Societat Catalana de BIOLOGIA

XV Jornada de Virologia

Organitzada per la Secció de Virologia de la SCB

INSTITUT D´ESTUDIS CATALANS

Carrer del Carme 47

Barcelona

28 de novembre de 2016

XV Jornada de Virologia
BCN Virology Meeting 2016

PROGRAMA

Coordinadores de la Secció i responsables de la coordinació de la Jornada:
Juana DÍEZ i Rosa M. PINTÓ

AMB EL SUPORT DE:

9.00 h
RECOLLIDA DOCUMENTACIÓ / REGISTRATION

9.15 h
BENVINGUDA / WELLCOME

SESSIÓ I / SESSION I
MODERADOR / CHAIR: Juana Díez

9.30 h

Opening Lecture

Mapping host shutoff during influenza viral infection
Noam Stern-Ginossar, Weizmann Institute of Science, UB, Israel.

10.00 h

A novel translational control mechanism involving RNA structures within coding sequences
Jennifer Jungfleisch, Universitat Pompeu Fabra, Barcelona

10:15h

Unravelling biological processes differentially involved in acute and persistent viral infections
Jordi Argilaguet, Universitat Pompeu Fabra, Barcelona

10.30-11h
PAUSA I CAFÈ / COFFE BREAK

SESSIÓ II/ SESSION II.
MODERADOR / CHAIR: Josep Quer

11h

HCV early kinetics and resistance-associated substitution dynamics during antiviral therapy with direct-acting antivirals
Elena Perpiñán, Hospital Clínic, Barcelona

11.15 h

HCV/HIV-1 patients with different stages of liver fibrosis treated with IFN-free DAAs
Sandra Franco, Institut recerca de la Sida, IrsiCaixa, Badalona

11.30 h

Whole-genome characterization of a new hepatitis C virus (HCV) genotype 1 subtype
Laura Ordeig, Vall d'Hebron Institute of Research, Barcelona

11:45 h

Enhancing the diagnosis of active hepatitis C virus (HCV) infection through molecular testing of dried blood spots in community-based centres in Barcelona
Verónica Saludes, Germans Trias i Pujol Health Sciences Research Institute (IGTP), Badalona

12 h

A high-throughput screening of an adenovirus encoded microRNA library identifies adenovirus with enhanced oncolytic activity in pancreatic tumors
Maria Rovira-Rigau, Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona

12:15 h

A43, a CD48 viral homolog with conserved ligand-binding features
Pablo Martínez-Vicente, Departament de Biomedicina, Universitat de Barcelona

12.30 h

The insertion site of HIV affects latency reversal
Guillaume Fillion, Center for Genomic Regulation, Barcelona.

13.00-14:30
DINAR / LUNCH

SESSIÓ III / SESSION III.
MODERADOR / CHAIR: Juan José López-Moya

14.30 h

The P25 gene product of a Spanish isolate of Cucurbit yellow stunting disorder virus acts as RNA silencing suppressor
Mariona Estapé, Centre for Research in Agricultural Genomics (CRAG), Bellaterra

14.45 h

Exploring the presence of hypothetical trans-framed gene products in Sweet potato mild mottle virus (SPMMV)
Maria Reñé, Centre for Research in Agricultural Genomics (CRAG), Bellaterra

15.00 h

FTA® cards: An approach for arbovirus detection
L. Birnberg, Centre de Recerca en Sanitat Animal (CRESA), Bellaterra

15.15-15.45 h
PAUSA

SESSIÓ IV/ SESSION IV.
MODERADOR / CHAIR: Susana Guix

15.45 h

Enterovirus: un "gap" en la vigilancia.
Andrés Antón, Vall d'Hebron Institut de Recerca, Barcelona

16.15h

Astrovirus MLB2 associated with meningitis: case report and exploratory prevalence study
Diem Lam Vu, Grup Virus Entèrics, Universitat de Barcelona.

16.30 h

Norovirus shedding among food and healthcare workers exposed to the virus in outbreak settings
Aurora Sabrià, Grup Virus Entèrics, Universitat de Barcelona.

16.45h

Closing Lecture

Emerging Coronaviruses: lessons from the past and future challenges

Isabel Sola, Departament of Molecular and Cell Biology, Centro Nacional de Biotecnología CNB-CSIC, Madrid.

17.15 h

SUMMARY OF THE MEETING AND BEST PRESENTATION AWARD

MAPPING HOST SHUTOFF DURING INFLUENZA VIRAL INFECTION

Noam Stern-Ginossar, Weizmann Institute of Science, UB, Israel.

Ribosome profiling is an emerging technique that uses deep sequencing to monitor translation in live cells. Studies using ribosome profiling have already provided novel insights into the identity and the amount of proteins that are being produced in cells, as well as novel insights into the mechanism of protein synthesis and translation regulation. In my talk I will discuss how application of ribosome profiling allowed us to explore the mechanisms that are being utilized by Influenza A virus (IAV) to induce host shutoff. We show that viral transcripts are not preferentially translated and instead the decline in cellular protein synthesis is mediated by viral takeover on the mRNA pool. Our measurements also uncover strong variability in the levels of cellular transcripts reduction, revealing that short transcripts are less affected by IAV. Interestingly, these mRNAs that are refractory to IAV infection are enriched in cell maintenance processes such as oxidative phosphorylation. Furthermore we show that the continuous oxidative phosphorylation activity is important for viral propagation. These results advance our understanding of IAV-induced shutoff, and suggest a mechanism that facilitates the translation of genes with important housekeeping functions.

A NOVEL TRANSLATIONAL CONTROL MECHANISM INVOLVING RNA STRUCTURES WITHIN CODING SEQUENCES

Jennifer Jungfleisch[1], Danny D. Nedialkova[2, 3*], Ivan Dotu[4*], Katherine E. Sloan[5], Neus Martinez-Bosch[6], Lukas Brüning[5], Emanuele Raineri[7], Pilar Navarro[6], Markus T. Bohnsack[5,8], Sebastian A. Leidel[2, 3, 9], Juana Díez[1†]

The impact of RNA structures in coding sequences (CDS) within mRNAs is poorly understood. Here we identify a novel and highly conserved mechanism of translational control involving RNA structures within coding sequences and the DEAD-box helicase Dhh1. Using yeast genetics and genome-wide ribosome profiling analyses we show that this mechanism, initially derived from studies of the *Brome Mosaic virus* RNA genome, extends to yeast and human mRNAs highly enriched in membrane and secreted proteins. All Dhh1-dependent mRNAs, viral and cellular, share key common features. First, they contain long and highly structured CDSs, including a region located around nucleotide 70 after the translation initiation site, second, they are directly bound by Dhh1 with a specific binding distribution and third, complementary experimental approaches suggest that they are activated by Dhh1 at the translation initiation step. Our results show that ribosome translocation is not the only unwinding force of CDS and uncover a novel layer of translational control that involves RNA helicases and RNA folding within CDS providing novel opportunities for regulation of membrane and secretome proteins.

UNRAVELLING BIOLOGICAL PROCESSES DIFFERENTIALLY INVOLVED IN ACUTE AND PERSISTENT VIRAL INFECTIONS

Argilaguet J[1], Pedragosa M[1], Esteve-Codina A[1,3], Riera G[1]. Peligero C[1], Heath S[1,3] and Meyerhans A[1,2]

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Viral infections can be fundamentally categorized as acute or persistent according to their temporal relationships with their hosts. Acute infections in humans are usually resolved within a few weeks. By contrast, persistent infections, such as the caused by HIV and HCV, are not resolved and develop when immune responses are not sufficient to eliminate the invading virus during the primary infection phase. There is a broad knowledge of individual viral and host factors that in the early infection phase are involved in the fate decision between an acute and a persistent infection outcome. However, a systemic view of the complexity of the host response that finally leads to the establishment of a persistent infection is missing. Thus, we used the Lymphocytic Choriomeningitis Virus (LCMV)-infection mouse model system, which enables us to follow the dynamics of the virus infection and the corresponding host responses with a systems biology approach *in vivo*. Acute or persistent infections were established by inoculating mice with different LCMV-virus doses, and spleen-specific transcriptomes of mice with different infection outcomes were determined. Here, we present the results obtained through a bioinformatic analysis that allows us to define the kinetics of the main biological processes induced in response to virus infection. We have identified transcriptional coexpression networks differentially implicated in acute and chronic infections. These analysis revealed two major observations associated with persistent infections: (i) an early attenuation of the inflammatory response, and (ii) a specific involvement of the Xcl1-Xcr1 communication axis after appearance of T-cell exhaustion. These results contribute to a more comprehensive understanding of the biological features that orchestrate the establishment of a persistent infection.

TITLE: HCV EARLY KINETICS AND RESISTANCE-ASSOCIATED SUBSTITUTION DYNAMICS DURING ANTIVIRAL THERAPY WITH DIRECT-ACTING ANTIVIRALS

Elena Perpiñán[1], Noelia Caro-Pérez[1], Josep Gregori[2,3], Patricia González[1], Concepción Bartres[1], Maria Eugenia Soria[2], Celia Perales[2], Sabela Lens[1], Zoe Mariño[1], George Koutsoudakis[1], Josep Quer[2], Xavier Forns[1], Sofía Pérez-del-Pulgar[1]
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Background&Aim:
Resistance-associated substitutions (RAS) can compromise efficacy of direct-acting antivirals (DAA). Most RAS are detected at baseline and relapse. Little is known, however, about RAS selection at early points during treatment. The aim of this study was to analyze the potential emergence of RAS immediately after the initiation of DAA therapy.

Material&Methods:
Seventy-one patients treated with different DAA regimens were included in the study. Serum samples were collected at baseline, during therapy (hours 4, 8 and 12; days 1-6; weeks 1-10) and follow-up. RAS in NS3, NS5A and NS5B were detected by ultra-deep pyrosequencing.

Results:
Of the 71 enrolled-patients, 63 achieved a sustained virological response, 7 relapsed, and 1 experienced a breakthrough. All non-responder patients, except one, showed RAS either at baseline or relapse. At baseline, L159F(NS5B) together with the fitness-associated substitution C316N, and Y93H(NS5A) were detected in 3, and 1 patients, respectively. These substitutions persisted at high frequency throughout treatment and reappeared at relapse. Occasionally, S122G(NS3), V170A(NS3) and V321A(NS5B) were detected at low frequency during the first 72h, but not selected afterwards. Interestingly, L31V(NS5A), Q80R(NS3), D168E/V(NS3) and R155K(NS3) were only identified at relapse.
Conclusion:
During DAA treatment, early selection of RAS was not observed. This suggests that selection may occur at later stages when HCV-RNA is undetectable but indeed, there is minimal HCV replication in hepatocytes. However, we cannot exclude that RAS selection takes place during the first hours of treatment, when HCV-RNA is still detectable, but these RAS are present in very low proportions below the sensitivity limit of UDPS.

HCV/HIV-1 PATIENTS WITH DIFFERENT STAGES OF LIVER FIBROSIS TREATED WITH IFN-FREE DAAs

Sandra Franco[1], Leire Díez, Juanjosé López[3], Maria Nevot[1], Bonaventura Clotet[1-4], Cristina Tural[2-4] and Miguel Ángel Martínez[1].
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HCV infection accelerates liver fibrosis in patients coinfected with HIV-1. New IFN-free DAA therapies increase the sustained viral response (SVR) in patients with different stages of liver fibrosis(LF), including cirrhotic patients with more than 90% of SVR. Different drug combinations against 3 proteins essentials for HCV viral replication (NS3, NS5A and NS5B) showed more than 95% of response in HCV mono- and co-infected with HIV-1 with different HCV genotypes(gt). Therefore, we decide to investigate the prevalence of resistance mutations(RM) in a well-characterized cohort of 89 HCV/HIV-1 patients that were treated with six IFN-free DAA combinations. Only 7/89(7.8%) patients failed therapy(NR), and RM were found at 12wkPT inNS3gt1a (R155K,D168V,Q80R), in NS3gt4 (A156G,T122I,D168Y) and NS5Agt3a(Y93H). In BL samples of 82patients with SVR(92%), we found RM in NS3 (gt1a:Q80R/L; gt1b:T54S) and in NS5A(gt1a:S122N/G; gt1b:R30Q,L31V,Q62R,Q54H,Y93H). No RM were observe in the NS5B in any of the samples analyzed. HCVgt did not correlate with the appearance of RM. 78/89(87.6%) patients with advanced fibrosis(F3-F4) had SVR and only 7/89(7.8%) with F4 were NRs. When we compare both groups, SVR vs NR, no significant differences were found in CD4+(587vs403,p=0.07), in logHCVVL(6.87vs6.41,p=0.41), ALT (75.4vs 9.3, p=0.85) and AST (89.3vs89.4, p=0.99); HIVVL undetectable in all patients. Resistant mutations at baseline do not predict the response to the new DAAs. Most failing patients developed resistance to targeted NS3 protease or NS5A, however, no SOF resistance was observed in NS5B. The high level of SVR(92%) found in cirrhotic patients urge the need of treat all the infected population to eradicate HCV.

WHOLE-GENOME CHARACTERIZATION OF A NEW HEPATITIS C VIRUS (HCV) GENOTYPE 1 SUBTYPE

Laura Ordeig[a,d], Damir Garcia[a,d], Josep Gregori[a,d,e], Maria Eugenia Soria[a], Leonardo Nieto[b], Celia Perales[a,d,f], Meritxell Llorens[a], Qian Chen[a], Rafael Esteban[a,c,d], Juan Ignacio Esteban[a,c,d], Francisco Rodriguez-Frias[b,c,d], Josep Quer[a,c,d]
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Background: In the last few years the combination of direct-acting antiviral (DAAs) have achieved cure levels of Hepatitis C virus (HCV) infection greater than 95%. It has been demonstrated that DAA therapy is subtype dependent. The methodology of high resolution subtyping based on next generation sequencing has been essential to identify the correct subtype of HCV infecting each patient with an efficiency of 100%. Using this technology, in Vall d'Hebron University Hospital (HUVH), one naïve sample from Guinea Equatorial could not be classified in any of the confirmed subtypes. The aim of this study was to characterize the complete genome of this sample into one pre-established subtype or into a new one.

Materials and methods: The amplification and sequencing of the complete genome was performed by Sanger sequencing and compared to the reference sequences of all subtypes. The NS3, NS5A and NS5B regions were analyzed in order to verify whether resistance associated substitutions (RAS) were naturally present in this sequence.

Results: The phylogenetic study of the whole genome shows that the sample could not be classified as any existing subtype. The sliding-window analysis along the whole genome discards any recombination phenomenon. Moreover, some RAS (Y93H among others) are naturally occurring in the NS5A region, conferring resistance to some inhibitors of NS5A.

Conclusion: This virus belongs to a new HCV genotype 1 subtype and some mutations that are present at basal level may confer resistance to certain DAAs, which could limit the possibilities of treatment for patients infected with this subtype.

ENHANCING THE DIAGNOSIS OF ACTIVE HEPATITIS C VIRUS (HCV) INFECTION THROUGH MOLECULAR TESTING OF DRIED BLOOD SPOTS IN COMMUNITY-BASED CENTRES IN BARCELONA

Verónica Saludes[1,2], Cinta Folch[2,3], Adriana Morales-Carmona[4], Montserrat Jiménez[1], Laura Fernández[2,3], Laia Ferrer[2,3], Adrián Antuori[1], Xavier Majó[5], Jordi Casabona[2,3] and Elisa Martró[1,2*]; HepCdetect I and II Study Groups.

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Background. Promoting HCV diagnosis is a global priority (in Spain, 70% undiagnosed), but the conventional algorithm (serology and molecular confirmation) hampers it in hard-to-reach populations at risk. Alternative strategies are essential to improve the diagnosis and epidemiological data.
Objectives. To set up and evaluate in the community an HCV-RNA detection assay from dried blood spots (DBS).
Methodology. A real-time RT-PCR assay for HCV detection in DBS was set up. Its acceptability and feasibility (versus rapid antibody test) was assessed in men who have sex with men (MSM), male (MSW) and transsexual women sex workers (TSW), and injection drug users (IDU). Epidemiological and behavioural data were collected.
Results. The assay showed a satisfactory detection limit, was precise/reproducible, 94% sensitive and 99% specific. DBS were stable at room temperature for at least 2 months. Acceptability was >95%.
Among the 569 participants at sexual risk (74% MSM), a 0.64% seroprevalence was self-reported. No HCV undiagnosed cases were detected, 6% were HIV positive, gonorrhoea and syphilis were prevalent, 32% reported condomless anal intercourse, and 58% the use of drugs for sex.
Regarding the 201 IDU recruited so far, we observed a 79.6% HCV seroprevalence, a 60.2% prevalence of active infection (20 new diagnoses); 17.9% had never been exposed to HCV.
Conclusions. DBS testing showed a good performance and acceptability. Given the high-risk behaviour and prevalence of other STIs in MSM, MSW and TSW, HCV spread should be monitored. The usefulness of this diagnostic strategy is warranted in IDU and will allow us to characterize the circulating HCV isolates.

A HIGH-THROUGHPUT SCREENING OF AN ADENOVIRUS ENCODED MICRORNA LIBRARY IDENTIFIES ADENOVIRUS WITH ENHANCED ONCOLYTIC ACTIVITY IN PANCREATIC TUMORS

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Pancreatic ductal adenocarcinoma (PDAC) is characterized by a high mortality rate due to late detection, metastatic capacity and resistance to conventional therapies. Patients with this neoplasia could benefit from oncolytic adenoviral (OA) therapy. Despite the progress in the generation of tumor-selective adenoviruses, more potent OA are needed. Cell/virus interactions are key determinants to impact the virus outcome. Cancer cells have acquired a hallmark of traits that may interfere with viral propagation. Since microRNAs (miR) influence many biological processes, and are profoundly deregulated in PDAC, we hypothesized that restoring specific miR activity in PDAC may enhance oncolytic activity.

In this study, we generated a library in an Adwt5 EGFP genome expressing up to 243 human microRNAs. The miR-adenoviral library was bioselected through different infection passages in PDAC cell lines and two microRNAs (miR-A and miR-B) were selected for their capacity to increase adenoviral fitness. Significant increase in viral release was obtained when compared to the parental virus. Interestingly, this effect was tumor-specific since it was not observed in the non-tumoral pancreatic cells HPDE. In line with increased viral production, enhanced cytotoxicity was also observed. In vivo bioselection in patient-derived xenografts confirmed that Adwt5 EGFP miR-A replicated better than the parental virus.

Bioinformatic analysis of miR-A and miR-B targets identified candidate genes susceptible to modulate adenoviral fitness, of special interest were genes related to the regulation of the adenovirus RNA splicing. Currently, we are studying the molecular mechanisms implicated in the miR-candidates adenoviral sensitization.

A43, A CD48 VIRAL HOMOLOG WITH CONSERVED LIGAND-BINDING FEATURES

Pablo Martínez-Vicente[1,2], Domènec Farré[1], Natàlia Pérez-Carmona[1], Pablo Engel[1,2], and Ana Angulo[1,2]

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During evolution, cytomegaloviruses (CMVs) have been capturing genes from their hosts, employing the derived encoded proteins to evade host immune defenses. We have discovered three homologs of CD48, a cell surface receptor of the signalling lymphocytic activation molecule (SLAM) family, in a CMV species (OMCMV) that infects owl monkeys. CD48 interacts with CD244, another SLAM family receptor, regulating target cell lysis by NK cells and cytotoxic T lymphocytes. We report that an ancestral gene of CD48 was captured by retrotrascription by OMCMV and subsequently duplicated forming this viral gene family. The protein products of these genes (A43, A44, and A45) display a significant amino acid identity in their ligand-binding N-terminal immunoglobulin domain with host CD48, notably reaching the 91% in A43. Remarkably, these viral proteins display distinctive features, which include higher N-glycosylated ectodomains and transmembrane regions instead of the characteristic GPI anchor of CD48. We have individually expressed and characterized these three viral CD48 homologs. A44 and A45 do not interact with host CD244, hypothesizing that they diverged to perform new functions. On the other hand, A43 is able to bind to the host receptor with the same intensity than host CD48. Since A43 is produced as a soluble molecule, we anticipate that it may act as a viral decoy receptor interfering with CD244 functions in effector cells. Altogether these results introduce viral CD48 homologs as convenient structural molds for viral evolution and an important new class of immune modulators.

THE INSERTION SITE OF HIV AFFECTS LATENCY REVERSAL

Guillaume Fillion, Center for Genomic Regulation, Barcelona.

To complete the infection cycle, HIV needs to be inserted in the genome of its host cell. This step is critical, as it allows the virus to become latent and to establish a a reservoir of infected cells without any sign of infection. For this reason, patients infected with HIV cannot interrupt the anti-retroviral therapy at any moment, otherwise the virus rebounds and all the symptoms of the infection quickly reappear. How and why HIV goes latent remains mostly speculative. Surprisingly little attention has been brought to the hypothesis that HIV may be silenced by the chromatin of the host. To test this idea, we have a developed a technology called B-HIVE (Barcoded HIV Ensembles) in which we tag viral genomes with a barcode to follow their insertion and expression in the host cell. Using B-HIVE, we have discovered that HIV latency depends on the insertion site. Insertions far from human enhancers are more likely to be latent. In addition, we have used B-HIVE to test several latency reversal drugs and have discovered that their typical targets have distinct insertion sites. Our results thus suggest that the chromatin of the host plays a key role in the fate of the HIV infection and in the perspective of treatment.

THE P25 GENE PRODUCT OF A SPANISH ISOLATE OF *CUCURBIT YELLOW STUNTING DISORDER VIRUS* ACTS AS RNA SILENCING SUPPRESSOR

Mariona Estapé, María Luisa Domingo Calap, Juan José López-Moya & Ana Beatriz Moreno

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Plant viruses are efficient pathogens that can cause severe symptoms and damages. The plant antiviral defence includes RNA silencing, and virus counteract mechanism includes the expression of RNA silencing suppressors (RSS).

Cucurbit yellow stunting disorder virus (CYSDV), (family Closteroviridae, genus *Crinivirus*) infects cucurbit crops and causes important yield losses in our country. In melon plants symptoms include reduction of the size and sugar content on fruits, what leads to a high negative economic impact on this culture. CYSDV is transmitted by whitefly (*Bemisia tabaci*) vectors, and it is often found in co-infection with other unrelated viruses. The genome of CYSDV consists of two molecules of single-stranded RNA of positive polarity (RNA 1 and 2).

Previous studies have shown RSS activity in different criniviruses gene products, including the P25 encoded in the RNA1 of CYSDV. Now, we have analysed two different gene products from the RNA1 of a Spanish isolate of CYSDV, P22 and P25. Using agroinfiltration assays in *Nicotiana benthamiana* plants followed by qRT-PCR quantification of the mRNA corresponding to the co-agroinfiltrated reporter gene GFP, we analysed the ability of both proteins to supress RNA silencing *in vivo*.

Our results confirmed that the P25 protein, but not p22, presents a clear RNA silencing suppression activity. Moreover, further assays showed that P25 cannot prevent the cell-to-cell spread of the silencing signal. Those findings can help to understand the pathogenicity of CYSDV and provide some clues to minimize its negative effect in cucurbits production, in particular when co-infected with other viral pathogens.

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EXPLORING THE PRESENCE OF HYPOTHETICAL TRANS-FRAMED GENE PRODUCTS IN *SWEET POTATO MILD MOTTLE VIRUS* (SPMMV)

Maria Reñé, Juan José López-Moya & Ares Mingot

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Most RNA viruses have small genomes, with sizes being limited by different constrains. To optimize their coding capacity, some viruses adopted peculiar strategies, like the presence of overlapping open reading frames leading to multiple proteins. This strategy is used by all members of the family *Potyviridae* to express an additional P3N-PIPO gene product from a conserved out-of-frame PIPO region starting at a G1-2A6-7 motif. In previous works, our group has characterized another trans-framed gene product in the potyvirus *Sweet potato feathery mottle virus* (SPFMV), which contains another overlapping reading frame named PISPO embedded near the 5' end of the genome. We also reported that P1N-PISPO is expressed through a polymerase slippage mechanism, which generates transcripts containing an extra A residue at a conserved G2A6 motif, and that P1N-PISPO plays an important role as RNA silencing suppressor able to block host defenses.
To gain insights about the possible presence of additional overlapping reading frames in other sweet potato viruses, we analyzed the disease phenotypes caused by two isolates of the ipomovirus *Sweet potato mild mottle virus* (SPMMV) in tobacco plants. The 5' region of the most aggressive isolate SPMMV-0900 was amplified by RT-PCR and sequenced, and compared to the genome of the milder SPMMV-130 isolate, focusing on regions preceded by sequences resembling G1-2A6-7. The presence of similar motifs in SPMMV, and their possible implication in the expression of hypothetical out-of-frame or truncated forms of viral gene products will be discussed.

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FTA® CARDS: AN APPROACH FOR ARBOVIRUS DETECTION

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Treated filter paper cards (FTA cards) have been used as sugar bait in arboviral surveillance. The main aim of the present study was to validate the FTA cards as a tool for arbovirus detection in experimental assays and in entomological surveys. In vector competence assays, laboratory colonies *Culex pipiens* (*molestus* and hybrid form) and *Aedes albpictus* were experimentally infected with medically important arboviruses (DENV, CHKV, WNV, RVFV). FTA cards were used to estimate transmission rate/efficiency. In entomological surveys, FTA cards were used for *Flavivirus* detection and for virome characterization. Entomological surveys were conducted at The Llobregat River´s Delta during the high mosquito abundance season. Sampling locations were selected according to previous evidence of viral circulation. EVS Traps supplemented with standard FTA cards were placed at each location for specimen captures. Captured specimens and FTA cards were molecularly analysed by conventional RT-nPCR and by New Generation Sequencing (NGS). In vector competence assays, positive FTA cards showed *Aedes albopictus* as a competent vector for CHKV_ITA, DENV_1, Wn_lin 2 and RVFV, and *Culex pipiens* hybrid form as a competent vector for Wn_lin 2 and RVFV. In entomological surveys, flaviviruses were detected in *Culex pipiens* and *Aedes albopictus* pools. On the other hand, a variety of viral families (*Flaviviridae, Bunyaviridae, Rhabdoviridae*, *Mesoniviridae*) were able to be detected in FTA cards pools using NSG. FTA cards showed their usefulness in arboviral detection, but further investigation is required to show their relevance as an alternative tool for mosquito sampling in zoonontic arbovirus endemic areas or areas with risk of arbovirus introduction.

ENTEROVIRUS: UN "GAP" EN LA VIGILANCIA

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Enteroviruses are small single-stranded RNA viruses in the Picornaviridae family. All viruses in the Picornaviridae family are small (18–30 nm), non-enveloped, and single-stranded positive-sense RNA viruses. Enteroviruses are major contributors to disease worldwide, with a wide range of clinical features ranging from asymptomatic or mild to fatal infections.

Enterovirus D68 (EV-D68) belongs to the enterovirus D species. EV-D68 is mainly related to respiratory infections. EV-D68 was first identified in 1962, and up to 2014 few cases were reported. However, the unprecedented outbreak of EV-D68 in 2014 in USA and Canada, which resulted in an upsurge of hospitalisations and admissions to intensive-care units, has prompted concern about a potential uncontrollable epidemic of severe lower respiratory diseases with neurological complications.

Enterovirus A71 (EV-A71) belongs to the enterovirus A species. It first appeared in California in the 1960s. Most symptomatic EV-A71 infections manifest as a self-limiting hand, foot and mouth disease (HFMD), and only a very small proportion of patients develop severe and life-threatening disease. The largest HFMD outbreaks have largely confined to the Asia-Pacific region. But EV-A71 spread across the world, and EV-A71 also circulated at low levels of activity in America, Europe, and Africa, producing sporadic cases or small local outbreaks, sometimes associated with severe neurological symptoms, and restricted mainly to young children, as recently in Catalonia (Spain).

The incidence of non-polio enterovirus infections in European countries is unknown. The reported cases of 2014 EV-D68 outbreak or recent EV-A71 outbreaks with severe neurological symptoms might only be the tip of the iceberg, with a potentially larger number of mild cases remaining undiagnosed, challenging the accuracy of estimates for severity and the burden of disease. The lack of knowledge about circulating enteroviruses indicates the need for better clinical and virological surveillance at community and hospital levels.

ASTROVIRUS MLB2 ASSOCIATED WITH MENINGITIS: CASE REPORT AND EXPLORATORY PREVALENCE STUDY

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Abstract
Introduction: Next-generation sequencing (NGS) has identified novel astroviruses in humans and animals. Their pathogenic role is not yet clearly defined, but some have been associated with encephalitis in immunocompromised hosts.
Methods: NGS was performed on cerebrospinal fluids (CSF) of patients with meningo-encephalitis. We identified an astrovirus MLB2 in CSF of an immunocompetent woman with acute meningitis. NGS and two astrovirus MLB2-specific RT-PCR assays were used on several biological specimens of the patient. We performed then a single-centre, retrospective astrovirus MLB2 prevalence study, in which real-time RT-PCR was performed on CSF and stool specimens collected from hospitalized patients.
Results: We detected RNA from *astrovirus MLB2 Geneva 2014* in the case patient's CSF, anal swab, urine, and plasma specimens during the acute phase of the disease; the anal swab specimen, where the whole viral genome sequence could be obtained, contained the highest viral load (25 cycle threshold (CT) value). Plasma and stool specimens collected eight months after resolution of the meningitis were negative. Phylogenetic analysis based on the full-length sequence identified an astrovirus MLB2 that shared 98.5% nucleotide sequence identity to "astrovirus MLB2 isolate MLB2/human/Stl/WD0559/2008".
Of the stool specimens collected from 615 additional patients, six specimens, mainly from immunocompromised children, contained MLB2 RNA, while of the CSF specimens collected from 404 patients, one was positive.
Conclusions: We have demonstrated that astrovirus MLB2 is a cause of acute meningitis in immunocompetent humans too. Its identification in stools and CSF of additional patients is relevant and supports that astrovirus MLB2 is circulating in our population.

NOROVIRUS SHEDDING AMONG FOOD AND HEALTHCARE WORKERS EXPOSED TO THE VIRUS IN OUTBREAK SETTINGS

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Noroviruses (NoV) are the leading cause of nonbacterial outbreaks of gastroenteritis worldwide. Individuals who are asymptomatically infected may facilitate the transmission of NoV. Our aim was to evaluate the occurrence of NoV infections among workers exposed to the virus in different outbreak settings. Percentages of subjects who were symptomatically or asymptomatically infected were determined for different type of outbreak and concentrations of NoV shedding were analyzed in correlation with symptoms.

We screened feces from food handlers and healthcare workers related with gastroenteritis outbreaks, and shedding concentrations over time were calculated from serial samples collected in approximately one-week intervals of infected individuals. Sequence analysis from different genomic regions were used to evaluate linkage between asymptomatic employees and outbreak cases. NGS approaches were also used in two selected outbreaks.

Of all employees, 59.1% were positive for NoV, and more than 70% of them were asymptomatic. Asymptomatic infections were significantly more frequent in foodborne compared to person-to-person transmitted outbreaks and in restaurant and hotels compared to nursing homes and healthcare institutions. Mean viral loads were similar between symptomatic and asymptomatic individuals, starting at 7.51±1.80 and 6.49±1.93 $\log_{10}$ genome copies/g, respectively, and after almost 3 weeks, average viral loads decreasing but were still 5.28±0.76 and 4.52±1.45 $\log_{10}$ genome copies/g.

In the setting of a NoV outbreak, workers show a high risk of becoming infected. Since the amount of viruses shed by asymptomatic subjects is also high, reinforcement of hygiene practices among workers is relevant to reduce the risk of virus secondary transmissions, even in the absence of symptoms.

EMERGING CORONAVIRUSES: LESSONS FROM THE PAST AND FUTURE CHALLENGES

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The emergence of viruses causing diseases in humans is constant in History. Most emergent viruses have been transmitted from animal hosts to humans (zoonosis). Coronaviruses (CoVs) have frequently crossed the species barriers and two novel coronaviruses have caused important zoonosis in the twenty-first century. Severe acute respiratory syndrome coronavirus (SARS-CoV) emerged in 2002 in South East China and Middle East respiratory syndrome coronavirus (MERS-CoV) emerged in 2012 in Saudi Arabia. Both viruses cause acute respiratory distress syndrome and are associated with high mortality rates, around 10% and 35% respectively. However, mortality rates higher than 50% are observed in the aged and immunosuppressed populations.
The identification of the genes involved in CoV virulence and in signaling pathways contributing to pathogenesis has been addressed using SARS- and MERS-CoVs in order to develop effective therapeutic and preventive strategies that can be readily applied to new emergent coronaviruses. Using a reverse genetics system, SARS-CoV envelope E gene (E) has been deleted leading to an attenuated phenotype (SARS-CoV-ΔE). The expression of proinflammatory cytokines was reduced in the lungs of mice infected with a mouse adapted SARS-CoV-MA15-ΔE compared to lungs infected with the wild type virus. In infections by SARS-CoV with and without E protein, NF-κB was the only proinflammatory pathway differentially activated. Interestingly, the addition of an inhibitor of NF-κB led to a reduced inflammatory response after SARS-CoV infection and to an increase in mice survival. Therefore, these inhibitors could serve, in principle, as antivirals. A reduction in neutrophil migration to lung-infected areas was observed in mice infected with SARS-CoV-MA15-ΔE, probably contributing to the lower degree of inflammation detected and to SARS-CoV-ΔE attenuation. SARS-CoV E protein is a viroporin with different functional domains: a transmembrane region with ion channel activity and a PDZ binding domain mapping at the most carboxy-terminus. Alteration of these domains attenuated the virus, and the mechanisms of attenuation have been studied. These attenuated mutants provided long-term protection both in young and elderly mice against the challenge with pathogenic SARS-CoVs. Deletion of E gene in MERS-CoV using a reverse genetics system, led to a replication-competent propagation-defective virus that is a safe vaccine candidate. These data indicated that SARS-CoV and MERS-CoV with E protein deleted or modified are promising vaccine candidates.

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