie-Laure Fogeron[1], Mathilde Briday[1], Maarten Schledorn[3], Thomas Wiegand[3], Laura Cole[1], Roland Montserret[1], Stéphane Bressanelli[4], Michael Nassal[2], Beat H. Meier[3], Anja Böckmann[1]

[1]Molecular Microbiology and Structural Biochemistry (MMSB), UMR 5086 CNRS/Université de Lyon, 69367 Lyon, France
[2]Dept. of Medicine II / Molecular Biology, University of Freiburg
[3]Physical Chemistry, ETH Zurich, 8093 Zurich, Switzerland
[4]Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris-Saclay, 91198 Gif sur Yvette Cedex, France

Viral hepatitis is growing into an epidemic illness, and it is urgent to neutralize the main culprit, hepatitis B virus (HBV), a small enveloped retrotranscribing DNA virus. The HBV viral particle assembles from core proteins dimers which, together with the pregenomic RNA and the polymerase, form the immature capsid. The matured, rcDNA containing capsid is then enveloped by the three surface proteins L, M and S.

An intriguing observation in HB virion morphogenesis is that rarely capsids with immature genomes are enveloped and secreted. This prompted in 1982 the postulate that a regulated conformation switch in the capsid triggers envelopment. Using solid-state NMR, and a bacterial expression/synthetic biology tool box able to provide a variety of different capsid forms, we identified a stable alternative conformation of the capsid (Lecoq *et al*, 2021). The structural variations focus on the hydrophobic pocket of the core protein, a hot spot in capsid-envelope interactions. We found that this structural switch is triggered by a specific, high affinity binding of a pocket factor. The conformational change induced by the binding is reminiscent of a maturation signal.

Our observations not only bring a new molecular view on the mechanism underlying capsid envelopment, but open a rationale way for its inhibition. through the development of new ligands specifically targeting this region.

Lecoq L, Wang S, Dujardin M, Zimmermann P, Schuster L, Fogeron ML, Briday M, Schledorn M, Wiegand T, Cole L, Montserret R, Bressanelli S, Meier BH, Böckmann A. (**2021**) "A pocket-factortriggered conformational switch in the hepatitis B virus capsid" Proc Natl Acad Sci U S A, 118(17).
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Lauriane LECOQ: lauriane.lecoq@ibcp.fr

Modélisation moléculaire du domaine C-terminal de la protéineCore du virus de l'hépatite B: Implications pour le trafic intracellulaire de la nucléocapside

Molecular modelling of hepatitis B virus Core protein C-terminaldomain: Implications for nucleocapsid trafficking

Jean-Charles Carvaillo[1], Stéphane Bressanelli[1], Yves Boulard[1]

[1] *Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), 91198, Gif-sur-Yvette, France.*

The Core protein of the hepatitis B virus is composed of an N-terminal structured assembly domain (NTD, residues 1-149) followed by an intrinsically disordered C-terminal domain (CTD,residues 150-183). Crystal structures of recombinant (*E. coli*-produced) NTD show that in theabsence of genetic material, residues 1-149 are organized into a helical domain. The NTD forms dimers with a protruding spike composed of a four-helix bundle, while lateral contacts between other helices and loops allow 120 dimers to assemble into an icosahedral capsid [1]. The positively-charged CTD is located inside this capsid, where it interacts with the pre- genomic RNA upon immature nucleocapsid assembly in the cytosol of the infected cell. However, the CTD also harbours the nuclear localisation signals (NLS) that direct mature nucleocapsids to the nucleus. Thus, some at least of the 240 CTD in a mature nucleocapsid must be displayed through the pores that fenestrate the capsid. This structural feature is not clearly visible, even in recent high-resolution cryo-EM structures of full-length recombinant nucleocapsid-like particles [2], so that there is some controversy even as to which type of poreallows display of the CTD [3,4] and how far the CTD exits the pore [5,6].

Using molecular dynamics simulations of Core dimers, we show that the CTD tends to interactwith its NTD in such a way that it would either sit below 3-fold and quasi-3-fold pores, or be threaded though the same pores up to the tip of the Core spike. Further molecular modelling in the context of the capsid, including biased simulations, shows atomic-level features of CTDegress through 3-fold-type pores. We discuss the agreement of our results with published functional and structural properties of CTD display by the mature HBV nucleocapsid.

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Jean-Charles Carvaillo : jean-charles.carvaillo@i2bc.paris-saclay.fr

Influence des modulateurs d'assemblage sur la structure et lacinétique d'assemblage de la capside du virus de l'hépatite B

Influence of assembly modulators on the structure and assemblykinetics of hepatitis B virus capsid

Kalouna Kra[1,2], Stéphane Bressanelli[1], Guillaume Tresset[2]

[1] Institut de Biologie Intégrative de la Cellule (I2BC), CNRS, Université Paris-Saclay, CEA, Saclay, France;
[2] Laboratoire de Physique des Solides (LPS), CNRS, Université Paris-Saclay, Orsay, France

Hepatitis B virus (HBV) is an enveloped virus with an icosahedral nucleocapsid. The constituent of the capsid is the Core protein. It is a dimeric protein where the 149 N-terminal residues (Cp149) can self-assemble *in vivo* but also *in vitro* into capsids containing mainly 120 dimers (Birnbaum et al., J Virol 1990). The CpAMs (for Capsid assembly modulators)that can disrupt HBV capsid assembly are new antiviral molecules currently in clinical trials. They can either lead to the formation of seemingly normal icosahedral capsids (class I CpAMs, e.g., sulfamoylbenzamide (SBA) derivatives such as JNJ-632), or to the formation ofaberrant (larger and malformed) assemblies (class II CpAMs, e.g., heteroarylpyrimidines (HAP) such as BAY 41-4109).

Our experimental approach relies on static light scattering kinetics, small-angle scattering (SAXS) and electron microscopy with recombinant Core protein. We will present here ourfirst results obtained on the assembly of Cp149 dimers, with an emphasis on the effects of BAY 41-4109 and JNJ-632. We confirm that with the CpAM-II BAY 41-4109, the resulting objects no longer have the morphology of a capsid and are much larger in size. We further show that the size and heterogeneity of the objects actually increases with BAY 41-4109 concentration. Indeed, the objects are 1.25 to 2 times the size of a normal capsid (35 nm)and their polydispersity index is 20% while that of normal capsids is negligible. Preliminary results also show that BAY 41-4109 destabilizes Cp149 capsids. In the presence of the CpAM-I JNJ-632, the objects formed from Cp149 dimers indeed have the size and general shape of normal capsids, but clear alterations in structure are discerned, that furthermore increase with increasing CpAM-I concentration. Moreover, in static light scattering kinetics, we found that assembly modulators seem to dictate the kinetics of hepatitis B virus capsid formation and assembly kinetics in the presence of modulators are up to 7 times faster thanin the absence of modulators.

We will present these effects of CpAMs, including previously uncharacterized features, and discuss the possible implications for their action on capsid assembly pathways.

Kalouna Kra : kalouna.kra@i2bc.paris-saclay.fr

Caractérisation des interactions hétérologues de la protéine coredu virus de l'hépatite B par RMN et ITC

Heterologous interaction characterization of Hepatitis B Virus coreprotein by NMR and ITC

Mathilde Briday[1], Lauriane Lecoq[1], François Halle[2], Sylvie Radix[2], Roland Montserret[1], Juliette Martin[1], Nadine Walchshoffer[2], Beat Meier[3], Michael Nassal[4], Thierry Lomberget[2], Anja Böckmann[1]

[1]Molecular Microbiology and Structural Biochemistry (MMSB), Université de Lyon/CNRSUMR5086, 69367 Lyon, France
[2]Faculté de Pharmacie-ISPB, Université de Lyon/CNRS UMR 5246, 69373 Lyon, France
[3]Physical Chemistry, ETH Zürich, 8093 Zürich, Switzerland
[4]Dept. of Medicine II / Molecular Biology, University of Freiburg

Chronic Hepatitis B Virus (HBV) is still a major global health issue. HBV is an envelopedvirus enclosing a partially double-stranded DNA, and the core protein forming the capsid is one of the main focus of research to understand the interaction process of the virus. This protein is composed of 183 amino acids and assembles into dimers, which then autoassemble into an icosahedral capsid formed by 120 dimers. Cp is responsible for variousfunctions in the virus replication, and therefore its heterologous interactions with different partners are of central interest.

Multiple binding sites have been highlighted in the Cp structure, including the interdimer interface where capsid assembly modulators bind, and also the spike tip. In addition, we have recently identified using solid-state Nuclear Magnetic Resonance (NMR) that Triton X- 100, a detergent used in Cp purification, binds in the hydrophobic pocket and induces a conformational switch of the capsid (Lecoq *et al.*, 2021). The residues in and around this pocket have previously been shown to be important for the virus envelopment. We now here report details on the binding mode of Triton-X-100 and homologs thereof. To investigate the chemical moieties which are central to the binding event, we have tested interactions of different modified versions of Triton X-100, by means of commercial and specially designed chemical components. The screening analysis was performed using NMR, Isothermal Titration Calorimetry (ITC) and in silico docking.

The obtained information on the binding modes of the molecules to the pocket can guide the design of antivirals which target hydrophobic pocket interactants, as potentially the preS envelope domain.

Lecoq L, Wang S, Dujardin M, Zimmermann P, Schuster L, Fogeron ML, Briday M, Schledorn M, Wiegand T,Cole L, Montserret R, Bressanelli S, Meier BH, Böckmann A. (**2021**) "A pocket-factor-triggered conformational switch in the hepatitis B virus capsid" Proc Natl Acad Sci U S A, 118(17). doi: 10.1073/pnas.2022464118

Mathilde BRIDAY: mathilde.briday@ibcp.fr

101 rue de Tolbiac
75013 Paris
www.anrs.fr