Societat Catalana de BIOLOGIA

XVII Jornada de Virologia

Organitzada per la Secció de Virologia de la SCB

INSTITUT D´ESTUDIS CATALANS

Carrer del Carme 47

Barcelona

30 d'octubre de 2018

XVII Jornada de Virologia
BCN Virology Meeting 2018

PROGRAMA

Coordinadora de la Secció i responsable de la coordinació de la Jornada:
SOFIA PÉREZ DEL PULGAR

Con la colaboración de: ciberehd
Centro de Investigación Biomédica en Red
Enfermedades Hepáticas y Digestivas

9:15 h
RECOLLIDA DOCUMENTACIÓ / REGISTRATION

9:30 h
BENVINGUDA / WELLCOME: ***Sofía Pérez del Pulgar***

SESSIÓ I / SESSION I
MODERADOR / CHAIR: ***Georgios Koutsoudakis***

9:45 h
O-1: Linking cell dynamics with gene coexpression networks to characterize key events in chronic virus infections
Jordi Argilaguet. Universitat Pompeu Fabra, Barcelona, Spain.

10:00 h
O-2: Erlin-1 protein is required for efficient hepatitis C virus infection
Urtzi Garaigorta. Centro Nacional de Biotecnología (CNB-CSIC), CIBERehd, Madrid, Spain; The Scripps Research Institute, La Jolla, California, USA.

10:15 h
O-3: CD32 expression is associated to T cell activation and upregulated by HIV
Roger Badia. IrsiCaixa, Badalona, Spain.

10:30 h
O-4: Implications of transgene codon usage optimization in armed oncolytic adenovirus
Estela Núñez-Manchón. IDIBAPS. Barcelona, Spain.

10:45 h
O-5: Hepatitis A virus replication modulates the dynamics of ALIX synthesis in hepatocytes
Montserrat de Castellarnau. University of Barcelona, Institute of Nutrition and Food Safety, Barcelona, Spain.

11:00 h
O-6: Clinical and virological predictors of response after antiviral therapy interruption in HBeAg-negative chronic hepatitits B
Mireia García-López. IDIBAPS, Hospital Clinic, CIBERehd, Barcelona, Spain.

11:15-11:45h
PAUSA I CAFÈ / COFFE BREAK

11:45 h

SESSIÓ II / SESSION II
MODERADOR / CHAIR: *Ana Angulo*

INVITED SPEAKER
Host factors at the crossroads of cell proliferation, immune responses, and virus restriction
José A. Esté. IrsiCaixa, Badalona, Spain.

12:15 h
O-7: Dissecting immune evasion strategies during cytomegalovirus infection of antigen-presenting cells
Guillem Angulo. University of Barcelona, Barcelona, Spain.

12:30 h
O-8: Probing lipid and protein dynamics at individual HIV-1 assembly sites
Jakub Chojnacki. Weatherall Institute of Molecular Medicine, University of Oxford, UK; IrsiCaixa, Barcelona, Spain.

12:45 h
O-9: Chikungunya viral infection favors translation of mRNAs with non-optimal codons at the endoplasmatic reticulum
René Böttcher. Universitat Pompeu Fabra, Barcelona, Spain.

13:00 h
O-10: Quasispecies conservation and complexity of Hepatitis B X gene in different clinical groups of chronic HBV infection
Maria Francesca Cortese. Vall d'Hebron Institut de Recerca, Hospital Vall d'Hebron, Barcelona, Spain

13:15 h
O-11: HIV-1 Protease Evolvability Is Affected by Synonymous Nucleotide Recoding
Maria Nevot. IrsiCaixa, Badalona, Spain.

13:30-14:15 h
DINAR / LUNCH

14:15-15:00 h
SESSIÓ DE PÓSTERS/ POSTER SESSION
15:00 h

SESSIÓ III/ SESSION III
MODERADOR / CHAIR: *Núria Busquets*

INVITED SPEAKER
"One health": from the concept to reality
Joaquim Segalés. IRTA-CReSA, Bellatera, Spain.

15:30 h
O-12: Targeted amplicon deep sequencing analysis: diversity of adenovirus, papillomavirus and enterovirus in raw sewage
Sandra Martínez-Puchol. University of Barcelona, Barcelona, Spain.

15:45 h
O-13: Vector susceptibility of *Aedes albopictus* and *Culex pipiens* from Catalonia for Zika virus
Ana Nuñez. IRTA-CReSA, Bellaterra, Spain.

16:00 h
O-14: Virological and clinical surveillance (2014-2017) of respiratory enteroviruses from paediatric patients attended at a tertiary hospital in Catalonia (Spain)
Cristina Andrés. Hospital Universitari Vall d'Hebron, Vall d'Hebron Research Institute, Universitat Autònoma de Barcelona, Barcelona, Spain.

16:15 h
ROUND TABLE: *FUTURE CHALLENGES FOR YOUNG VIROLOGISTS*
MODERADOR / CHAIR: *Rosina Gironés*
PARTICIPANTS / PARTICIPANTS: *José A. Esté, Joaquim Segalés, Urtzi Garaigorta, Júlia Vergara-Alert, Elena Perpiñán*

17:00 h
SUMMARY OF THE MEETING AND BEST PRESENTATION AWARDS
Prizes for the best oral and best poster presentations are sponsored by CIBERehd (Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas)

POSTER SESSION

P-1: Gradual restoration of HCV-specific CD8+ T-cell frequency accompanied by altered phenotype in cirrhotic HCV-infected patients after successful interferon-free therapy

***Elena Perpiñán**, Sofía Pérez-Del-Pulgar, María-Carlota Londoño, Patricia González, Zoe Mariño, Sabela Lens, Concepción Bartres, Mireia García-López, Xavier Forns, George Koutsoudakis*

Liver Unit, Hospital Clinic, University of Barcelona, IDIBAPS and CIBERehd, Barcelona, Spain.

P-2: A hypothesis driven shRNA-based screening identifies aquarius and senataxin as restriction host factors for hepatitis B virus infection

*Andoni Gomez-Moreno[1], Christina Whitten-Bauer[2] and **Urtzi Garaigorta[1,2,3]***

[1]Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain. [2]The Scripps Research Institute, La Jolla, California, USA. [3]CIBER de Enfermedades Hepáticas y Digestivas (CIBERehd), Madrid, Spain.

P-3: Evaluation of a novel microfluidic nanoparticle-based immunoassay method (Virotrack Dengue Acute) for detection of dengue NS1 antigen in travelers

***Navero-Castillejos J**[1], Alejo-Cancho I[2], Peiró A[1,2], Albarracín R[2], Barrachina J[2], Gonzalo V[2], Pastor V[2], Martínez MJ[1,2]*

[1] ISGlobal, Barcelona Centre for International Health Research (CRESIB), Hospital Clinic, Universitat de Barcelona, Barcelona, Spain. [2] Department of Clinical Microbiology, Hospital Clinic, Barcelona, Spain

P-4: Improvement of the diagnosis of HCV and HIV infection through alternative strategies in people who inject drugs

***Adrián Antuori**[1], Verónica Saludes[1,2], Cinta Folch[2,3], Núria Ibáñez[4], Vincent Montoya[5], Richard Harrigan[5], Joan Colom[4], María Alma Bracho[2,6], Julia Hillung[6], Fernando González-Candelas[2,6], Jordi Casabona[2,3], and Elisa Martró[1,2]; HepCdetect II Study Group**

1. Microbiology Service, Germans Trias i Pujol University Hospital and Research Institute (IGTP), Badalona, Spain. 2. Biomedical Research Networking Centre in Epidemiology and Public Health (CIBERESP), Instituto de Salud Carlos III, Madrid, Spain. 3. Centre for Epidemiological Studies on Sexually Transmitted Infections and HIV/AIDS of Catalonia (CEEISCAT), Catalonia Public Health Agency (ASPCAT), Badalona, Spain. 4. Program on Substance Abuse, ASPCAT, Barcelona, Spain. 5. BC Centre for Excellence in HIV, Vancouver, Canada. 6. Joint Research Unit Infection and Public Health FISABIO-CSISP/ University of Valencia, Spain.

P-5: Molecular characterization of HCV resistance-associated substitutions after interferon-free treatment failure by massive parallel sequencing

***Qian Chen**[1,2], Celia Perales[1,2], Javier Crespo[3], Maria Buti[2,4], José Luis Calleja[5], Josep Gregori[2,6], Francisco Rodríguez-Frías[1,2], Leonardo Nieto[4], Jordi Niubó[7], Manuel Rodríguez[8], Isabel Conde[9], Maria Luisa García Buey[10], Inmaculada Fernández[11], Javier Torras[12], Rosa Morillas[13], Moisés Diago[14], Federico Sáez-Royuela[15], Montserrat Forné[16], Juan Tunes[17], Javier García Samaniego[18], José Antonio Carrión[19], Juan Ignacio Arenas[20], Xavier Forns[21], Zoe Mariño[21], Raúl Andrade[22], Silvia Montoliu[23], Miguel Ángel Simón[24], Gloria Sánchez Antolín[25], Juan José Sánchez-Ruano[26], Juan Manuel Pascasio[27], Juan Ignacio Esteban[2,4], Josep Quer[1,2].*

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P-6: Could HDV induce variability on HBV? Characterization of HBV quasispecies in HBV/HDV coinfected patients

Cristina Godoy[1], *Sara Sopena*[1,2], *Josep Gregori*[2], *David Tabernero*[1,2], *Maria Francesca Cortese,* [1] *Rosario Casillas*[1], *Marçal Yll*[1], *Carolina González*[1], *Ariadna Rando*[1], *Rosa Maria López*[1], *Josep Quer*[2], *Rafael Esteban*[3], *Mar Riveiro-Barciela*[3], *Francisco Rodríguez-Frías*[1,2], *Maria Buti*[3]

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P-7: Evaluation of sperm wash techniques for the elimination of Zika Virus in semen

A Peiró[1,2], *M Guimerà*[3], *M Dalmau*[3], *S Cívico*[3], *V Gonzalo*[1], *G Casals*[3], *V Moreno*[3], *JM Calafell*[3], *E Vidal*[3], *MJ Martínez*[1,2]

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P-8: Dynamics of HIV-1 reservoir decay in early-treated individuals

Ángel Bayón-Gil[1], *Víctor Urrea*[1], *Beatriz Mothe*[1,2,3], *Christian Brander*[1,2,3,4,5], *María C. Puertas*[1], *Javier Martínez-Picado*[1,2,3,4]

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P-9: Qsutils: an r package to study viral quasispecies complexity with ngs data

Mercedes Guerrero-Murillo[1], *Josep Gregori i Font*[1,2,3], *Francisco Rodríguez-Frias*[2,4],*Josep Quer Sivila*[1,2]

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P-10: The influence of warthog fecal microbiota transplantation on susceptibility of domestic pig to African Swine Fever

Zhang, Jinya[1]*; Navas, María Jesús*[1]*; López, Elisabet*[1]*; Bosch, L*[1]*; Acensi, Francesc*[1,2]*; Rodríguez Fernando*[1]*; Correa-Fiz F*[1]*; Martínez, Jorge*[1,2].

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P-11: Human astrovirus (hastv) alters the intestinal epithelium barrier

Lluïsa Miró[1,3]*, Cristina Fuentes*[2,3]*, Albert Bosch*[2,3]*, Rosa M. Pintó*[2,3]*, Miquel Moretó*[1,3]*, Susana Guix*[2,3]*, Anna Pérez-Bosque*[1,3]

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P-12: Cytomegalovirus-encoded CD48 homologs, a novel class of immunevasins

Pablo Martínez-Vicente[a]*, Domènec Farré*[a]*, Carolina Sánchez*[b]*, Antonio Alcamí*[b]*, Pablo Engel*[a]*, and Ana Angulo*[a]

Immunology Unit, Department of Biomedical Sciences, Medical School, University of Barcelona, C/ Casanova 143, 08036 Barcelona, Spain[a]; Centro de Biología Molecular Severo Ochoa, C/ Nicolás Cabrera 1, 28049 Madrid, Spain[b]

P-13: Middle East Respiratory Syndrome coronavirus (MERS-CoV) vaccination efficacy in a direct-contact transmission model in llamas

Rodon, Jordi[1]*; Te, Nigeer*[1]*; Saporiti, Viviane*[1]*; Okba M.A., Nisreen*[2]*; Pujols, Joan*[1]*; Abad, Xavier*[1]*; Haagmans, Bart*[2]*; Bosch, Berend-Jan*[3]*; van Dieren, Brenda*[3]*; Vergara-Alert, Júlia*[1]*; Bensaid, Albert*[1]*; Segalés, Joaquim*[1,4]

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P-14: Human metapneumovirus: are the new duplications within the *G* gene responsible for doubling its prevalence?

***Piñana M.**, Andrés C., Gimferrer L., Codina MG., Martín MC., Esperalba J., Fuentes F., Rubio S., Alcubilla P., Pumarola T. and Antón A.*

Respiratory Viruses Unit, Virology Section, Microbiology Department, Hospital Universitari Vall d'Hebron, Vall d'Hebron Research Institute, Universitat Autònoma de Barcelona, Barcelona, Spain

P-15: Lack of hepatitis E transmission during a hepatitis A outbreak exclude in men who have sex with men

***Sergio Rodríguez-Tajes**, Elena Perpiñán, Patricia González, Thais Leonel, Zoe Mariño, Sofía Pérez-del-Pulgar, George Koutsoudakis Xavier Forns*

Liver Unit, Hospital Clinic, University of Barcelona, IDIBAPS and CIBERehd, Barcelona, Spain.

P-16: New HCV genotype 1 whole-genome characterization

*Georg von Massow[a], **Damir Garcia-Cehic**[a,d], Josep Gregori[a,e], María Dolores Macià[c], Juan Ignacio Esteban[b,d], Josep Quer[a,d]*

Liver Diseases Unit, Vall d'Hebron Institute of Research (VHIR), Barcelona[a]; Liver Unit, HUVH-UAB, Barcelona[b]; Unidad de Microbiología Molecular, Servicio de Microbiología, Instituto de Investigación Sanitaria de les Illes Balears (IdISBa)[c]; Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Instituto de Salud Carlos III, Madrid[d]; Roche Diagnostics S.L., Sant Cugat del Vallès[e]

P-17: Co-localization of Middle East respiratory syndrome coronavirus (MERS-CoV) and dipeptidyl peptidase-4 in the respiratory tract and lymphoid tissues of pigs and llamas

***Te, Nigeer**[1]; Vergara-Alert, Júlia[1]; Lehmbecker, Annika[2]; Pérez, Mónica[1]; Haagmans, Bart[3]; Baumgärtner, Wolfgang[2]; Bensaid, Albert[1]; Segalés, Joaquim[1,4]*

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SPEAKERS' ABSTRACTS

INVITED SPEAKER

Host factors at the crossroads of cell proliferation, immune responses, and virus restriction

José A. Esté

IrsiCaixa, Badalona, Spain.

Viruses are intracellular parasites requiring the cellular machinery to replicate. Intracellular virus host dependency factors may promote infection (cofactors) or prevent (restriction factors) virus replication and disease. Additionally, virus infections trigger cell defense mechanisms aimed at inhibiting virus replication, initiating and innate immune response and inflammation. There are a number of intracellular proteins with antiviral properties that detect and recognize virus infections by identifying foreign proteins and/or genomic materials though pathogen pattern recognition receptors. Viruses have developed multiple mechanisms to counteract the host restriction factors and evade the immune system.

In this lecture, we will review the cellular mechanisms controlling and regulating virus replication using HIV-1 as a model. I will provide evidence to indicate how virus restriction factors affect virus replication. I will highlight the relevant role of SAMHD1, a HIV restriction factor, in the control of cell proliferation, the antiviral immune response and virus replication and how its activity may be modulated to combat virus infections and disease.

SAMHD1 is a triphosphohydrolase enzyme that controls the intracellular level of deoxyribonucleoside triphosphates (dNTPs) and plays a role in innate immune sensing and autoimmune disease. SAMHD1 has also been identified as an intrinsic virus restriction factor, inactivated through degradation by HIV-2 Vpx or through a post-transcriptional regulatory mechanism. Phosphorylation of SAMHD1 by cyclin-dependent kinases has been strongly associated with inactivation of the virus restriction mechanism, providing an association between virus replication and cell proliferation. Tight regulation of cell proliferation suggests that viruses, particularly HIV-1 replication, latency, and reactivation, may be similarly controlled by multiple checkpoint mechanisms that, in turn, regulate dNTP levels.

INVITED SPEAKER

"One health": from the concept to reality

Joaquim Segalés[1,2]*, Núria Busquets*[1]*, Júlia Vergara-Alert*[1]

[1]Centre de Recerca en Sanitat Animal (CReSA, IRTA-UAB), Campus de la Universitat Autònoma de Barcelona, and [2]Departament de Sanitat Anatomia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193 Bellaterra Spain

The concept One Health was born as such in 2004. Specifically, it referred to a worldwide strategy for expanding interdisciplinary collaborations and communications in all aspects of health care for humans, animals and the environment. That year, the Wildlife Conservation Society published the 12 Manhattan Principles, which summarize the global demands to world's leaders, civil society, the global health community and institutions of science towards the achievement of a global agenda on One Health. However, the implementation of this strategy is not devoid of hurdles and the need of breaking down barriers among agencies, individuals, specialties and sectors is crucial. By looking back at the last 20-30 years, outbreaks of West Nile, Ebola Hemorrhagic Fever, Severe Acute Respiratory Syndrome, Middle-East Respiratory Syndrome (MERS), Monkeypox, Mad Cow Disease, Dengue, Zika, Chikungunya, Rift Valley Fever and Avian Influenza have occurred around the world, and implementation of the One Health strategy has not been straightforward. The current presentation will focus on particular initiatives that the Centre de Recerca en Sanitat Animal (CReSA, at IRTA) is pursuing on a number of public health issues and their connection with animal diseases or pathogens. Specifically, the virological surveillance in mosquitoes as vectors of disease transmission and potential ways to prevent transmission of MERS-coronavirus from animals to humans will be tackled.

ORAL ABSTRACTS

O-1

Linking cell dynamics with gene coexpression networks to characterize key events in chronic virus infections

Jordi Argilaguet[1], *Mireia Pedragosa*[1,¶], *Graciela Riera*[1,¶], *Anna Esteve-Codina*[2], *and Andreas Meyerhans*[1,3]

[1]Infection Biology Laboratory, Department of Experimental and Health Sciences (DCEXS), Universitat Pompeu Fabra, Barcelona. [2]CNAG-CRG, Center for Genomic Regulation (CRG), Barcelona Institute of Science and Technology & Universitat Pompeu Fabra, Barcelona. [3]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona.

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The host immune response against a virus infection requires the coordinated action of many diverse cell subsets that dynamically adapt to a pathogen threat. Due to the complexity of such a response, most immunological studies have focused on a few genes, proteins, or cell types. With the development of “omic”-technologies and computational analysis methods, attempts to analyze and understand complex system dynamics are now feasible. However, the decomposition of transcriptomic data sets generated from complete organs remains a major challenge. Here, we combined Weighted Gene Coexpression Network Analysis (WGCNA) and Digital Cell Quantifier (DCQ) to analyze time-resolved mouse splenic transcriptomes in acute and chronic Lymphocytic Choriomeningitis Virus (LCMV) infections. This enabled us to generate hypotheses about complex immune functioning after a virus-induced perturbation. This strategy was validated by successfully predicting several known immune phenomena, such as effector cytotoxic T lymphocyte (CTL) expansion and exhaustion. Furthermore, we predicted and subsequently verified experimentally that virus-specific CD8+ T cells with an early effector transcriptome profile participate in the host adaptation to an overwhelming virus threat. Thus, the linking of gene expression changes with immune cell kinetics provides novel insights into the complex immune processes within infected tissues.

O-2

Erlin-1 protein is required for efficient hepatitis C virus infection

Urtzi Garaigorta[1,2,3], *Christina Whitten-Bauer*[3], *Josan Chung*[3], *Michael D. Huber*[3], *Larry Gerace*[3] *and Francis V. Chisari*[3]

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Hepatitis C virus (HCV) is an enveloped virus belonging to the *Flaviviridae* family. It is estimated that around 71 million people are chronically infected worldwide, and 399.000 die every year from complications such as cirrhosis and hepatocellular carcinoma. The development of a hepatitis C virus (HCV) infection cell culture system has permitted the identification of cellular factors that regulate different aspects of the HCV life cycle. Some of these factors affect steps in the viral life cycle that are tightly associated to intracellular membranes derived from the endoplasmic reticulum (ER). Erlin proteins are endoplasmic reticulum membrane lipid raft-associated proteins that belong to a larger family of proteins containing a conserved stomatin, prohibitin, flotillin, HflK/C (SPFH) domain, which is proposed to organize membrane microdomains. Erlin proteins have been implicated in cholesterol homeostasis and the ER-associated degradation pathway of several substrates. Given the tight dependency of HCV on lipid metabolism and ER membranes and the functions and localization of Erlin proteins we decided to investigate the role of Erlin proteins during HCV infection. In this study, we describe the discovery of Erlin-1 protein as a cellular factor that regulates HCV infection. Silencing of Erlin-1, but not Erlin-2, protein expression by siRNA led to a decrease infection efficiency characterized by a modest reduction in intracellular RNA accumulation and stronger suppression of HCV protein expression and virus production. Mechanistic studies revealed that Erlin-1 protein is required early in the infection, downstream cell entry and primary translation, specifically to initiate RNA replication. Experiments performed in subgenomic replicon cells and persistently infected cells indicated that Erlin-1 protein is not required to maintain the ongoing HCV RNA replication. Moreover, our data is compatible with the hypothesis that Erlin-1 protein might be required to maintain the steady-state levels and right concentration of HCV proteins in lipid droplets facilitating virus assembly. Collectively, this study identifies Erlin-1 protein as an important cellular factor regulating the HCV infection.

O-3

CD32 expression is associated to t cell activation and upregulated by HIV

***Roger Badia**, Ester Ballana, Marc Castellví, Edurne García-Vidal, Maria Pujantell, Bonaventura Clotet, Miguel A. Martínez, Eva Riveira-Muñoz, José A. Esté*

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The overexpression of the gene encoding for the transmembrane protein FCGR2A (CD32a+) has been proposed as a potential marker of HIV+ latently infected cells. We aim to identify the role of CD32 as molecular signature of resting, latently infected cells to facilitate the development of new therapeutic approaches. CD32 and activation markers HLA-DR and CD69 were measured in PBMC and CD4+ T lymphocytes from HIV+ individuals or healthy donors stimulated with or without IL-2 (16 U/ml), PHA (4 µg/ml), αCD3/CD28 or IL-7 (CONC) and/or infected with an HIV-1 NL4-3-GFP virus carrying Vpx. Contribution of CD32+ cells to the viral reservoir was determined in sorted CD4+ T cells from healthy donors infected in vitro or HIV+ patients by qPCR of integrated HIV-1 DNA.

Activation of CD4+ T cells irrespective of the stimuli, upregulated CD32, HLA-DR and CD69. Productive infection of CD4+ T cells increased CD32 expression in CD4 T cells. CD32 expression in CD4+ T cells from HIV+ individuals under antiretroviral treatment indicated that a mean of 85% of cells were CD32+/HLA-DR+. Proviral DNA copies/cell in resting was higher in CD4+/CD32- T cells infected with HIV-1 NL4-3*GFP-Vpx. There were no statistically significant differences in the mean viral DNA copies/cell in CD32+ or CD32- CD4+ T cells from HIV+ individuals under therapy. In conclusion, CD32 expression is a marker of CD4+ T cell activation in healthy donors and HIV+ patients. The viral reservoir lay outside the CD32+ component and therefore HIV-1 latency may not be preferentially associated to CD32+ cells.

O-4

Implications of transgene codon usage optimization in armed oncolytic adenovirus

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Oncolytic adenoviruses (OA) are viruses engineered to selectively replicate and kill cancer cells. In the past few years, multiple preclinical and clinical trials have been developed using these viruses as therapies for the treatment of solid tumors. These studies have shown the safety of the treatment but the need to generate improved OA to increase clinical benefits. Arming OA with transgenes that complement viral activity is an interesting strategy to enhance the therapeutic efficacy. Nevertheless, armed viruses very often interfere with viral replication.

We have recently demonstrated that a balanced codon usage in viral genes is key to guarantee efficient viral fitness. Here we show that transgenes, when expressed in the late phase of adenoviral infection, compete with viral genes for cellular resources, affecting viral activity in a codon usage-dependent manner. Transgenes with high codon usage optimization can lead to an enormous reduction on viral proteins and thus to a considerable impairment of viral fitness. Codon adaptation Index (CAI) and tRNA Adaptation Index (tAI) analysis of HAd5 late genes have allowed us to identify codons whose overuse by transgenes negatively impact viral activity. Thus, analyzing and adapting the codon usage of transgenes to viral genes might be a critical parameter to consider when designing armed OA.

O-5

Hepatitis A virus replication modulates the dynamics of ALIX synthesis in hepatocytes.

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HAV is a unique picornavirus with very special molecular features. Among these features, an unexpected exit pathway through its envelopment into exosomes has been recently described. The mechanism of HAV sorting into the exosome-containing multi-vesicular bodies involves the interaction with the ALIX protein. In the present work using two HAV strains, which differ in their replication capacity, we show that indeed ALIX plays a role in the formation of HAV quasi-enveloped particles in human hepatocytes. Human hepatocytes (Huh7-AI cell line) show an increased level of expression of ALIX over time, and they respond to virus replication by increasing the levels of ALIX mRNA synthesis. Huh7-AI cells infected with a fast-growing strain of HAV (HM175-HP; HP) showed an increase of ALIX expression compared to cells infected with a slow-growing strain (HM175-L0; L0). Additionally, the HP strain showed to be much less sensitive to ALIX silencing than the L0 strain. Analysis of quantitative confocal microscopy of the capsid protein and ALIX, in Huh7-AI cells, revealed a higher proportion of HP capsids rather than L0 capsids co-localizing with ALIX. These results point out to a more efficient interaction of HP capsids with ALIX, which correlates with a higher release of capsid containing-exosomes in the HP population. In conclusion, the fast-growing phenotype of the HP strain respond to a combination of features including a more efficient IRES, an optimized codon usage and a more efficient system to egress from the cell.

O-6

Clinical and virological predictors of response after antiviral therapy interruption in HBeAg-negative chronic hepatitits B

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Background and aims: The possibility of stopping nucleos(t)ide analog (NA) therapy in virologically suppressed HBeAg-negative (HBeAg-) patients has been considered in the recent European Clinical Guidelines. However, the variables predicting a successful discontinuation of NA in this population have not been defined yet. The aim of this study was to investigate the epidemiological, virological and clinical factors associated to a successful NA therapy discontinuation in HBeAg- patients.

Methodology: Prospective study including non-cirrhotic patients with HBeAg- chronic hepatitis B with viral suppression for more than 3 years under NA therapy. Patients underwent a liver biopsy at inclusion to exclude the presence of advanced fibrosis and to analyze total intrahepatic HBV-DNA (iHBV-DNA) and cccDNA by an optimized qPCR. Standard liver tests with viral serum markers (HBV-DNA, qHBsAg, HBcrAg) were performed at 1-month intervals after NA discontinuation.

Results: Twenty patients have been included so far. Most (85%) were male; median age was 52 years-old. The median duration of NA therapy was 9 years, 16 (80%) received tenofovir. Median (IQR) qHBsAg levels at baseline correlated significantly with iHBV-DNA levels (rho = 0.7, $p < 0.01$) but not with cccDNA levels. After a median follow-up of 43 weeks, 16 patients (80%) remained off-therapy and 5 (25%) presented HBsAg loss. ALT (>3UNL) and DNA peaks (>20,000 IU/ml) were frequently observed during follow-up in 40% and 50% of patients, respectively. Patients with HBsAg loss had significantly lower baseline qHBsAg (63 vs 2122 IU/ml, $p < 0.01$) and iHBV-DNA levels (0.04 vs 0.98 copies/cell, $p < 0.01$). There were no adverse events related to therapy discontinuation.

Conclusion: Antiviral therapy discontinuation is feasible in a high proportion of HBeAg-Caucasian patients under NA therapy. Interestingly, low qHBsAg levels are associated with a high chance of HBsAg loss, particularly if associated with low iHBV-DNA (but not cccDNA) levels

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O-7

Dissecting immune evasion strategies during cytomegalovirus infection of antigen-presenting cells

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Through millions of years of coevolution with their hosts, cytomegaloviruses (CMVs) have achieved an exquisite adaptation to their immune system, establishing latent infections with sporadic reactivations. This is largely due to the multiple immune evasion mechanisms CMVs have evolved. Antigen presenting cells (APCs) play a main role in the induction and maintenance of protective T cell immunity; accordingly, CMVs invest many efforts in manipulating the function of these cells. In this study we have analyzed the effect of murine CMV (MCMV) infection on the expression of several immune relevant molecules on the surface of APCs. We found that MCMV markedly depletes a ligand of a key T-cell cosignaling molecule from the cell surface of infected cells. Using UV-inactivated virus we determined that these effects required viral gene expression. The screening of a panel of murine CMV deletion mutants led to the identification of a single viral gene product responsible for the downregulation of this cell surface ligand. Furthermore, we show that when ectopically expressed on APCs, this viral gene product is sufficient to efficiently function by itself. During infection, MCMV promotes the degradation of the cellular protein. Finally, we report that human CMV and other human herpesviruses can employ this immunomodulatory strategy using the same or a different mechanism. A better understanding of the immunomodulatory activities evolved by herpesviruses, and in particular by human CMV, to persist in the host may aid the future design of new strategies to control infections by these pathogens.

O-8

Probing lipid and protein dynamics at individual HIV-1 assembly sites

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Human Immunodeficiency Virus type 1 (HIV-1) assembly at the plasma membrane brings together individual virus components in a process organized by the viral structural protein Gag. The protein distribution at virus assembly sites has been studied in depth by both electron and super-resolution microscopy approaches. However, currently little is known about the dynamics of participating lipids and proteins during assembly, with information only available from studies on model membrane systems. Here we present a novel approach to study the molecular dynamics of *bona fide* virus assembly sites based on a combination of super-resolution STED microscopy and Fluorescence Correlation Spectroscopy (FCS). STED-FCS allows for the investigation of diffusion dynamics of lipids and proteins at subdiffraction scales and has already been applied to study protein dynamics on the surface of individual virus particles[1]. We have adopted STED-FCS to probe lipid and protein diffusion inside and outside virus assembly sites in Jurkat T-cells infected with fully infectious HIV-1. We found trapping of Env and MHC-I proteins as well PI(4,5)P2 and cholesterol inside these sites whereas sphingomyelin or phosphatidylethanolamine did not interact with the Gag assembly sites. Our experiments introduce a powerful tool for future studies of lipid and protein diffusion and interaction dynamics at individual virus assembly sites in a fully infectious virus model. Furthermore, these results indicate that instead of budding from pre-existing lipid domains Gag may instead create its own specialised lipid environment, by selectively trapping lipids at virus assembly sites.

References: [1]Chojnacki, J. *et al.* Envelope glycoprotein mobility on HIV-1 particles depends on the virus maturation state. *Nat Commun. 2017 Sep 15;8(1):545.*

O-9

Chikungunya viral infection favors translation of mRNAs with non-optimal codons at the endoplasmatic reticulum

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The codon sequence of multiple viral RNA genomes, including the emerging and highly expressed Chikungunya virus (CHIKV), Dengue virus and Zika virus, exhibits a low codon adaption index (CAI) predicted to hamper their translation. Whether and how such viral RNAs efficiently outcompete cellular mRNAs remains largely unknown. We combined subcellular fractionation with two genome-wide techniques, RNA-seq and ribosome profiling, to study spatial translational control under CHIKV infection conditions. We found that translation efficiency of CHIKV RNAs at the endoplasmatic reticulum (ER) is dramatically higher than that in the cytosol. Interestingly, whereas the cytosolic translation landscape of cellular mRNAs is not affected by CHIKV infection, the ER translation landscape is modulated and adapted to the codon usage of CHIKV RNAs. At the ER, translation efficiency (TE) of cellular mRNAs with low CAI increases to levels similar to CHIKV RNAs, whereas TE of cellular mRNAs with high CAI decreases. Importantly, this viral-induced alteration of the host translational machinery is exploited by other viruses such as Dengue and Zika. Our results uncover a novel viral strategy to favor translation of the viral RNAs at the ER by a specific and spatial adaptation of the codon usage.

O-10

Quasispecies conservation and complexity of Hepatitis B X gene in different clinical groups of chronic HBV infection.

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Background: The X protein (HBx) plays a key role in hepatitis B virus (HBV) replication and disease progression, thus being a feasible therapeutic target in a curative anti-HBV therapy. The aim of this study is to examine the quasispecies conservation and complexity of the 5'-end of HBx at different clinical stages.

Methods: 54 treatment-naïve HBV-infected patients with negative HBeAg and HBV-DNA >1000 IU/ml were included. They were grouped according to their clinical stages: Chronic hepatitis B, CHB (15), Cirrhosis, CRR (6), Hepatocellular Carcinoma, HCC (17) and Chronic Infection, CI (16). The region between nucleotides (nt) 1255-1611 has been analyzed by Next-Generation Sequencing (MiSeq, Illumina). Conservation was evaluated by calculating the Information Content (IC) of each nt position in a multiple alignment of all haplotypes (unique sequences) obtained. A Wilcoxon test was implemented to compare the deviation of each group from the general IC mean. Quasispecies complexity was defined by Shannon entropy, Gini-Simpson, Mutation frequency and Nucleotide Diversity indices.

Results: A similar pattern of conservation was observed in all groups with 4 hyper-conserved nt regions (nts 1255-1286, 1411-1435, 1519-1543, 1575-1605), as previously published (González et al., WJG 2018). CHB showed higher variability related to the CI group (p-value=0.005). By studying the quasispecies complexity, we observed that CRR and CI showed higher level of Mutation frequency (median [IQR] = 30.4[12.1-51.9] and 17.5[9.5-35.6] for CRR and CI respectively) than the other two groups (median [IQR] = 3.1[2.7-11.6] and 3.6[1.6-8.0] for CHB and HCC respectively). Similarly, CRR group showed higher value of nucleotide diversity (median [IQR] = 0.04[0.02-0.056]) related to CHB and HCC (median [IQR] = 0.005[0.004-0.02] and 0.005[0.003-0.013] respectively).

Conclusions: More complex viral quasispecies were observed in patients at low viral replication state, CRR and CI. Patient groups with higher viral replication rates, i.e. CHB, showed less conservation, thus suggesting that in CRR and CI groups the higher complexity is determined by the presence of highly mutated haplotypes at very low frequency. The identified hyper-conserved regions could be potential targets for gene therapy.

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O-11

HIV-1 Protease Evolvability Is Affected by Synonymous Nucleotide Recoding

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One unexplored aspect of HIV-1 genetic architecture is how codon choice influences population diversity and evolvability. Here we compared the levels of development of HIV-1 resistance to protease inhibitors (PIs) between wild-type (WT) virus and a synthetic virus (MAX) carrying a codon-pair-reengineered protease sequence including 38 (13%) synonymous mutations. The WT and MAX viruses showed indistinguishable replication in MT-4 cells or peripheral blood mononuclear cells (PBMCs). Both viruses were subjected to serial passages in MT-4 cells, with selective pressure from the Pis atazanavir (ATV) and darunavir (DRV). After 32 successive passages, both the WT and MAX viruses developed phenotypic resistance to PIs (50% inhibitory concentrations [IC50s] of 14.6 ± 5.3 and 21.2 ± 9 nM, respectively, for ATV and 5.9 ± 1.0 and 9.3 ± 1.9, respectively, for DRV). Ultradeep sequence clonal analysis revealed that both viruses harbored previously described mutations conferring resistance to ATV and DRV. However, the WT and MAX virus proteases showed different resistance variant repertoires, with the G16E and V77I substitutions observed only in the WT and the L33F, S37P, G48L, Q58E/K, and L89I substitutions detected only in the MAX virus. Remarkably, the G48L and L89I substitutions are rarely found in vivo in PI-treated patients. The MAX virus showed significantly higher nucleotide and amino acid diversity of the propagated viruses with and without PIs ($P = 0.0001$), suggesting a higher selective pressure for change in this recoded virus. Our results indicate that the HIV-1 protease position in sequence space delineates the evolution of its mutant spectrum. Nevertheless, the investigated synonymously recoded variant showed mutational robustness and evolvability similar to those of the WT virus.

O-12

Targeted amplicon deep sequencing analysis: diversity of adenovirus, papillomavirus and enterovirus in raw sewage

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Purpose/Objectives: Current protocols used for the detection of viruses in food/water give indication only on the specific pathogens, but will not provide information on other pathogens or variants.

In this study, Deep Amplicon Sequencing has been applied to evaluate DNA and RNA of emergent viruses in sewage. Enteroviruses (EV) presence and diversity were evaluated during an important outbreak in Catalonia causing neurologic affection to children. Also, human papillomaviruses (HPV), recently described to occur in water and potentially carcinogenic, were studied. Human adenoviruses (HAdV) were investigated as fecal indicators always present in urban sewage.

Materials/Methods: Viral particles present in sewage were concentrated by ultracentrifugation during a monthly sampling from April 2016 to March 2017. Viral concentrates were pooled per season and nucleic acids were extracted. The presence of different viral pathogens was assessed by nested (RT)-PCR. Amplicons produced were deep sequenced using Illumina Miseq 2x300bp.

Results: Around 45 EV species were detected. Interestingly, EV A71 with sequences highly similar to the C1 variants related to the outbreak were observed during this period of high incidence of clinical cases. In addition, a high variety of species resulted from the HPV amplification, being the *Alphapapillomavirus* HPV 6 and 66, described previously as oncogenic with low and high risk respectively, the more abundant types found. HAdV 40, 41 and 31, associated to gastroenteritis, were the HAdV species producing more reads in the urban sewage studied.

Conclusions: In this study, deep amplicon sequencing protocols have been developed for surveillance purposes pointing all viral variants within a given viral family in a single assay. This study supports the idea that sewage can be used for surveillance, delivering additional information for early warning related to circulating emerging strains and outbreak tracing and investigation.

O-13

Vector susceptibility of *Aedes albopictus* and *Culex pipiens* from Catalonia for Zika virus

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The Zika virus (ZIKV) was isolated for the first time in 1947 in the Zika forest (Uganda). It belongs to *Flavivirus* genus (*Flaviviridae* family). The virus is maintained in a zoonotic cycle between mosquitoes and non-humans primates and others mammals in Africa. *Aedes aegypti* mosquito is the main vector to transmit ZIKV in urban populations. *Aedes albopictus*, which is an widespread invasive mosquito species in Europe, is an opportunistic feeder and highly anthropophilic, and some *Ae. albopictus* populations have showed to be vector of several viruses, such as the ZIKV. In the Mediterranean countries, *Culex pipiens* is widely distributed and has been shown to play a critical role in the transmission of West Nile virus (WNV) and Usutu virus (USUV) but it seems not to be able to transmit ZIKV. The last two mosquito species are present in Catalonia with a high activity during summer period. The vector competence in mosquitoes depends on populations and not only on mosquito species. Herein, the vector competence of two *Ae. albopictus* mosquito populations (from Rubí and El Prat de Llobregat municipalities) and one *Cx. pipiens* population (from Cerdanyola del Vallés municipality) for different strains of ZIKV was evaluated. Females were artificially fed with blood containing ZIKV under BSL3 conditions. After a period of 7, 14 and 21 days post exposure to infectious blood, infection, disseminated infection and transmission rates and transmission efficiency were estimated. The virus was able to induce a disseminated infection and transmission in both tested *Ae. albopictus* populations. However, the virus was unable to replicate and be transmitted by *Cx. pipiens*. The results of the present study demonstrated that it is unlikely that *Cx. pipiens* mosquitoes are involved in the transmission of ZIKV but confirm that *Ae. albopictus* mosquito populations established in Catalonia can sustain virus transmission in case of ZIKV introduction. Our results provide helpful information to health authorities in order to establish efficient surveillance and vector control programs for ZIKV.

O-14

Virological and clinical surveillance (2014-2017) of respiratory enteroviruses from paediatric patients attended at a tertiary hospital in Catalonia (Spain)

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Background: Enterovirus (EV) infections are usually asymptomatic or mild, but symptomatic infections can evolve to severe complications. EV-A71 and EV-D68 outbreaks have recently been reported worldwide, sometimes related to severe outcomes. The aim was to describe the EV genetic diversity and the clinical features from paediatric cases attended at a tertiary hospital in Barcelona from 2014 to 2017.

Material and methods: Respiratory tract specimens for laboratory-confirmation of respiratory viruses were collected from paediatric cases with suspicion of respiratory tract infection. Specific EV laboratory-confirmation was performed by specific real-time RT-PCR assays. Partial VP1 coding sequences were additionally sequenced for genetic characterisation.

Results: A total of 376 (7%) from 5,703 cases were EV laboratory-confirmed. Phylogenetic analyses of VP1 (210; 81%) sequences distinguished up to 27 different EV types distributed within EV-A (82; 40%), EV-B (90; 42%), EV-C (5; 2%), and EV-D (33; 15%) species, in addition to 50 (19%) rhinoviruses. The most frequently detected EVs were EV-A71 (37; 45%) and EV-D68 (32; 99%). EV-A71 was highly related to neurological complications (25/39, 63%), of which 20/39 were rhombencephalitis. While, most EV-D68 (28/32, 88%) were associated with low respiratory tract infections (LRTI), but one (3%) with acute flaccid paralysis.

Conclusions: EV-A71 and EV-D68 were the most detected EV. EV-A71 was highly related to neurological disease because of the 2016 rhombencephalitis outbreak in Catalonia. Despite EV-D68 was mostly associated with LRTI, its potential relatedness to neurological diseases makes its monitoring mandatory. The potential neurotropism of all EVs reinforces the need for a better virological and clinical surveillance.

POSTER ABSTRACTS

P-1

Gradual restoration of HCV-specific CD8+ T-cell frequency accompanied by altered phenotype in cirrhotic HCV-infected patients after successful interferon-free therapy

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Background & Aim: Chronic hepatitis C virus (HCV) infection impairs either HCV-specific or other virus-specific CD8+ T-cell phenotypes (Owusu Sekyere, S. *et al*. Front. Immunol. 2015), which can be restored following interferon (IFN)-free therapy. Here, we focused on the potential restoration of HCV-specific CD8+ T-cells in patients with established cirrhosis after successful IFN-free therapy.

Methods: HCV-specific CD8+ T-cells obtained from peripheral blood of 29 cirrhotic HCV-infected patients were analyzed after *in vitro* expansion at the beginning of treatment (BL), at week 4 during therapy (W4) and at weeks 12 and 48 after end-of-therapy (FU12 and FU48, respectively). As controls, Cytomegalovirus- (CMV) and Flu-specific CD8+ T-cells were analyzed in the HCV-infected patients and in 12 healthy individuals.

Results: Frequency of HCV-specific CD8+ T-cells increased gradually from BL to FU48 (P=.0001) in the majority of patients (72 %). The expression of PD-1 and CTLA-4 in this subset remained stable during and after therapy, although Tim-3 expression decreased at FU48 (P=.02).

Interestingly, the frequency of CMV-, but not Flu-specific CD8+ T-cells, was higher in HCV-infected patients than controls even at FU48 (P=.03). Of note, higher PD-1, Tim-3 and CTLA-4 expression was detected on both specific CD8+ T-cells in HCV-infected patients compared to controls (P<.05).

Conclusions: Despite the significant increase in the frequency of HCV-specific CD8+ T-cells in cirrhotic patients after viral elimination, the expression of co-regulatory markers remained altered even in other virus-specific CD8+ T-cells. Therefore, HCV may leave a sustained impact in CD8+ T-cell responses, whose clinical relevance requires further investigation.

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P-2

A hypothesis driven shRNA-based screening identifies aquarius and senataxin as restriction host factors for hepatitis B virus infection

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Hepatitis B virus (HBV) represents an important human pathogen causing both acute and chronic hepatitis. It is estimated that over 240 million people are chronically infected and more than 780,000 people die every year due to complications of HBV, including liver cirrhosis and hepatocellular carcinoma. Currently approved therapies for the treatment of chronic HBV are very effective in suppressing virus replication and viremia but they are not curative because they do not eliminate the long-lasting nuclear episomal DNA form of HBV, known as covalently-closed circular DNA (cccDNA), that re-establishes infection upon interruption of therapy. Despite of our understanding of many aspects of the HBV life cycle, details of the HBV cccDNA biology including the identity and function of cellular and viral factors regulating its formation and homeostasis are poorly understood. The recent identification of the HBV receptor (NTCP), the establishment of a new cell culture HBV infection system (i.e. HepG2-NTCP) and the development of specific and quantitative methods for HBV cccDNA analysis have been instrumental for undertaking cccDNA-focused studies. In this context, our group is pursuing a hypothesis driven loss-of-function shRNA-based screening approach to identify cellular factors regulating cccDNA biogenesis and homeostasis. We hypothesized that proteins belonging to the DNA damage repair pathway (DDR) could regulate the formation and/or maintenance of cccDNA. To test this hypothesis we performed a lentivirus-driven shRNA screening targeting a total of 80 different DDR proteins and we identified 10 DDR factors that regulate positively or negatively the HBV infection. Two of those proteins, Aquarius (AQR) and Senataxin (SETX), were subsequently validated in independent experiments as factors restricting the HBV infection. Silencing of AQR or SETX led to an increase infection efficiency that was characterized by higher intracellular levels of HBV cccDNA, HBV mRNA and HBV core protein as well as an increased HBe antigen accumulation in the supernatant of the infected cells. Moreover, silencing of AQR or SETX did not significantly affect the expression levels of the HBV receptor, NTCP, suggesting that the efects observed were probably not due to an increased entry of HBV into target cells. Collectively these results are compatible with AQR and SETX restricting early steps in the HBV life cycle, downstream HBV entry, afecting cccDNA accumulation.

P-3

Evaluation of a novel microfluidic nanoparticle-based immunoassay method (Virotrack Dengue Acute) for detection of dengue NS1 antigen in travelers

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Dengue is the most important arbovirus and the fastest growing vector borne disease worldwide. A rapid diagnostic test is useful to improve patient management and for arbovirus surveillance programs.

We aimed to evaluate a new rapid and semi-quantitative microfluidic dengue NS1 immuno-magnetic agglutination assay (IMA) based on aggregation of magnetic nanoparticles detected by an electronic reader (Virotrack Dengue Acute and Blubox, Blusense diagnostics). The assay is a simple rapid test that provides results in 10 minutes.

A panel of 135 acute serum samples characterized by specific real-time PCR (74 dengue positive samples including the four serotypes, 25 positive chikungunya samples, 26 positive zika samples, 5 malaria samples and 5 negative samples.) was tested with ViroTrack Dengue Acute and with two available NS1 detection assays: SD Dengue NS1 Ag ELISA and NS1 Dengue Duo rapid test (immunochromatographic method).

ViroTrack Dengue Acute showed a sensitivity of 91.9% and specificity of 98.4%, only slightly lower values than the results obtained by SD Dengue ELISA NS1 (97.2% and 100% respectively). SD Dengue Duo showed a high specificity (100%) but a lower sensitivity than the other assays (68.1%). ViroTrack Dengue Acute is a sensitive and specific assay for dengue NS1 detection. It provides faster results than the ELISA method and a better performance than the rapid immunochromatographic tests. ViroTrack Dengue Acute could be a valuable tool to rapidly diagnose dengue in returning travellers from endemic countries.

P-4

Improvement of the diagnosis of HCV and HIV infection through alternative strategies in people who inject drugs

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Background: The early diagnosis of HCV and HIV in people who inject drugs (PWID) is a challenge because their access to the health system is limited. We developed an alternative screening strategy in PWID who attend harm reduction centers (HRC) in Catalonia with the aim of (i) estimating the prevalence of HCV and HIV (ii) evaluating the self-knowledge of the infection status and (iii) epidemiologically characterizing circulating viruses.

Material & Methods: A cross-sectional study among 410 active injectors in four HRC of Barcelona province was performed. Bio-behavioral data was collected. Rapid tests for the detection of HCV antibodies (TürkLab) and HIV antigen/antibody (HIV Combo test, Alere) were used. In parallel, HCV RNA was detected from dried blood spots (DBS) with an *in-house* assay (96.1% sensitivity and 100% specificity according to a field validation in PWID). HIV infection was confirmed in plasma specimens by routine serological testing (VITROS, Ortho-Clinical Diagnostics). The HCV NS5B region and the HIV protease/retrotranscriptase region were amplified form DBS and plasma, respectively, sequenced and subjected to phylogenetic analysis with reference sequences.

Results: The participants were 85.4% men, with an average of 40.5 years of age and of 17.7 years of injection. 19.0% were recent injectors (≤ 5 years) and 28.0% were immigrants (61.7% were from Eastern Europe). The HCV seroprevalence was 79.8% and the prevalence of active infection was 58.5%. Among the latter, 38.2% of recent injectors were unaware of their disease (9.5% in non-recent injectors). Regarding the country of origin, 20.9% of immigrants were unaware of their disease vs. 11.3% in native population (p<0.001). The prevalence of HIV and HCV/HIV coinfection were significantly lower in immigrants vs. native population (11.2 vs. 28.5%, and 16.4 vs. 28%, respectively). The HCV subtypes found were mainly 3a (38.7%) and 1a (36.5%), and B (81.3%) for

HIV. Out of 181 participants, 49 belonged to HCV transmission clusters. Among participants with an HIV diagnosis and detectable viral load (n=16), no cluster was identified.

Conclusions: Decentralized screening represents a simple and feasible strategy to improve the rate of diagnosis and self-knowledge of HCV and HIV in PWID who attend HRC, as well as to characterize these epidemics.

P-5

Molecular characterization of HCV resistance-associated substitutions after interferon-free treatment failure by massive parallel sequencing

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Introduction: At present, more than 60.000 chronically Hepatitis C infected patients have started Direct-Acting Antiviral (DAA) Interferon-free treatments in Spain. It has been estimated that around 2% will fail and viral resistant-associated substitutions (RASs) are usually selected, thus conditioning future therapeutic options due to the cross-resistance with inhibitors of the same family. In this scenario, the best accurate retreatment strategy depends on the type, frequency and how RASs are combined in the same genome. The aim of the study is to detect RASs among treatment failure patients, the frequency at which they are found and their combination in the same genome using massive parallel sequencing.

Methods: More than 160 treatment failure patients were recruited from 29 Spanish hospitals included in the National HCV Strategic Plan with collaboration of CIBER of Liver and Digestive Diseases (Ciberehd). All serum samples were genotyped and RASs were analyzed by Next Generation Sequencing (NGS).

Results: The vast majority of patients got a relapse of viral infection once several weeks after stopping DAA treatment. 90% of the patients show RASs in at least one analyzed regions (NS3, NS5A and/or NS5B). The subtype distribution among all treatment failure samples was 53% G1b, 16% G1a, 17% G3a, 9% G4d, 1% G2j, 1% G2c, 1% G4a and 2% mixed infections. The most prevalent RAS were detected in positions 80, 122, 155 and 168 in NS3 region, positions 28, 30, 58 and 93 in NS5A region, positions 159 and 316 in NS5B region. Moreover, resistance substitution S282T, which has been directly associated with resistance to Sofosbuvir (SOF), despite causing a dramatic viral fitness decay in cell culture experiments, has been detected in 4% of SOF treated patients. The combination Q80R + D168E and L31M + Y93H, have been shown in 6.3% and 16.3% of G1b patients, respectively. Among all analyzed samples, 18% of patients did not show any RAS despite relapse of viral load in plasma.

Conclusion: The extensive characterization of the spectrum of substitutions by massive parallel sequencing is an essential tool to identify resistance-associated substitutions, combination of variants in the same sequence and their frequency during direct-acting antiviral based treatment failures. This information will help design the most accurate retreatment option for these patients.

P-6

Could HDV induce variability on HBV? Characterization of HBV quasispecies in HBV/HDV coinfected patients

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Background and aims: In presence of Hepatitis D Virus (HDV), Hepatitis B virus (HBV) is less replicative, but little is known about their mechanism of interaction. Studying the HBV Quasispecies (QS) in HBV/HDV infection could provide valuable information in this sense, but it has not been analysed up today. The aim of this study was to evaluate the complexity of HBV QS in serum samples from chronic HBV/HDV infected patients (HDV superinfection) and compare it with the viral QS complexity in chronic HBV-monoinfected patients either in the phase of chronic infection (CI) or chronic hepatitis (CHB).

Material and Methods: A total of 24 untreated patients were included: 7/24 (29.2%) with HBeAg-negative chronic HBV infection (previously defined as “inactive carrier”, CI), 8/24 (33.3%) with HBeAg-negative chronic hepatitis B (CHB) and 9/24 (37.5%) patients with chronic HDV superinfection. A serum sample from each patient was collected and tested for HBV DNA (COBAS 6800, Roche Diagnostics) levels. HBV QS complexity and genotype were analysed in the 5’ region of the HBV X gene (nucleotides 1255-1611) by Next-generation sequencing (MiSeq, Illumina, USA). Viral QS complexity was evaluated by using the number of haplotypes (nHpl) and the number of mutations (nMuts) as incidence-based indices, the Hill numbers of order (q=1 and q=2) as abundance-based indices and the mutation frequency (Mf) and nucleotide diversity (Pi) as functional indices.

Results: HBV patients with chronic hepatitis showed higher median HBV-DNA levels [5.4 (3.5-7.9) logIU/mL] than HDV superinfection [3.4 (3-7.6) logIU/mL] ($p<0.05$) and chronic infection [3.2 (2.3-3.5) logIU/mL] ($p<0.01$) patients. By characterizing the viral genotype in the HBV genome region analyzed, we observed that 15/24 patients (62.5%) presented a complex mixture of genotypic variants, mostly A/D/C genotypes. HBV QS complexity in CI patients was 1.97 and 2.69 fold higher than in CHB patients for respectively nHpl and nMuts (p-value <0.05) and 3.21 and 3.51 fold higher than CHB in terms of Hill numbers (q=1 and q=2 respectively, p-value <0.05). A similar trend was observed in HBV/HDV superinfected patients (fold related to CHB = 1.38 and 2.56 for nHpl and nMuts and 2.48 and 3.16 for Hill numbers, p-value >0.05). Of note, no differences were observed in the functional-based indices (Mf and Pi) among groups.

Conclusions: For the first time, HBV QS complexity was analyzed in HDV superinfection. The less replicative HBV groups (HDV superinfected and CI) seemed to show a trend to higher complexity than the CHB group, thus suggesting that external factors could influence the higher variability with the prevalence of variants with low fitness. In case of HBV/HDV infected patients, the HDV-related innate immunity activation and the interaction between HDAg and the host’s RNA polymerase II could affect the HBV genetic diversity. Further studies with larger cohorts of patients are required to confirm the HBV QS results. In addition, *in vitro* phenotypical assays could be useful to understand the mechanisms of interaction between HDV and HBV QS and how it could influence HBV replication.

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P-7

Evaluation of sperm wash techniques for the elimination of Zika Virus in semen

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Zika Virus (ZIKV) is an arbovirus mainly transmitted throught the bites of infected *Aedes* mosquitoes, although sexual and mother-to-child transmission have also been described. Zika virus can induce microcephaly and other anomalies in the fetus when women are infected during pregnancy. Persistance of ZIKV in seminal fluid has been documented for up to 6 months after the onset of symptoms. This has important implications for assisted reproduction (AR) techniques in patients that have travelled to endemic areas.

We aimed to evaluate the usefulness of sperm washing techniques, commonly used in AR for other viruses such as HIV, for the elimination of ZIKV in semen samples.

A ZIKV strain from the 2016 epidemic isolated from a ZIKV induced miscarriage was inoculated into semen samples. Three different dilutions mimicking ZIKV in clinical samples as well as negative control samples were used. Two different techniques, a standard sperm wash and a double wash-gradient based protocol, were evaluated. The different fractions obtained were analyzed by specific real-time RT-PCR for ZIKV and by cell culture. ZIKV was detected by RT-PCR in all fractions analyzed and in, addition was isolated from samples obtained in the standard sperm wash protocol.

These results indicate that standard sperm washing techniques might not be useful to eliminate ZIKV from semen samples, at least when the virus is present in high amount.

P-8

Dynamics of HIV-1 reservoir decay in early-treated individuals

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Background: The establishment of the viral reservoir is a key limitation to achieve an HIV/AIDS cure. Different studies show that initiation of combination antiretroviral therapy (cART) within the first 6 months after infection (early-treated patients) limits the size of the viral reservoir. However, these studies on early-treated patients reflect controversial results regarding the duration of cART needed before the viral reservoir is stabilized. This factor is pivotal in the design of therapeutic interventions aiming to achieve a functional cure.

Objective: In this longitudinal study we characterized the decay dynamics of the HIV reservoir in early-treated individuals, and determined the timespan required to relative stabilization of the viral reservoir after treatment initiation.

Methods: This ambispective study comprises 21 patients who initiated an integrase inhibitor-based cART treatment within 102 days since estimated time of infection. Regular blood sampling was performed during 4 years. HIV-1 reservoir measurement was based on total HIV-DNA quantification in peripheral CD4+ T-cells by droplet digital PCR (ddPCR). Individual dynamics were adjusted to average global dynamics using a statistical linear mixed model.

Results: The viral reservoir was already well-established within the first 3 months of infection, and it showed two-phase global decay dynamics. We observed a total HIV-DNA reduction in 1.39 $\log_{10}$ units after 4 years under treatment and our mathematical model predicted that, in our study population, the stabilization of the viral reservoir is reached after 1.7 years on cART. Additionally, we observed a positive correlation between HIV-DNA levels and plasma viremia before cART initiation and viral reservoir size at the relative setpoint.

Conclusions: In early-treated populations with similar characteristics to our study population, therapeutic interventions with a potential effect on the HIV-1 reservoir size should be introduced at least 1.7 years after cART initiation, when the viral reservoir is already stabilized, to avoid misinterpretation of the results.

P-9

Qsutils: an r package to study viral quasispecies complexity with ngs data

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Summary: RNA and DNA viruses that replicate by low fidelity polymerases generate viral quasispecies, a collection of closely related viral genomes. This variability contributes greatly to the adaptive potential of the virus. We define complexity of a viral quasispecies as the intrinsic property that quantifies the diversity and frequency of haplotypes, independently of the population size that contains them. Quasispecies complexity can describe viral behaviour by predicting viral disease progression and/or response to treatment; hence, it has an obvious interest for clinical reasons. The complexity can be estimated through diversity indices, which may be classified as incidence-based, focused on the number of observed entities irrespective of their abundances; abundance-based, indices that take into account the observed or estimated abundance of each entity and; functional, that are computed on differences among traits of the observed entities. Part of the diversity indices are adapted from ecology.

We developed and R package, that we have named "QSutils", intended for use with quasispecies data obtained by next-generation sequencing (NGS) of highly mutated viral populations. QSutils offers a set of utility functions for viral quasispecies analysis, there are three main types: (1) data manipulation and exploration: functions useful for converting reads to haplotypes and frequencies, repairing reads, intersecting strand haplotypes, and visualizing haplotype alignments; (2) diversity indices: functions to compute diversity and entropy, in which incidence, abundance, and functional indices are considered; (3) data simulation: functions useful for generating random viral quasispecies data.

Availability: https://github.com/VHIRHepatiques/QSutils

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P-10

The influence of warthog fecal microbiota transplantation on susceptibility of domestic pig to African Swine Fever

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Background: African Swine Fever (ASF) is a devastating and highly contagious disease that, in the acute form, results in 100% mortality in naïve pigs. Warthogs and bush pigs from Africa are known as reservoirs to ASFV exhibiting no clinical signs, African indigenous pigs are less susceptible to specific ASFV genotypes infection than improved international breeds. Here we examined how warthog fecal microbiota transplantation (FMT) to domestic pig affects host responses systemically to ASF susceptibility.

Material and Methods: 48 piglets (3-weeks-old) were divided into four groups. All groups were orally inoculated for three consecutive days with PBS (Group 1), with a solution of domestic pig feces (Group 2), or with a solution of warthog feces collected from Barcelona zoo (Groups 3 and 4), group 4 was previously treated with a non-absorbing cocktail of antibiotics. Fecal samples were collected at 0, 8, 15, 22 and 30 days post FMT. QIIME software was used to analyze microbial composition through 16s rDNA sequencing. Six animals per group were moved to the CReSA´s BSL-3 facility on 30dpfmt and challenged with ASFV. Pigs from groups 1 and 3 were challenged with virulent E75 strain (10^4 HAU), groups 2 and 4 were challenged with attenuated E75CV1 strain ($3.3x10^4$ PFU). Temperature and clinical signs were recorded daily. Virus titration was measured through qPCR in serum, nasal and rectal swabs on 0, 4, 7, 13 and 24 days post challenge (dpc). Specific antibody level was checked in sera.

Results: Pigs inoculated with E75 showed acute clinical signs. After inoculation pigs from group 2 developed consistently moderate/high fever from 17dpc to 24dpc (3/6), highly viremia on 24dpc (4/6), higher antibody (4/6); group 4 showed slight/moderate fever from 17dpc to 23dpc (1/5), highly viremia on 24dpc (1/5), reduced nasal shedding and antibody (1/5). Microbiota composition analysis from feces showed no statistical differences among treatments at α-diversity and β-diversity at 8, 15dpfmt, while group 4 showed a tendency (p= 0.157) of higher diversity.

Conclusions: Although no differences were found on microbiota composition among treatments, 80% of pigs from group 4 showed no fever, no viremia and reduced virus shedding after challenge. Furthermore, group 3 (5/12) and 4 (6/12) produced more IgM against PCV2 on 4dpft than group 1(3/12) and 2 (1/12). which suggests a potential beneficial role of warthog FMT on domestic pigs, not only against ASFV, also other diseases. The mechanisms exert by the FMT still need to be elucidated.

P-11

Human astrovirus (hastv) alters the intestinal epithelium barrier

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Astroviruses are non-enveloped icosahedric small viruses with a positive-sense single-stranded RNA genome. Human astroviruses (HAstV) are one of the leading agents of acute viral gastroenteritis in children worldwide. The infection by HAstV causes abdominal pain and vomiting. Although its impact on human health is obvious, the mechanism by which HAstV cause diarrheal syndrome is unknown. The aim of the present study was to know how HAstV destabilizes the intestinal epithelial barrier that results in changes in the water fluxes across the epithelium. Caco-2 cells (ATCC; passages 21-32) were seeded on *transwell* inserts (0.4 µm, Corning) and formed a stable barrier after 21 days. The barrier function was estimated from measurements of transepithelial electrical resistance (TEER) and FITC-dextran (4 kDa) permeability while its integrity was evaluated by determining the abundance of tight-junction proteins like zonula occludens-1 (ZO-1), β-catenin and occludin by Western blot. The expression of several ion channels such us cystic fibrosis transmembrane conductance regulator (CFTR), chloride-bicarbonate exchangers SLC26A3 (DRA), Na+/H+ exchangers (NHE2 and NHE3), epithelial sodium channel (ENaC-γ) as well as the expression of aquaporins (1,3 and 4) were analyzed by real time PCR 24 h after inoculation. HAstV infection decreases 30% the TEER at 28 h after infection ($P<0.05$) which is consistent with a lower abundance of tight-junction proteins (near 60% of E-cadherin and occludin and 35% of ZO-1 abundance, all $P<0.05$). HAstV infection increased more than 3-fold the expression of DRA, NHE2, NHE3 and EnaC-γ and 1.7-fold that of CFTR (all, $P<0.05$). HAstV infection did not modify the expression of aquaporins. HAstV infection decreased epithelial permeabilty by lowering the abundance of tight-junction proteins. Moreover, infection increased the expression of the sodium and chloride channels.

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P-12

Cytomegalovirus-encoded CD48 homologs, a novel class of immunevasins

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Cytomegaloviruses (CMVs) deploy multiple strategies to elude the host immune response. Some of these strategies are mediated by viral genes originally acquired from their hosts. CD48 is a GPI-linked molecule expressed on most hematopoietic cells that contains an ectodomain composed by an N-terminal immunoglobulin (Ig) variable-like (IgV) domain followed by an Ig constant-like (Ig-C2) domain. Via its IgV domain, CD48 recognizes the cell surface receptor 2B4. Ligation of 2B4 by CD48 leads to signal transduction events that regulate target cell lysis by NK cells and CTLs. Previous work from our group led to the identification of several CD48 homologs (vCD48) encoded by the genomes of different viruses, including cytomegaloviruses. Here, we present a detailed characterization of the three vCD48s of owl monkey CMV, A43, A44, and A45, which derive from a common host CD48 ancestor by two duplication events. We show that these molecules are highly glycosylated type I transmembrane proteins with unique features, differing in structure, biochemical properties and cellular localization. Among them, only A43, the viral CD48 that exhibits the highest amino acid identity with CD48, recognizes host 2B4, with the two other vCD48s having evolved to create alternative binding specificities. Consistent with these observations, sequence comparisons and molecular modeling of the N-terminal Ig domains of these vCD48s evidence that just A43 closely mimics CD48. Interestingly, A43, a soluble protein released from the cell after being proteolytically processed through its stalk region, also interacts with human 2B4. Indeed, surface plasmon resonance assays revealed that this viral molecule binds human 2B4 with higher affinity and remarkable slower dissociation rates than human CD48. Accordingly, we demonstrate that purified soluble A43 is capable to interfere with 2B4-mediated human NK cell adhesion to target cells and drastically reduce NK cell cytotoxicity. Thus, our findings emphasize the relevance of the CD48:2B4 axis in antiviral responses and place viral CD48 homologs as a new class of immunevasins.

P-13

Middle East Respiratory Syndrome coronavirus (MERS-CoV) vaccination efficacy in a direct-contact transmission model in llamas

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Background: Middle East Respiratory Syndrome (MERS) is caused by MERS-coronavirus (MERS-CoV), and dromedary camels are the main reservoir of the virus. MERS-CoV and related viruses are endemic in these animals in East Africa and Middle East. Thus, animal vaccination to prevent virus shedding might be a convenient way to prevent zoonotic transmission. However, since working with dromedaries under biocontainment environment is difficult, other camelid species may act as surrogates. In this study, the efficacy of a recombinant S1-protein vaccine against MERS-CoV in a direct-contact transmission model in llamas was explored.

Methods: The experiment was conducted in two separate boxes. In box 1, a group of llamas (n=3) were intranasally inoculated with 107 TCID50 MERS-CoV Qatar (2015) strain. At 2 days post-inoculation (dpi) naïve llamas (n=5) were put in contact with challenged llamas. In box 2, one group of llamas (n=5) were primo vaccinated with 35 µg of a recombinant S1 protein, and with 50 µg (second immunization) 3-weeks later, both by intramuscular administration. Two weeks after the booster, vaccinated llamas were housed in the same box with MERS-CoV inoculated llamas (n=3), following the same schedule indicated for box 1. Virus nasal shedding was monitored by RT-qPCR during all the experiment, and sera was collected at immunizations, day of inoculation and weekly after challenge for seroneutralization assays and anti-MERS-CoV S1 protein antibody titres. Animals were euthanized 3-weeks after infection.

Results: Intranasally infected llamas from both boxes shed virus for a period of 2-weeks. In-contact naïve llamas from box 1 got infection as soon as 4-5 days after contact, with similar viral loads and periods of shedding observed in the inoculated animals. In box 2, inoculated llamas found the same infection pattern than those from box 1, but only one out of five vaccinated llamas got infection levels comparable to non-vaccinated in-contact animals. Sera from the S1-vaccinated llamas showed virus neutralization and antibodies against S1 capacity already at 3-weeks after the first immunization.

Conclusions: (i) The in-contact llama model was very efficient for transmission of MERS-CoV between animals, representing a more natural route of infection, (ii) the vaccine efficacy was proved by using the animal-to-animal MERS-CoV transmission model.

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P-14

Human metapneumovirus: are the new duplications within the *G* gene responsible for doubling its prevalence?

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Background: HMPV is an important aetiologic agent of respiratory tract infection (RTI). This virus belongs to *Pneumoviridae* family and is classified into genotypes A and B, which generally alternate in predominance biannually. G protein is one of the major envelope glycoproteins, and the most used for genetic characterisation.

Methods: Respiratory specimens from patients with RTI-suspicion at Hospital Universitari Vall d'Hebron (Barcelona, Spain) were collected from October 2014 to May 2018 for laboratory-confirmation of respiratory viruses. Partial *G* gene from all laboratory-confirmed HMPV was sequenced for molecular characterisation with MEGA v6.0.

Results: A total of 29,556 specimens (21,926 patients) were collected, of which 761 (2.6%) samples (729 patients, 3.3%) were HMPV laboratory-confirmed. HMPV prevalence increased from 2.4% to 5.0% throughout the seasons, alternating the predominant genotype (HMPV-B in 2014-2015; HMPV-A in 2015-2016; 50% each in 2016-2017 and HMPV-A in 2017-2018). Novel HMPV-A variants carrying a 180-nucleotide and 111-nucleotide duplications in the *G* gene were characterised, whose prevalence also increased from 6% to 93%. Moreover, these duplications resulted on the acquisition of new potential O-glycosylated sites, but no new potential N-glycosylation sites.

Conclusions: HMPV has doubled its prevalence in the population of a tertiary hospital in Barcelona (Spain). The expected shift of predominance from HMPV-A to –B during the last season surely did not occur due to the significant increase of HMPV-A in the last season, especially of those viruses carrying *G* gene duplications. These duplications might be an evolutionary advantage, probably contributing to the evasion of the host immune response.

P-15

Lack of hepatitis E transmission during a hepatitis A outbreak exclude in men who have sex with men

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Background and Aims: Hepatitis A spreads through the faecal-oral route, via contaminated food or water or through person-to-person contact, including sexual contact. Men who have sex with men (MSM) are at high-risk of hepatitis A virus (HAV) infection due to elevated number of sexual partners and risky sexual behavior, mostly unprotected oral sex. In Europe, hepatitis E virus (HEV) transmission is mainly zoonotic. However, HEV shares HAV transmission route since it is eliminated in faces. The aim of this study was to investigate the presence of HEV during a hepatitis A outbreak mainly affecting MSM in Barcelona.

Methods: We prospectively analyzed all cases of acute hepatitis A diagnosed in our hospital between January 2017 and July 2018. We evaluated demographic data, risk factors, clinical symptoms, sexual orientation and additional STD. We collected serum samples of the patients during the acute hepatitis A (AHA) and determined both HEV IgG and IgM (Reconwell-Mikrogen GmBH) and HEV-RNA by RT-PCR.

Results: One hundred and two patients were diagnosed of AHA. Sixty-nine (68%) were MSM, 75% of whom had sexual risk behavior and 46% had suffered previous STD: Syphilis (n=19), Chlamydia trachomatis (n=3), Neisseria gonorrhea (n=9), hepatitis B (n=5) or HIV (n=12). We collected serum from 85 (83%) patients. Six (7%) patients were HEV-IgG positive (no differences between MSM (n=4; 7%) vs no-MSM (n=2; 8%)); two were HEV-IgM positive, one MSM, whereas HEV-RNA was negative in all samples. Elisa Test had both a 98.9% sensitivity and a 98.5% specificity. Other viral IgM were also positive during AHA as Ebstein-Barr Virus, parvovirus-B19, Citomegalovirus or Herpes virus 6. Noteworthy, 86% of the patients had an increase in gammaglobulin proportion (median 23.4), also 86% had elevated total IgM and 75% showed any positive (>1/80) autoantibodies such as antinuclear (ANA) or smooth muscle (SMA).

Conclusions: HEV-IgG prevalence in our cohort was similar to the general population in our area and there were no differences between MSM and no-MSM. Importantly, HEV-RNA was negative in all samples including both patients with positive IgM. These results support that despite the HEV elimination by feces, the HEV sexual transmission in Europe is unlikely. In fact, positive HEV IgM positivity may be explained by a high no selective IgM production during AHA.

P-16

New HCV genotype 1 whole-genome characterization

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Background: Hepatitis C virus (HCV) is a single stranded, positive sense RNA virus that infects an estimated 71 million people worldwide resulting in a global prevalence of around 1-3%. HCV is divided into 7 genotypes and 86 subtypes according to the ICTV. In order to determine HCV subtypes correctly, high-resolution subtyping based on deep sequencing (HRCS) has been implemented at the Vall d'Hebron Research Institute (VHIR-HUVH). This method, with the use of phylogenetic analysis is used to identify the 86 HCV subtypes and additionally can detect new subtypes. Due to its high number of reads (2000-5000) mixed infections in patients with different genotype or subtype can be detected as well. Using this technology, one naïve sample form Equatorial Guinea send from IdISBa, could not be classified in any of the subtypes known. The aim of this study was to characterize the complete genome of this virus and the RASs present at the naïve sample.

Materials and methods: The whole-genome was amplified by RT-PCR-Nested using degenerated primers and random primers to cover 3' end, and Sanger sequenced. The sample was compared to the reference sequences of all subtypes accepted by the HCV classifying committee lead by Prof. Simmonds, and 8 not yet assigned isolates, using a phylogenetic analysis. The profile of resistance associated substitutions (RAS) was studied using deep sequencing of the antiviral targeted amino acid regions (NS3, NS5A and NS5B) using MiSeq (Illumina) platform.

Results: The phylogenetic study and the heatmap analysis couldn't classify the new isolate into any of the existing genotype 1 subtypes. The sliding-windows analysis along the whole-genome ruled out recombination phenomena. Moreover, deep-sequencing analysis has revealed some RAS occurring in the NS3 (D168E and M175L) and NS5A (Y93L) that confer resistance to some direct acting antivirals (DAAs).

Conclusions: This isolate did not belong to any of the existing genotype 1 subtypes. Three RASs (two at NS3 and one at NS5A) have been found to be naturally present in the genome, which could make the virus resistant to several DAAs.

P-17

Co-localization of Middle East respiratory syndrome coronavirus (MERS-CoV) and dipeptidyl peptidase-4 in the respiratory tract and lymphoid tissues of pigs and llamas

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Background: Middle East respiratory syndrome coronavirus (MERS-CoV) threaten animal and human health. The present study investigated for the first time the co-localization of the MERS-CoV and its receptor dipeptidyl peptidase-4 (DPP4) across respiratory and lymphoid organs of experimentally MERS-CoV infected pigs and llamas by immunohistochemistry (IHC). Also, scanning electron microscopy (SEM) was performed to describe the ciliation integrity of respiratory epithelial cells in both species.

Materials and Methods: Two-month-old pigs and 6 to 8-month-old llamas were intranasally inoculated with 10^7 50% tissue culture infective dose of MERS-CoV. Four pigs were euthanized on day 2 post-inoculation (p.i.), and 4 animals of each species were sacrificed on day 4 p.i. Finally, 6 pigs and 4 llamas were euthanized on day 24 p.i. Necropsies were performed and the co-localization of the MERS-CoV/DPP4 across respiratory and lymphoid organs of pigs and llamas was analyzed by double IHC. Formalin-fixed samples of nasal turbinate, trachea and lung were also used for SEM studies.

Results: In pigs, on day 2 p.i., MERS-CoV/DPP4 co-localization was detected in epithelial cells of medial turbinate and bronchus-associated lymphoid tissue. On day 4 p.i., the virus/receptor co-localized in frontal and medial turbinate epithelia and cervical lymph node in pigs; however, many infected-cells did not display DPP4 in their surface. Infected-epithelial cells were distributed unevenly through the whole nasal cavity and cervical lymph node in llamas. MERS-CoV viral nucleocapsid was mainly detected in upper respiratory tract sites on days 2 and 4 p.i. in pigs and 4 p.i. in llamas. While pigs showed severe ciliary loss in the nasal mucosa both on days 2 and 4 p.i. and moderate loss in trachea on days 4 and 24 p.i., ciliation of respiratory organs in llamas was not significantly affected.

Conclusions: The present work provides evidence that MERS-CoV preferably infects respiratory epithelial cells expressing DPP4 in llamas, supporting that DPP4 is necessary for virus entry. However, the role of DPP4 in regulating virus entry in respiratory organs of pigs and lymphoid tissues of both species may not be sufficient. Although pigs showed a significant expression of DPP4, the number of cells permissive for MERS-CoV in this species was lower than that of llamas. Since a very low amount of MERS-CoV antigen was found in nasal turbinates of pigs, the severe ciliary loss was probably due to a bystander effect.

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