



Societat Catalana
de **BIOLOGIA**

XVI Jornada de Virologia

*Organitzada per la Secció de Virologia de la
SCB*

INSTITUT D'ESTUDIS CATALANS

Carrer del Carme 47
Barcelona

4 de desembre de 2017



**XVI Jornada de Virologia
BCN Virology Meeting 2017**

PROGRAMA

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

AMB EL SUPORT DE:



9:00 h

RECOLLIDA DOCUMENTACIÓ / REGISTRATION

9:15 h

BENVINGUDA / WELCOME

SESSIÓ I/ SESSION I

MODERADOR / CHAIR: Andreas Meyerhans

9:30 h

OPENING LECTURE

HBV minichromosome regulation: a chromatin proteomics approach

Barbara Testoni, INSERM U1052, CNRS UMR-5286, Cancer Research Center of Lyon (CRCL), Lyon, France.

10:00 h

Synonymous recoded env gene induce lethality and loss of protein expression in HIV-1

Ana Jordan-Paiz, AIDS Research Institute IrsiCaixa, Badalona (Spain); Universitat Autònoma de Barcelona UAB (Spain)

10:15 h

Normalization of receptor expression on NK cells is delayed after successful interferon-free therapy in cirrhotic patients with chronic HCV infection

Elena Perpiñán, Liver Unit, CIBERehd, Hospital Clínic, IDIBAPS, Barcelona.

10:30 h

Study of the hepatitis B virus quasiespecies in the protein X transactivating region in patients with progression to hepatocellular carcinoma

David Tabernero, Vall d'Hebron Institut de Recerca, Hospital Vall d'Hebron, Barcelona.

10:45-11:15h

PAUSA I CAFÈ / COFFE BREAK

11:15 h

SESSIÓ II/ SESSION II

MODERADOR / CHAIR: Miguel Ángel Martínez

INVITED SPEAKER

Opportunities and challenges of oncolytic viruses for cancer treatment

Cristina Fillat, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona

11:45 h

Evasion of NK cell responses by a cytomegalovirus encoded decoy molecule that mimics the immune receptor CD48

Pablo Martínez-Vicente, Immunology Unit, Department of Biomedical Sciences, Medical School, University of Barcelona.

12:00 h

Characterization of immunization-induced innate immune changes in protection against a chronic LCMV infection

Graciela Riera, Universitat Pompeu Fabra, Barcelona.

12:15 h

Improvement of the diagnosis of hepatitis C through molecular testing of dried-blood spots in people who inject drugs

Adrián Antuori, Microbiology Service, Germans Trias i Pujol University Hospital and Research Institute (IGTP), Badalona, Spain.

12:30 h

Epidemiological study of patients with hepatitis E virus infection in Catalonia: a translational study of genotype 3 subtypes

Sofia Piriz-Ruzo, Vall d'Hebron Institut de Recerca, Hospital Vall d'Hebron, Barcelona.

12:45 h

Enhancing oncolytic adenovirus anticancer activity by codon usage optimization of armed transgenes

Estela Núñez-Manchón, IDIBAPS, CIBERER, Barcelona, Spain.

13:00 h

Viral load at 15 days after liver transplantation as a good predictor of fast liver fibrosis progression

Meritxell Llorensa, Vall d'Hebron Institut de Recerca, Hospital Vall d'Hebron, Barcelona.

13:15-14:30

DINAR / LUNCH

14:30 h

SESSIÓ III/ SESSION III

MODERADOR / CHAIR: Georgios Koutsoudakis

INVITED SPEAKER

Diagnosis and surveillance of imported arboviral diseases

Miguel J. Martínez, ISGlobal, Hospital Clínic, Barcelona.

15.00 h

Influence of Insect Specific Flaviviruses on the vector competence of *Aedes vexans* (Meigen) and *Culex pipiens* for Rift Valley fever phlebovirus

Lotty Birnberg, IRTA, Centre de Recerca en Sanitat Animal (CRESA, IRTA-UAB), Campus de la Universitat Autònoma de Barcelona, Bellaterra, Spain.

15:15 h

Fecal microbiota characterization in animals with differential susceptibility to African Swine Fever Virus

Florencia Correa-Fiz, Centre de Recerca en Sanitat Animal (CRESA)-IRTA, Campus de la Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain

15:30 h

Metagenomics Public Health Surveillance: From sewage to vegetables

Sandra Martínez-Puchol, Department of Genetics, Microbiology and Statistics, University of Barcelona.

15:45 h

Soraphen A is a potent broad-spectrum antiviral natural product against emerging flaviviruses

Marc Talló, Molecular Virology group, Universitat Pompeu Fabra, Barcelona, Spain

16:00 h

CLOSING LECTURE

Emerging Flaviviruses: The West Nile virus as a model

Juan Carlos Saiz, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid.

16:30 h

SUMMARY OF THE MEETING AND BEST PRESENTATION AWARD

HBV MINICHROMOSOME REGULATION: A CHROMATIN PROTEOMICS APPROACH

Barbara Testoni

INSERM U1052, CNRS UMR-5286, Cancer Research Center of Lyon (CRCL), Lyon, 69008, France

Hepatitis B virus (HBV) chronic infection relies on the persistence of covalently-closed-circular (ccc)DNA in the nucleus of infected hepatocytes. Histones, HBV core (HBc) protein and cccDNA are closely linked together to build a dynamic chromatin structure that translates into different levels of biological activity. Current available therapies control viral replication but are not able to eliminate cccDNA from the nucleus of infected cells. We analyzed different cohorts of long-term treated patients by droplet digital (dd)PCR and chromatin Immunoprecipitation (ChIP) to assess their effect on cccDNA quantity and epigenetic modulation.

While ChIP experiments allow to identify viral and host factors involved in the regulation of HBV cccDNA transcriptional activity, a comprehensive knowledge of cccDNA-network of interacting partners during chronic infection is still lacking.

To this aim, we developed a new approach to identify and characterize the global cccDNA-associated proteome combining cccDNA-specific ChIP to high throughput Mass Spectrometry (MS) analysis, named chromatin proteomics (ChroP). Efficiency of ChroP methodology was confirmed by the high HBc peptide coverage across experiments (>70%). Gene Set Enrichment Analysis indicated that Nucleotide binding processes, RNA splicing and Cell-cycle regulation were the most significantly enriched pathways. Functional silencing of selected network members by RNA interference severely affected viral replication, supporting their crucial role in HBV biology.

Altogether, we developed new methodologies applied to HBV infectious models and human liver specimens to identify host factors and epigenetic signatures essential for cccDNA biological activity. The new generated knowledge will help unveiling possible mechanisms underlying cccDNA silencing or reactivation and revealing potential new therapeutic targets.

SYNONYMOUS RECODED ENV GENE INDUCE LETHALITY AND LOSS OF PROTEIN EXPRESSION IN HIV-1

Ana Jordan-Paiz*, Maria Nevot, Sandra Franco and Miguel Angel Martinez

AIDS Research Institute IrsiCaixa, Badalona (Spain); Universitat Autònoma de Barcelona UAB (Spain)

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The early expressed transinducer Rev is needed for the transport and expression of HIV-1 late genes, such as Env-mRNA. The genetic code is redundant and codon usage differs among different species. Protein expression is affected by the presence of “rare” codons. We aim here to explore the impact of synonymous codon usage on HIV-1 ENV expression and virus replication capacity.

Six codons, AGG, GAG, CCT, ACT, CTC and GGG of HIV-1 env gene were synonymously changed to CGT, GAA, CCG, ACG, TTA and GGA, respectively, generating a new env (recoded-Env).

After transfection, we observed that the mutated virus variant was lethal; no syncytia and no p24 production were observed. WB analysis of Env expression revealed that, in contrast to WT, the recoded-Env mRNA was not being translated although there were not significant differences in Env-mRNA production. Additional mutants were designed to see which mutations were responsible for this phenotype. All new mutant env genes, changed by total number of substitutions or CpG content, generated replicative viruses. Remarkably, mutations in the 3' end were affecting, to a greater extent, the virus phenotype.

We show here that the HIV-1 replication capacity is affected by the codon usage. Our results also indicate that mutations in the 3' coding region of Env can lead to lethality. Ex vivo expression experiments demonstrated that Env-mRNA translation was affected. However, it remains to be elucidated whether the interaction of Env-mRNA and Rev is affected. Overall, our results emphasize the relevance of synonymous substitutions in shaping virus phenotype.

NORMALIZATION OF RECEPTOR EXPRESSION ON NK CELLS IS DELAYED AFTER SUCCESSFUL INTERFERON-FREE THERAPY IN CIRRHOTIC PATIENTS WITH CHRONIC HCV INFECTION.

Elena Perpiñán^{1,2}, Sofía Pérez-Del-Pulgar^{1,2}, María-Carlota Londoño^{1,2}, Zoe Mariño^{1,2}, Sabela Lens^{1,2}, Concepción Bartres^{1,2}, Anna Pla^{1,2}, Patricia González^{1,2}, Mireia García-López^{1,2}, Elisa Pose¹, Xavier Fornés^{1,2}, George Koutsoudakis^{1,2}

¹Liver Unit, Hospital Clinic, University of Barcelona, IDIBAPS, Barcelona, Spain. ²CIBEREHD, Barcelona, Spain.

Background & aim: chronic hepatitis C virus (HCV) infection alters natural killer (NK) cell phenotype, which may be normalized rapidly during interferon-free therapy. Here, we focused on potential changes in NK cells induced by viral eradication in a patient population with established liver cirrhosis.

Materials & methods: NK cells of 22 cirrhotic HCV-infected patients were analyzed by flow cytometry at baseline (BL), at week 4 during therapy (W4) and at weeks 12 and 48 after end-of-therapy (FU12 and FU48). Samples obtained from 12 healthy individuals served as controls.

Results: in HCV-infected patients at BL, W4 and FU12, frequencies of CD56dim and CD56bright cells were decreased or increased, respectively, in comparison to those in healthy controls. At the same time points, frequencies and expression levels of activating NKp30, NKp46, HLA-DR and inhibitory KIR2DL2/L3, NKG2A, CD85j receptors on NK cells were also increased compared to those in healthy controls.

However, at FU48, CD56dim and CD56bright cell frequencies were restored. Moreover, there was a clear decline in the frequency of NKp30+ CD56bright cells and in the frequencies of NKp46+, HLA-DR+, KIR2DL2/L3+, NKG2A+ total NK cells, reaching levels similar to those in healthy controls. Likewise, a similar restoration pattern was observed for the expression levels of NKp46, KIR2DL2/L3 and NKG2A within the NK cells, except CD85j.

Conclusions: normalization of CD56dim and CD56bright cell frequencies and NK cell receptors expression occurs at FU48 and not immediately after viral eradication in cirrhotic HCV-infected patients, suggesting a delayed recovery of NK cells in this patient population.

STUDY OF THE HEPATITIS B VIRUS QUASIESPCIES IN THE PROTEIN X TRANSACTIVATING REGION IN PATIENTS WITH PROGRESSION TO HEPATOCELLULAR CARCINOMA

Maria Francesca Cortese^{1,2}, Marçal Yll¹, David Tabernero^{1,3}, Josep Gregori^{2,3,4}, Rosario Casillas^{1,2}, Mar Riveiro-Barciela^{3,5}, Josep Quer^{2,3}, Rafael Esteban^{3,5}, Maria Buti^{3,5}, Francisco Rodríguez-Frías^{1,3}

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Introduction: Hepatitis B virus (HBV) infection is one of the main causes of hepatocellular carcinoma (HCC). The pleiotropic protein HBx, encoded by the HBV-X gene, plays a key role in carcinogenesis thanks to the transactivating function of its C-terminal end.

Objective: To study the variability of the HBV-X gene 3' end, encoding the transactivating region, in a group of patients with HBV-associated HCC.

Methods: In one serum sample from 9 patients with HBV-associated cirrhosis and HCC (characteristics in table), HBV-X gene 3' end (nucleotides 1600-1884) was analyzed by next generation sequencing (MiSeq, Illumina). Amino acid (aa) sequence of the different haplotypes (unique sequences) obtained was analyzed by aligning them with their respective genotype consensus (determined by phylogenesis).

Results: 737476 sequences were obtained (75088 sequences/sample, interquartile range 33950). The genotyping and the most frequent mutations were reported in attached table. All patients showed the double mutation K130M/V131I and 7/9 (77.8%) the mutation I127T/N/M, both with a median frequency of 100% in viral quasispecies. In 4/9 (44.4%) patients, variants with insertions and deletions (Indels) were detected with a median frequency of 5.3% (0.5-6.7).

Conclusions: K130M/V131I and I127T/N/M were the main HBx mutations observed in our group of patients, whereas variants with Indels were infrequent. A control group including patients with different stages of chronic hepatitis B is necessary to establish the relationship between the observed variants and the progression to HCC.

Funding: Instituto de Salud Carlos III (PI15/00856), co-financed by the European Regional Development Fund (ERDF).

OPPORTUNITIES AND CHALLENGES OF ONCOLYTIC VIRUSES FOR CANCER TREATMENT

Cristina Fillat

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Oncolytic viruses (OV) represent a modality of cancer treatment based on viruses that selectively infect and lyse tumor cells, spread within tumors and activate anti-tumor immunity. Several viruses are being developed as oncolytic viruses through genetic engineering of the viral genome. Some of them are showing clinical promise and a Herpes Virus-derivative was granted marketing approval for intratumoral delivery of non-resectable metastatic melanoma.

In this presentation, we will review the state-of-the art of cancer virotherapy and discuss about the approaches that are being pursued for next generation of oncolytic viruses and their use in combination with other anti-cancer therapies.

EVASION OF NK CELL RESPONSES BY A CYTOMEGALOVIRUS ENCODED DECOY MOLECULE THAT MIMICS THE IMMUNE RECEPTOR CD48

Pablo Martínez-Vicente, Domènec Farré, Elena Gracia-Latorre, Pablo Engel, and Ana Angulo
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In order to ensure their survival, cytomegaloviruses (CMVs) hinder the host immune response through a wide range of tactics. To that end, they encode a large collection of immune modulator genes, some of which derive from their host genomes after being captured at different points during host-virus co-evolution. CD48 is a GPI-anchored protein that contains an ectodomain composed by 2 immunoglobulin (Ig) domains. Via its N-terminal Ig domain, CD48 recognizes the cell surface receptor 2B4. Engagement of 2B4 by CD48 results in the regulation of cytotoxic T lymphocyte and NK cell functions. We have recently reported the presence of a number of CD48 homologs encoded by different CMVs. Here, we present the structural and functional characterization of A43, the viral CD48 that exhibits the highest amino acid identity with host CD48. We show that A43, which is encoded by owl monkey CMV, is a soluble molecule with distinctive structural attributes. Remarkably, A43 maintains the ability to interact with host 2B4, and also binds with exceptional high affinity human 2B4 (h2B4). In fact, our kinetics studies revealed that A43:h2B4 interactions have a K_d at least 3-fold lower and a K_{off} drastically reduced (at least 10-fold lower) as compared with those established between human CD48 (hCD48) and h2B4. Furthermore, we show that soluble A43 is capable to efficiently abrogate hCD48:h2B4 interactions and severely impair 2B4 mediated-NK cell cytotoxicity, thereby representing the first functional virally-encoded CD48 decoy receptor described to date. Finally, the identification of potential CD48 homologs in other large DNA viruses, such as poxviruses, adenoviruses, and some additional herpesviruses, suggests that exploitation of CD48 mimicry might constitute a widespread viral immune evasion strategy.

CHARACTERIZATION OF IMMUNIZATION-INDUCED INNATE IMMUNE CHANGES IN PROTECTION AGAINST A CHRONIC LCMV INFECTION

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Vaccination is an important tool to prevent viral infections by generating virus-specific adaptive immune responses. However, despite the efforts made in the past, there is still no successful vaccine against some important chronic infections, highlighting the lack of knowledge of the specifically required immunological components. During the last years, several studies have called attention to the importance of vaccine-triggered innate immunity inducing a protective pathogen-specific immune response. Moreover, it has been shown that innate immune cells can exhibit a non-specific memory-like behavior, opening the possibility of exploiting the innate immune response for development of efficient vaccines. The objective of the present work is to elucidate novel innate immune mechanisms triggered by a successful immunization that prevent the establishment of a chronic infection. Mice were immunized with a 2x10² pfu of LCMV-Docile strain. 60 days later, immunized and naïve mice were challenged with 2x10⁶ pfu of LCMV-Docile to establish an acute or chronic infection, respectively. Transcriptomes from spleens at early time-points post-challenge were obtained by RNAseq. Gene Ontology analysis revealed a differential innate immune signature in immunized animals, suggesting a specific role of neutrophils in the immunization-induced protection. FACS analysis confirmed the increase of neutrophils but not monocytic and dendritic cells at 24 hours post-challenge. Subsequent work will characterize the mechanisms involved in this rapid recruitment of neutrophils and their relevance in the immunization-conferred protection against a chronic infection. These results might contribute to identify novel vaccine strategies focused on inducing an effective innate immune response against a chronic viral infection.

IMPROVEMENT OF THE DIAGNOSIS OF HEPATITIS C THROUGH MOLECULAR TESTING OF DRIED-BLOOD SPOTS IN PEOPLE WHO INJECT DRUGS

Adrián Antuori¹, Verónica Saludes^{1,2}, Cinta Folch^{2,3}, Núria Ibáñez⁴, Vincent Montoya⁵, Richard Harrigan⁵, Joan Colom⁴, Jordi Casabona^{2,3}, and Elisa Martró^{1,2*}; HepCdetect II Study Group.

HepCdetect II Study Group: Laia Gasulla⁴, Xavier Majó⁴, Noemí González⁶, Sonia Cebrián⁷, Jaume Minguell⁸, Aitor Remírez⁹, Laura Fernández^{2,3}, Rafael Muñoz³, Jordi Hernández¹, Lurdes Matas^{1,2}.

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Background: The collective of people who inject drugs (PWID) is characterized by a high HCV prevalence and a low diagnosis rate. We aimed to identify silent hepatitis C cases and to characterize the epidemic in PWID through the implementation of an alternative one-step screening and confirmatory assay based on HCV-RNA detection from dried blood spots (DBS).

Material & Methods: Cross-sectional study of current injectors attending four harm-reduction centers in the province of Barcelona (n=410). Each participant underwent rapid HCV antibody testing and fingerprick DBS collection. HCV-RNA was assessed using an in-house RT-PCR assay. Plasma was also collected in one center for viral load quantification (Abbott). Next-generation sequencing and phylogenetic analysis were implemented for HCV intra and inter-host variability analyses. Epidemiological and behavioral data were collected.

Results: The HCV seroprevalence (83.9%) was higher than previous estimates. The prevalence of active infection was characterized for the first time in this setting to be 58.6% (33% of participants <30 years old were not aware of their status). The DBS assay was 95% sensitive and 100% specific in comparison with the conventional two-step algorithm (rapid antibody test and viral load assay). Transmission networks and recent infections have been virologically identified.

Conclusions: This one-step diagnosis strategy presents a simple and reliable way of detecting HCV infections among PWID, as a first step to treatment. The implementation of this screening strategy at a national level would facilitate increasing the diagnosis rate and monitoring HCV epidemics in this collective, in order to fulfill the elimination goals set by the World Health Organization.

EPIDEMIOLOGICAL STUDY OF PATIENTS WITH HEPATITIS E VIRUS INFECTION IN CATALONIA: A TRANSLATIONAL STUDY OF GENOTYPE 3 SUBTYPES

Sofia Piriz-Ruzo, Francisco Rodriguez-Frias, Damir Garcia-Cehic, Josep Gregori, Meritxell Llorens, Qian Chen, Maria Eugenia Soria, Celia Perales, Maria Buti, Juan Ignacio Esteban, Mar Riveiro-Barciela, Josep Quer

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Hepatitis E virus (HEV) is a non-enveloped single stranded RNA virus member of the Hepeviridae family and genus Orthohepevirus, which is a common cause of acute hepatitis worldwide.

An epidemiological study from Hospital Universitari Vall d'Hebron (HUVH) HEV Data Base was carried out to study which is the most probable source of infections, risk factors, and diagnosis confidence test based on clinical data. Our results suggest that HEV infection is associated with old age and/or immunocompromised status, and HEV subtyping diagnosis classification should be improved, especially to classify HEV genotypes 3 infected patients. Sliding windows analysis was used to identify a short region of 400 nucleotides able to confidently classify genotype 3 genomes into subtypes.

From the clinical analysis, we identified an HEV food-borne infected patient who got infected in 2012 and developed a long-lasting chronic infection. Surprisingly, this patient had HEV-RNA serum negativization periods. To study whether this patient had got multiple infections or whether it was the result of reactivation of the original infection, pre-treatment and post-treatment samples were compared using Next Generation Sequencing (NGS).

Reactivation of the original infection was demonstrated by phylogenetic and genetic distance studies. This result has important clinical implications, since any patient that develop a chronic infection should be followed up for several months or years with highly sensitive test before assuring that HEV infection has been completely resolved.

This study is a translational study which has a clinical relevance and which opens new lines of research for new future projects.

ENHANCING ONCOLYTIC ADENOVIRUS ANTICANCER ACTIVITY BY CODON USAGE OPTIMIZATION OF ARMED TRANSGENES

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Arming oncolytic adenoviruses (OA) with therapeutic genes is a well-established strategy to increase their antitumor effect. The possibility to express molecules with additional functions provides an opportunity to modulate the tumor microenvironment and maximize the therapeutic output. However, this strategy is challenging since encoding transgenes often attenuates virus activity.

We have recently demonstrated that a balanced codon usage in viral genes is key to adequately exploit cellular resources for efficient viral fitness. We hypothesize that introducing transgenes into the adenoviral genome may modify this balance and contribute to attenuate viral activity.

In order to assess the transgene codon usage impact on viral fitness, we have studied the effects of different codon usages in transgenes, on viral proteins expression and viral production.

Our data suggest that transgenes, when expressed in the late phase of viral infection compete with viral genes for cellular resources affecting viral fitness in a codon usage-dependent manner. Thus, adapting the codon usage of the transgenes to not compete with the virus might be a critical aspect to consider when designing armed OA, and may be used as a tool for maximizing viral fitness.

VIRAL LOAD AT 15 DAYS AFTER LIVER TRANSPLANTATION AS A GOOD PREDICTOR OF FAST LIVER FIBROSIS PROGRESSION

Meritxell Llorens^a, Josep Gregori^{a,d,e}, Cristina Dopazo^f, Francisco Rodriguez-Frías^{b,c,d}, Damir Garcia^{a,d}, Maria Eugenia Soria^a, Celia Perales^{a,d}, Silvia Bergamo^b, Gerardo Ruiz^b, Qian Chen^a, Laura Ordeig^{a,d}, Rafael Esteban^{a,c,d}, Juan Ignacio Esteban^{a,c,d}, Josep Quer^{a,c,d*}, Itxarone Bilbao^{c,f*}

Viral Hepatitis, Liver Unit, Vall d'Hebron Institute of Research (VHIR)-Hospital Universitari Vall d'Hebron (HUVH), Barcelona^a; Liver Pathology Unit, Departments of Biochemistry and Microbiology, HUVH^b, Universitat Autònoma de Barcelona (UAB), Barcelona^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, Hepatobiliopancreatic Surgery and Transplant Unit, HUVH^f.

Background: The recurrence of Hepatitis C virus (HCV) infection after a liver transplantation is about 100% and 20-40% of them develop cirrhosis in a 5 years period. To avoid the fast progression to cirrhosis, antiviral therapy using direct acting antiviral (DAA) is required as fast as possible. However, the immunosuppressant state at which they are subjected to avoid liver rejection, and the clinical instability after the liver transplantation, makes difficult starting an antiviral therapy the first weeks after the liver transplantation without any unforeseen complication. The aim of this study was to evaluate the changes on the quasispecies complexity after transplantation and to find a predictive index of fibrosis evolution at one year post liver transplantation in order to objectively choose the best moment to start the antiviral therapy.

Patients and methods: Baseline and 15th day post liver transplantation samples of 10 patients were processed in the 454/GS-Junior sequencing platform and the viral load was measured. Sequences were filtered using own made software. Samples were compared by phylogenetic analysis, and the variability was analyzed using several complexity measures. Single test, principal component analysis and the adjusted R2 value of each complexity measures was calculated. The association between viral load and fibrosis degree was carried out by ANOVA analysis.

Results: Our results suggest that viral bottlenecking is not a generalized effect after reinfection of the donor liver, homogenization of the viral population after reinfection is not universal, and that complexity measures displayed low adjusted R2 values, which mean low predictive capacity of liver damage evolution at one year post transplantation. Interestingly, the viral load increased significantly between basal and 15th day post liver transplantation samples in patients with advanced stage of fibrosis measured one year after liver transplantation.

Conclusions: The clear association between an increase of viral load at 15th day post and advanced liver disease state (F3-F4) at one year post liver transplantation suggest that the difference between viral loads before and after liver transplantation could be an adequate predictive index of fast liver fibrosis progression.

DIAGNOSIS AND SURVEILLANCE OF IMPORTED ARBOVIRAL DISEASES

Miguel J. Martínez, ISGlobal, Hospital Clínic, Barcelona.

Arthropod-borne viruses (arboviruses) are a large group of viral pathogens transmitted mainly by infected mosquitoes, ticks and sandflies. They have an enormous impact on human health and economy and are distributed around the globe. As an example, dengue, the most important arbovirus and the fastest growing vector-borne disease worldwide, may cause up to 400 million infections annually. The emerging potential of arboviruses is well known and recent examples can be found in the latest outbreaks of chikungunya and Zika viruses in the Americas. The expansion of competent vectors such as *Aedes albopictus* (the tiger mosquito) and the increase of travelers to arboviral endemic areas make autochthonous transmission possible in non-endemic areas, as it has been recently documented in Italy or France. We will review the main clinical, epidemiological and diagnostic features of the most relevant arboviruses, with recent data on the impact of the Zika virus outbreak in our setting, including the persistence of zika in body fluids, pregnancy outcomes and laboratory preparedness. The risk of autochthonous arboviral transmission in Catalonia and the challenges of laboratory diagnosis of arboviral diseases will be analyzed.

INFLUENCE OF INSECT SPECIFIC FLAVIVIRUSES ON THE VECTOR COMPETENCE OF *Aedes vexans* (MEIGEN) AND *Culex pipiens* FOR RIFT VALLEY FEVER PHLEBOVIRUS

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Background: Rift Valley fever (RVF) is a mosquito – borne zoonotic disease caused by the Rift Valley fever phlebovirus (RVFV), which affects mainly domestic ruminants and humans. Due to globalization and climate change, the European continent is threatened by its introduction. Two Catalan autochthonous mosquito species (*Aedes vexans* and *Culex pipiens*) were evaluated for RVFV vector competence. This is the first study that evaluates the transmission of RVFV in a naturally infected *Ae. vexans* population and in artificially infected *Culex pipiens* mosquitoes with an insect specific flavivirus (ISF).

Methods: *Aedes vexans* field collected larvae were transported to the laboratory and reared under summer simulated conditions. A 50% (25/50) natural infection of ISF was observed for the studied *Ae. vexans* population. Laboratory *Culex pipiens* specimens were intrathoracically inoculated with a *Culex* flavivirus (CxFv). Adult females from both populations were exposed to artificial blood-meals doped with the virulent Rift Valley fever phlebovirus strain (RVFV 56/76). Infection and dissemination rates and transmission efficiency were estimated.

Results: In both mosquito populations, RVFV was capable of infecting and disseminating. Infectious viral particles were isolated from the saliva of disseminated specimens showing their capacity to transmit the disease. No significant differences in terms of infection, dissemination or transmission rates were observed between co – infected and non co - infected (ISF/RVFV and CxFv/RVFV) specimens.

Conclusions: Findings demonstrate that ISFs present in Spanish mosquito populations do not seem to affect the vector competence of *Ae. vexans* and *Cx. pipiens* and therefore the potential risk of an autochthonous outbreak in case of RVF introduction to Europe.

FECAL MICROBIOTA CHARACTERIZATION IN ANIMALS WITH DIFFERENTIAL SUSCEPTIBILITY TO AFRICAN SWINE FEVER VIRUS

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African swine fever (ASF) is a devastating swine disease (100% mortality in domestic pigs *Sus scrofa*) against which there is no efficient vaccine. African swine fever virus (ASFV) is a DNA virus derived from a virus of soft tick that can also infect wild swine, such as warthogs (*Phacochoerus africanus*), where infection is asymptomatic. In our group, it was found an extreme susceptibility of specific pathogen free (SPF) pigs to attenuated ASFV strains that provoked none or minor ASF clinical signs in genetically identical conventional pigs. The composition of the microorganisms colonizing the intestine, the fecal microbiota, has demonstrated to be associated to many diseases. The hypothesis was, that together with genetic differences, the microbiota could be play a role in these differential ASFV-susceptibilities. The objective was to unravel the microbiota composition to find out possible differences related to the differential ASFV susceptibility, with the final goal of finding potential candidates to use as probiotics in future therapeutic or vaccine formulations. The microbial composition of feces from (1) African (wild) and (2) Spanish (Zoo) warthogs, (3) European- and (4) SPF- domestic pigs and (5) African indigenous pigs was characterized at different taxonomical levels through 16S massive sequencing, and compared among groups. The alpha diversity was estimated, where SPF-pigs showed the lowest diversity indexes. A core diversity analysis was performed to extract particular taxa present in ASF-resistant animals and absent/diminished in susceptible ones. Our group is currently evaluating if these differences play a role in ASF protection.

METAGENOMICS PUBLIC HEALTH SURVEILLANCE: FROM SEWAGE TO VEGETABLES

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Foodborne illnesses suppose an important public health problem. Among all the microbial agents, viruses are the most important causative agents of food-borne outbreaks. Raw-eaten vegetables are susceptible to cause food-related diseases and the microbiological quality of irrigation water is of importance when considering the risk of contamination. The use of metagenomics to study the viruses circulating in a population gives a promising information for public health surveillance.

The aim of this study was to evaluate by using metagenomics, the viral presence in sewage, different sources of irrigation water (tertiary treated effluents, river water and groundwater) and lab-grown parsley irrigated with fecally tainted river water. To address possible contamination due to sample manipulation or library preparation, parsley plants irrigated with nutritive solution were used as a negative control.

The application of a concentration method based on organic flocculation of viruses using skimmed milk (SMF) in sewage has allowed the detection of many viruses, producing very valuable information on the virome of urban sewage in different seasons. Urban raw sewage consists of the excreta of thousands of inhabitants; therefore, it is a representative sample for epidemiological surveillance purposes and risk evaluation. Most of the viral reads detected in all types of irrigation waters were related to the algae viral family *Phycodnaviridae* (15%), the phage viral families *Myoviridae* (14%), *Siphoviridae* (5%) and *Microviridae* (8%), the plant viral families *Virgaviridae* (10%) and *Tombusviridae* (6%), the fish viral family *Iridoviridae* (7%) and the insect viral families *Dicistroviridae* (7%). Less than 1% of the viral reads were related to viral families that infect humans, including members of the *Adenoviridae* and *Caliciviridae* families. On the surface of the parsley plants, most of the viral reads that were detected were assigned to the *Dicistroviridae* family (41.52%). The high diversity of viral sequences found in the parsley plants suggests that irrigation on fecally-tainted food may have a role in the transmission of a wide diversity of viral families. The study of the metaviromes of sewage, irrigation water and ready-to-eat vegetables can provide important information of public health significance, highlighting the presence of significant viral strains circulating within a population.

SORAPHEN A IS A POTENT BROAD-SPECTRUM ANTIVIRAL NATURAL PRODUCT AGAINST EMERGING FLAVIVIRUSES

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OBJECTIVE: Soraphen A (SorA) is a myxobacterial metabolite that inhibits the acetyl-CoA carboxylase, a key enzyme in lipid biosynthesis. We have previously shown SorA to efficiently inhibit the hepatitis C virus (HCV) by interfering with the formation of the membranous web, the HCV replication site. The aim of the present study was to evaluate the capacity of SorA and analogues to inhibit the important emerging viruses Dengue virus (DENV), West Nile virus (WNV), Chikungunya virus (CHIKV) and Zika virus (ZIKV). All of them induce and depend, as HCV, on membranous web formation.

MATERIAL AND METHODS: SorA antiviral activity was evaluated *in vitro* using DENV, WNV, CHIKV and ZIKV virus derivatives carrying the luciferase gene. Infection studies were performed in HuH7/Scr cell line for DENV and WNV, in BHK21 cell line for CHIKV and in Vero cell line for ZIKV.

RESULTS: SorA potently inhibits DENV, WNV and ZIKV infection at nanomolar concentrations. Obtained EC50 values were 21.8 nM, 20nM and 150 nM, respectively. However, no antiviral activity was detected against CHIKV. Finally, a series of natural and synthetic SorA analogues were tested to increase the solubility of SorA without affecting its antiviral activity.

CONCLUSIONS: The natural product SorA has a very potent antiviral activity against DENV, WNV and ZIKV, constituting a very promising anti-Flavivirus molecule for further development. SorA does not inhibit the Togavirus CHIKV, indicating the unique and exquisite use of cellular lipid metabolism by viruses.

EMERGING FLAVIVIRUS: THE WEST NILE VIRUS AS A MODEL

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Flaviviruses (*Flaviviridae* family) are arboviruses (**arthropod-borne** viruses) that constitute a genus containing more than 50 different species, many of which are important human and animal pathogens (Dengue, West Nile, Usutu, Japanese encephalitis, Yellow fever, Tick-borne encephalitis, or Zika viruses), as they can infect the host nervous system, thus inducing severe neurological diseases that can lead to death. Most flaviviruses are maintained in nature in an infectious cycle that includes transmission between wild animals, which act as their natural host, and insects (mosquitoes and ticks) that act as their vector. These vectors can transmit the virus to other animals and to humans causing zoonoses. Climate and landscape conditions directly affect arthropod populations, density and stability, thus playing a pivotal role in arboviral dissemination and evolution. The flaviviruses genome is constituted by a single-stranded RNA molecule of positive polarity ($\approx 11,000$ nt) flanked by two untranslated regions at the 5' and a 3' ends. The genomic RNA encodes a polyprotein in a single open reading frame that is co- and post-translationally processed by viral and cellular proteases into 10 mature proteins: 3 structural primarily involved in viral particle formation, and 7 non-structural responsible for viral replication and virion assembly. One of the clinical most relevant example of (re)emerging flavivirus is the West Nile virus (WNV). In fact, during recent years WNV has caused a very high mortality in birds, horses, and humans in North America and Europe. Here, using the WNV as the guiding thread, different aspects of flaviviruses pathogenesis and clinical consequences, ecology, and control measures will be summarized, with especial focus on the search for innovative antiviral strategies.

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