Societat Catalana de BIOLOGIA

XIV Jornada de Virologia

Organitzada per la Secció de Virologia de la SCB

INSTITUT D´ESTUDIS CATALANS

Carrer del Carme 47

Barcelona

30 de novembre de 2015

XIV Jornada de Virologia
BCN Virology Meeting 2015

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: Albert Bosch

9.30 h
Opening 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.

10.10h

Dromedary camel as an experimental animal model for MERS-CoV
Júlia Vergara, Centre de Recerca en Sanitat Animal IRTA-UAB, Campus UAB Bellaterra.

10.25 h
Waterborne and foodborne human emerging viruses
Albert Blanco, Enteric Virus Laboratory, Department of Microbiology, University of Barcelona and Department of Botany and Microbiology, College of Science, King Saud University, Saudi Arabia.

10.40h
Vector Competence of European *Culex pipiens* (Diptera: Culicidae) mosquitoes for Rift Valley fever Virus
Marco Brustolin, Centre de Recerca en Sanitat Animal IRTA-UAB, Campus UAB Bellaterra, Centro de Investigación en Sanidad Animal, CISA-INIA, Valdeolmos.

10.55h
Bats: an important reservoir hosts for zoonotic viruses
Jordi Serra-Cobo, Animal Biology Department, University of Barcelona, Barcelona.

11.25-12.00 h
PAUSA I CAFÈ / COFFE BREAK

SESSIÓ II/ SESSION II.
MODERADOR / CHAIR: José Esté

12.00 h
The PD-1/PD-L1 pathway and its impact on regulatory T cells from HIV-infected individuals
Cristina Peligero, Infection Biology Laboratory, Universitat Pompeu Fabra, Barcelona.

12.15 h
HIV-1 induces p21-mediated cellular senescence in human primary macrophages
Eva Riveira-Muñoz, AIDS Research Institute – IrsiCaixa and Health Research Institute Germans Trias i Pujol, Hospital Germans Trias i Pujol, Universitat Autònoma de Barcelona, Badalona.

12.30 h

A novel HIV-1 latency reversing bromodomain inhibitor

Erik Abner, Institut de Biologia Molecular de Barcelona, CSIC, Barcelona.

SESSIÓ III / SESSION III.
MODERADOR / CHAIR: Sofia Pérez del Pulgar

12.45 h

Synonymous virus genome recoding as a tool to impact viral fitnes

Miguel Angel Martínez, AIDS Research Institute – IrsiCaixa and Hospital Germans Trias i Pujol, Badalona.

13.15 h

Phylogenetic analysis of an epidemic outbreak of acute hepatitis C in HIV-infected patients by massive sequencing

Noelia Caro Pérez, Liver Unit, Hospital Clínic, IDIBAPS, CIBERehd, Infectious Diseases Service, Hospital Clínic, IDIBAPS, Liver Unit, Vall d'Hebron Institut de Recerca-Hospital Universitari Vall d'Hebron, CIBERehd, Roche Diagnostics SL. , Sant Cugat del Vallès, Centre Diagnòstic Biomèdic, Hospital Clínic.

13.30 h

Reassessing the hepatitis A virus enterohepatic cycle: a new take on HAV transcytosis in polarized hepatocytes

Montserrat de Castellarnau, Enteric Virus Laboratory, Department of Microbiology, University of Barcelona, Barcelona.

13.45-15:15
DINAR / LUNCH

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

15.15 h

Studies with brome mosaic virus reveal a new role of the exonuclease Xrn1 in viral and cellular translation

Bernat Blasco-Moreno, Virology Unit, CEXS, Universitat Pompeu Fabra , Barcelona and RNA Biology Group, Max Planck Institute for Molecular Biomedicine, Münster, Germany.

15.30 h

Positional cloning of *cmv1*, a gene for resistance to *Cucumber mosaic virus* in melon

Laura Pascual, Centre de Recerca en Agrigenòmica, CSIC-IRTA-UAB-UB, Campus UAB Bellaterra.

15.45 h

Construction of full-length clones of the genomic RNAs of the crinivirus *Cucurbit yellow stunting disorder virus* (CYSDV)

Bader Arouisse, Centre de Recerca en Agrigenòmica, CSIC-IRTA-UAB-UB, Campus UAB Bellaterra.

16.00 h

Immunization with a deletion mutant of African Swine fever virus confers protection against homologous and heterologous lethal challenges

Paula López-Monteagudo, Centre de Recerca en Sanitat Animal, IRTA-UAB, Campus UAB Bellaterra ,

Boehringer Ingelheim Veterinary Research Center, Hannover, Germany, Centro de Biología Molecular Severo Ochoa CSIC-UAM, Madrid.

16.15-16.45 h
PAUSA I CAFÈ / COFFE BREAK

SESSIÓ IV/ SESSION IV.
MODERADOR / CHAIR: Andreas Meyerhans

16.45 h
Closing Lecture
Role of alphavirus replicase in innate antiviral responses
Andrés Merits, University of Tartu, Tartu, Estonia.

17.30 h
SUMMARY OF THE MEETING AND BEST PRESENTATION AWARD

EMERGING CORONAVIRUSES: LESSONS FROM THE PAST AND FUTURE CHALLENGES

Isabel Sola, J. L. Nieto-Torres, J. M. Jimenez-Guardeño, J. A. Regla-Nava, C. Castaño-Rodriguez, R. Fernandez-Delgado, Javier Canton-Bailon, Javier Gutierrez-Alvarez, S. Zuniga, and Luis Enjuanes.
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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.

DROMEDARY CAMEL AS AN EXPERIMENTAL ANIMAL MODEL FOR MERS-COV

Vergara-Alert, J[a]; Solanes, D[a]; Bensaid, A[a]; Segales, J[a,b]

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Middle East respiratory syndrome coronavirus (MERS-CoV) infections cause ongoing outbreaks in humans, and dromedary camels (DC, *Camelus dromedaries*) have been shown to be a potential zoonotic risk. Through October 2015 it has caused more than 1,611 cases and 35% fatalities. Similar to other zoonotic viruses, control by vaccination of the reservoir animal species may be one of the first choices to limit the spread of MERS-CoV to humans.

After initial observations showing that DC may have neutralizing antibodies to MERS-CoV, a recent study indicates a link between MERS-CoV infection in camels and in humans in Qatar. Here, the benefits and problems of using DC to study MERS-CoV infections will be described. Moreover, the protective efficacy of an experimental candidate vaccine in this animal model will be discussed. Briefly, a recombinant vaccinia virus MVA expressing the spike protein of MERS-CoV conferred protection against this viral infection and camel pox in DC.

Acknowledgements:

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WATERBORNE AND FOODBORNE HUMAN EMERGING VIRUSES

Albert Blanco[1*], Islem Abid[2], Susana Guix[1], Francisco J. Pérez-Rodríguez[1], Cristina Fuentes[1], Nawal Al-Otaibi[2], Rosa M. Pintó[1], Albert Bosch[1]

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Emerging viruses include the highly pathogenic Ebola filovirus, SARS-CoV, MERS-CoV, and Hepatitis E virus, but also include new strains of well-known viruses such Hepatitis A virus (HAV) and Human Astrovirus (HAstV).

Traditionally, water concentration techniques focus on non-enveloped virus recovery since these are the main targets in environmental virology. However, some relevant emerging pathogens such as Ebola, and SARS or MERS coronaviruses may be excreted in feces and urine, and potentially become water contaminants. With the aim to recover both enveloped and non-enveloped viruses, we developed and optimized a technique based on glass wool filtration, using Transmissible Gastroenteritis virus (TGEV) and HAV as models for enveloped and non-enveloped viruses, respectively. This method enabled the recovery of 7.41% and 7.47% (final recovering efficiency) of TGEV and HAV, respectively.

Using our optimized glass wool filtration technique, surface and well water samples from Saudi Arabia were concentrated to recover viral pathogens that were then analyzed by molecular techniques. HAV and Human Norovirus GI and GII were detected using a multiplex qRT-PCR and all positive samples were subsequently quantified with a monoplex qRT-PCR. Additionally, for the detection of MERS-CoV, we used a Pan-CoV seminested PCR followed by MERS-specific real-time PCR. Although some samples were positive for HAV, NoV GI and/or NoV GII, all samples were negative for CoV.

THE PD-1/PD-L1 PATHWAY AND ITS IMPACT ON REGULATORY T CELLS FROM HIV-INFECTED INDIVIDUALS

Cristina Peligero[1], Jordi Argilaguet[1], Roberto Güerri-Fernandez[2], Berta Torres[3], Carmen Ligero[3], Pilar Colomer[2], Montserrat Plana[4], Hernando Knobel[2], Felipe García[3] and Andreas Meyerhans[1,5]

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Human immunodeficiency virus (HIV) infection causes a progressive impairment of effector immune responses contributing to virus persistence. The restoration of these responses is essential to achieve a drug-free control over HIV. One strategy that could restore effector immune responses is the relief of the inhibitory signal displayed by the PD-1/PD-L1 pathway on effector cells. Most reports have studied the impact of the PD-L1 blockade on effector cells but neglected possible effects on regulatory T cells (Treg), which are essential players balancing immunopathology and antiviral effector responses. We investigated PD-1 and PD-L1 expression on Treg cells and the impact of *ex vivo* PD-L1 blockade on Treg cells from HIV-infected individuals. We observed that HIV infection led to an increase in the frequency of PD-1+ and PD-L1+ Treg, which correlated with disease progression. *Ex vivo* PD-L1 blockade increased the proliferative capacity of Treg from viremic individuals and increased viral reactivation. In contrast, PD-L1 blockade had no significant effect on the proliferative capacity of Treg from individuals that control viremia, but significantly increased effector CD8 T cell proliferation in all HIV-study groups. Taken together, these data suggest that the net gain of T cell effector function after PD-L1 blockade may critically depend on the plasma viremia of the host.

HIV-1 INDUCES P21-MEDIATED CELLULAR SENESCENCE IN HUMAN PRIMARY MACROPHAGES

Eva Riveira-Muñoz, Roger Badia, María Pujantell, Bonaventura Clotet, Ester Ballana and José A. Esté

AIDS Research Institute – IrsiCaixa and Health Research Institute Germans Trias i Pujol (IGTP), Hospital Germans Trias i Pujol, Universitat Autònoma de Barcelona, Badalona, Spain

Cellular senescence (CS) represents a state of permanent cell cycle arrest in response to a variety of stressors in which cells irreversibly stop dividing but remain metabolically active. HIV-1 infection is a known external inducer of cell cycle arrest and cellular senescence. This state is maintained by either or both the p53/p21 and p16INK4a/pRb tumor suppressive pathways that cross-regulate each other. Here, we show that monocyte differentiation into macrophages with M-CSF led to cell proliferation and susceptibility to HIV-1 infection that, in turn, induced cell cycle arrest. Established HIV-1 infection activated STAT1 phosphorylation, transcription of interferon-stimulated genes and production of β-galactosidase, a marker of senescent cells. In addition, there was an increased expression of p21 and subsequent inactivation of cyclin-CDK2 activity leading to a hypo-phosphorylated active retinoblastoma protein (pRb) and deactivation of E2F1-dependent transcription. Additionally, HIV infection led to downregulation of the ribonucleotide reductase subunit R2 (RNR2) and reactivation of the HIV-1 restriction factor SAMHD1. In conclusion, we demonstrate that G2-M arrest induced by HIV-1 infection is the consequence of a process of cellular senescence mediated by p21 inhibition of CDK2 kinase activity.

A NOVEL HIV-1 LATENCY REVERSING BROMODOMAIN INHIBITOR

Erik Abner, Ming-Ming Zhou, Albert Jordan Vallès

Upon HIV-1 infection, a reservoir of latently infected resting T-cells prevents the eradication of the virus from patients. To achieve eradication, the current repressive therapy must be combined with drugs that reactivate the dormant viruses. Our group has found potential therapeutic agents that are capable of reactivating latent HIV. A combination of biological and virtual screenings of 10,000 compounds provided a hit, a substance initialled MMQO, which does not cause any harmful global T-cell activation and has a lower toxicity than other initial hits.

We also established that the drug does not activate HIV transcription through the canonical transcription factors like Sp1, AP1, NFAT or NFκB. Furthermore, in combination with commonly known viral activators such as PMA, *TNFα* or Prostratin, MMQO induces viral transcription synergistically. RNA expression microarrays with different treatment times on native Jurkat lymphocytes with MMQO confirmed that no specific pathways were saturated, while hundreds of unspecific genes were up-and downregulated. Based on existing data and additional NMR assays, we are fairly confident that the drug's highest affinity is towards the HIV-1 transcription prohibiting first bromodomain of the protein Brd4.

A major downside with the current state of bromodomain inhibitors is the lack of diversity – almost all of the described compounds are derivatives of the benzodiazepine skeleton structure of JQ1 and thus function almost identically and share the similar characteristics. A larger range of basic chemical structures, e.g. MMQO, will prove to be useful in both research and clinical settings to treat a variety of diseases.

SYNONYMOUS VIRUS GENOME RECODING AS A TOOL TO IMPACT VIRAL FITNESS

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Synthetic genome recoding is a novel method of generating viruses with altered phenotypes, whereby many synonymous mutations are introduced into the protein coding region of the virus genome without altering the encoded proteins. Virus genome recoding with large numbers of slightly deleterious mutations has produced attenuated forms of several RNA viruses. Virus genome recoding can also aid in investigating virus interactions with innate immune responses, identifying functional virus genome structures, strategically ameliorating cis-inhibitory signaling sequences related to complex viral functions, to unravel the relevance of codon usage for the temporal regulation of viral gene expression and improving our knowledge of virus mutational robustness and adaptability. The present review discusses the impacts of synonymous genome recoding with regard to expanding our comprehension of virus biology, and the development of new and better therapeutic strategies.

PHYLOGENETIC ANALYSIS OF AN EPIDEMIC OUTBREAK OF ACUTE HEPATITIS C IN HIV-INFECTED PATIENTS BY MASSIVE SEQUENCING

Noelia Caro Pérez* [1], María Martinez-Rebollar [2], Josep Gregori[3, 4], Josep Quer[3], Patricia González[1], Martina Gambato[1], Juan I. Esteban[3], Josep Costa [5] Josep Mallolas[2], Xavier Forns[1], Montserrat Laguno[2] Sofía Pérez-del-Pulgar[1]and Liver Unit, Hospital Clínic, IDIBAPS, CIBERehd, Barcelona, Spain.

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Background and Aim: The incidence of acute hepatitis C (AHC) among HIV-infected men who have sex with men (MSM) has significantly increased in recent years. This increase may be due to factors such as high HCV viral load in blood and semen and the presence of concomitant ulcerative sexually-transmitted diseases. The aim of our study was to investigate the dynamics of HCV transmission in an outbreak of AHC in HIV-infected MSM in Barcelona.

Methods: Between 2008 and 2013, 113 cases of AHC in HIV-infected MSM were diagnosed in the Infectious Diseases Unit, Hospital Clínic, Barcelona. Phylogenetic analysis of the HCV NS5B gene was performed in 80 cases. Viral RNA was extracted from serum samples collected at the time of AHC diagnosis. Massive sequencing was performed using the Roche 454 GS Junior platform. To define possible transmission networks, phylogenetic trees were constructed by maximum-likelihood.

Results: The prevalence of HCV genotypes was: 4d 51% (n=41), 1a 41% (n=33), 1b 6% (n=5) and 3a 1% (n=1). Phylogenetic analysis showed the existence of at least 15 monophyletic groups: 7 clusters of genotype 1a, 7 clusters of genotype 4d and one of genotype 1b. Genotype 4d patients clustered independently from the local controls suggesting the existence of a single source of infection .

Conclusions: HCV infection spreads rapidly among HIV-positive MSM through a local transmission network in Barcelona. The implementation of public health campaigns and treatment interventions with the new direct-acting antivirals will allow the development of strategies to reduce HCV transmission within these groups.

REASSESSING THE HEPATITIS A VIRUS ENTEROHEPATIC CYCLE: A NEW TAKE ON HAV TRASCYTOSIS IN POLARIZED HEPATOCYTES

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Hepatitis A virus (HAV) is a prominent cause of fecal-orally transmitted acute viral hepatitis in spite of an efficient vaccine and improvements in hygienic conditions. HAV is one of the most frequent causes of large-scale outbreaks of foodborne infection; patient age at the time of infection is the most important factor influencing disease severity.

HAV is a picornavirus with unique molecular features, recently shown to exist in a “quasienveloped” form. This study explores the exit process in polarized Huh7-A1 cells, which represent a natural hepatocyte model. The preferential exit route of HAV was through the apical membrane and most viruses present in the canalicular side were quasienveloped particles. Furthermore, we took advantage of two HAV mutants bearing a VP2 replacement that potentially affects its binding to ALIX and thus its invagination into multivesicular bodies and the subsequent generation of the quasienveloped particles. Overall, extracellular release of quasienveloped particles was higher in Huh7-A1 than in FRhK-4 cells, which represent the traditional cell culture system utilized to grow HAV *in vitro*, particularly with mutant viruses. An updated model of HAV entry and exit from human hepatocytes will be presented.

STUDIES WITH BROME MOSAIC VIRUS REVEAL A NEW ROLE OF THE EXONUCLEASE XRN1 IN VIRAL AND CELLULAR TRANSLATION

B. Blasco-Moreno[1], D. Nedialkova[2], S. Leidel[2] and J. Díez[1]

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The highly conserved exonuclease Xrn1 degrades messenger RNAs (mRNAs) after they have been decapped. As the genomes of positive-strand RNA ((+)RNA) viruses mimic cellular mRNAs, Xrn1 has been shown to restrict the expansion of many of these viruses. In contrast, by using a model system that allows the replication of brome mosaic virus (BMV) in yeast here we show that Xrn1 promotes translation of BMV RNA genome. In polysome profile analyses Xrn1 preferentially associates to 40s ribosomal subunit and promotes viral translation initiation. Moreover, this function is linked to the BMV 5'UTR and to a stem-loop structure located in the ORF.

It was previously described that Xrn1 shuttles to the nucleus where it acts on transcription. Analyses of different Xrn1 mutants demonstrate that Xrn1 function in BMV RNA translation is independent of its function in transcription; however an intact exonuclease activity is required. Expression of the nuclear exonuclease Rat1 in the cytoplasm (Rat1ΔNLS) complements cell growth in Xrn1-depleted cells but no BMV RNA translation indicating the requirement of specific Xrn1 features besides its exonucleolytic function.

Importantly, Xrn1 function in translation is extended to some cellular mRNAs. Employing genome-wide translatome studies we identify a set of specific cellular mRNAs that are translationally activated by Xrn1. These mRNAs, as the BMV RNA genome, present a long 5`UTR and structured sequences in the ORFs. Together our results challenge the classical view of mRNA translation and degradation as a linear process and uncovers an unexpected cross-talk between both processes exploited by viruses.

POSITIONAL CLONING OF *CMV1*, A GENE FOR RESISTANCE TO *CUCUMBER MOSAIC VIRUS* IN MELON

Laura Pascual, Ana Giner, Gabor Gyetvai, Michael Bourgeois, Jordi Garcia-Mas, Ana Montserrat Martin-Hernandez
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Infections of *Cucumber mosaic virus* (CMV), type member of the Cucumovirus genus, cause harvest loss in more than 1000 plant species, including important crop plants. The search for naturally resistant cultivars is a successful control measurement against viral infections. In the melon cultivar Songwhan Charmi (SC), the resistance to CMV (with strains classified in subgroups I and II differing in 70% of nt sequence and virulence) is mediated by a complex mixture of qualitative and quantitative genes. One single gene, *cmv1*, confers by itself total recessive resistance to strains of subgroup II by preventing virus transport from the bundle sheath cells, that surround the vein, to the phloem. To confer resistance to the subgroup I strains, at least two other QTLs must act together and cooperatively with *cmv1*.

Here, we report the fine mapping and cloning of the melon *cmv1*, encoding the Vacuolar Protein Sorting 41(VPS41), a gene conserved among plants, animals and yeast, required for post Golgi vacuolar trafficking.

We have screened a recombinant population of 2500 F2 plants and narrowed the region carrying *cmv1* to 132 Kb including a VPS41. We have validated the VPS41 as *cmv1* by generating susceptible SC transgenic lines, expressing the VPS41 allele from a susceptible genotype. Besides, analyzing a collection of melon genotypes, we identified by association the responsible mutation that confers resistance to CMV.
We hypothesize that the virus somehow recruits VPS41 at the bundle sheath cell level, which possibly facilitates its transport to plasmodesmata, allowing encroachment on the phloem.

CONSTRUCTION OF FULL-LENGTH CLONES OF THE GENOMIC RNAs OF THE CRINIVIRUS CUCURBIT YELLOW STUNTING DISORDER VIRUS (CYSDV)

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The crinivirus *Cucurbit yellow stunting disorder virus* (CYSDV) is an emergent plant pathogen frequently found in combination with other viruses in cucurbits. However, its contribution to synergistic diseases during mixed infections is largely unknown. CYSDV is particularly difficult to manage because it is restricted to the phloem (the usual rub-inoculation method cannot be applied), and its propagation is only feasible by grafting or transmission with *Bemisia tabaci* whiteflies.

In our laboratory we aim to characterize in melon plants mixed infections of CYSDV with other viruses, like aphid-transmitted potyviruses. Full-length infectious clones (FLCs) of potyviruses are available, but for CYSDV we must use viruliferous vectors, a time-consuming and poorly efficient procedure. Thus, we began the construction of molecular tools for CYSDV management, using both *in vivo* recombination in yeast, and *in vitro* single-step cloning. Since the genome of CYSDV is bipartide (two RNAs above 8 Kb in length), we adopted a strategy to place cDNA copies of each one in separate plasmid constructs, using plant-specific promoter and terminator cassettes to transcribe the viral RNAs. So far, we succeeded in obtaining the RNA2 clone, corroborating the efficacy of the strategy.

While the first purpose of these clones is to facilitate the maintenance of the virus without insect vector inoculation, additional uses can be envisaged for these tools. For instance, they can serve to analyze by reverse genetics some unknown aspects of the virus biology. Also, they can be useful for rapid and efficient inoculation, allowing screening plant germplasm accessions for virus resistance.

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IMMUNIZATION WITH A DELETION MUTANT OF AFRICAN SWINE FEVER VIRUS CONFERS PROTECTION AGAINST HOMOLOGOUS AND HETEROLOGOUS LETHAL CHALLENGES

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The absence of safe and efficient vaccines against African swine fever virus (ASF), difficult its control. Experimental immunizations with Live Attenuated Viruses have demonstrated to induce efficient protective immune responses, albeit most of the times circumscribed to homologous ASFV challenges. The main objective of our work was to explore the protective potential of BA71ΔfX: a genetically modified LAV deficient on a key virulence factor. Groups of pigs were intramuscularly inoculated once with different amounts of BA71ΔfX and 28 days later were challenged using different ASFV stains. PBS-inoculated pigs were always used as controls in our assays. While 10^3pfu of BA71 killed 100% of the pigs within a week, the same dose of BA71Δfx did not provoke any clinical signs compatible with ASF. 2 out of the 6 pigs immunized with this low dose of BA71ΔfX survived the lethal challenge with the homologous virulent BA71 but the protection afforded by BA71ΔfX was dose-dependent since increasing 30 times the vaccine dose yielded 100% of protection against BA71 lethal challenge and also against the heterologous E75 lethal challenge. In consonance with these results, all pigs intramuscularly inoculated with 10^6pfu of BA71Δfx also survived, in this occasion showing no viremia at any time after challenge. A final experiment was performed, 10 pigs were intramuscularly inoculated with 10^6pfu of BA71ΔfX and then challenged with a lethal dose of Georgia07 and all BA71ΔfX-immunized pigs survived. In spite of these impressive protection results, further work is needed to increase the safety of our vaccine. In conclusion, BA71ΔfX can confer a very solid protection against experimental challenge with lethal homologous and heterologous ASF viruses.

ROLE OF ALPHAVIRUS REPLICASE IN INNATE ANTIVIRAL RESPONSE

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Alphaviruses (family *Togaviridae*) are enveloped positive-strand RNA viruses that are transmitted by mosquito vectors. In their vertebrate hosts alphaviruses cause different diseases. Chikungunya virus (CHIKV) is important human pathogen; symptoms of CHIKV infection include fever, joint pain and arthritis. Semliki Forest virus (SFV) is related to CHIKV but does not cause disease in humans. In mice several stains of SFV cause fatal encephalitis.

In cells and in infected organisms alphavirus infection is first limited by innate immune response. Both SFV and CHIKV induce high levels of type-I interferons (IFN) and in animals, lacking such response, their infection is rapidly fatal. It is also known that alphaviruses possess multiple mechanisms that interfere with IFN response; thus there is complicated interplay between alphavirus and host defense systems.

The precursor of alphavirus replicase is expressed directly from viral RNA genome. This so-called ns-polyprotein is processed into mature proteins by its own protease activity and is responsible for synthesis of viral RNAs. As several forms of replicase-made viral RNAs lack elements characteristic for cellular mRNAs they are recognized by cellular patter-recognition receptors such as RIG-I and MDA5. Surprisingly, it was found that SFV replicase is capable for production of RNAs, recognized primarily by RIG-I, even when the replicase is expressed from mRNA that cannot replicate. It was found that SFV replicase uses cellular RNAs to synthesize partial complementary RNA strand leading to molecules that are recognized by RIG-I. Importantly, this process also occurs in SFV infected cells and almost certainly also *in vivo*. Furthermore, it was found that neurovirulence of SFV correlates perfectly with ability of virus replicase to synthesize these non-canonic RNAs and elicit high level of IFN. This finding demonstrates that IFN response is not only protective; if over-activated it may lead to immune pathology and facilitate alphavirus replication in central nervous system.

The ability to convert cellular RNAs into IFN-inducing molecules was not universal. Thus, it was found that replicase of CHIKV completely lacks such function; this finding is in line with observation that compared to SFV CHIKV is much less efficient IFN inducer and more susceptible to antiviral effects of IFN. Mutations, known to prevent shutdown of host antiviral responses, did not result in ability of CHIKV replicase to produce IFN-inducing RNAs from cellular RNAs. Comparison of SFV and CHIKV replicases indicated that different speed of replicase complex maturation may represent the major difference between these two viruses. Coherently, mutations that slowed down CHIKV replicase maturation (processing of replicase precursor protein) converted CHIKV replicase into enzyme capable for synthesis of non-canonical IFN inducing RNAs. This indicates that ability to use cellular RNAs to induce IFN response is intrinsic for immature, but not mature, replicase complexes of alphaviruses.

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